

TRANSACTIONS
of the
NATIONAL ACADEMY of
SCIENCE and TECHNOLOGY
Philippines

2000
Volume 22



**National Academy of Science and
Technology Philippines
Department of Science and Technology**

**TRANSACTIONS OF THE
NATIONAL ACADEMY OF SCIENCE
AND TECHNOLOGY PHILIPPINES**

22nd ANNUAL SCIENTIFIC MEETING

Addressing Challenges in the New Millennium for Science and Technology

5-6 July 2000, Manila Hotel

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ISSN 0115-8848

Trans. Nat. Aca. Sci. Tech. Philippines
Vol. 22 (2000)

Published by the National Academy of Science and
Technology Philippines
2/F Philippine Science Heritage Center
DOST Complex
Bicutan, Taguig, Metro Manila 1631 Philippines
nast@mozcom.com
<http://www.dost.gov.ph/nast>
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TABLE OF CONTENTS

	Page
Welcome Address	
Addressing Challenges in the New Millennium for Science and Technology	
Acad. Perla D. Santos Ocampo	1
Keynote Address	
Human Reason, Science and Philosophy	3
Acad. Onofre D. Corpuz	
Resolutions	7

PLENARY PAPERS

The Detection of Climate and-Environmental Changes from Satellite and in situ Observations	11
Josefino C. Comiso	
A Look at the Transportation Situation in Metro Manila and Mitigating Measures to Alleviate the Impacts of Traffic	37
Hussein S. Lidasan	
Sustainably Productive Agriculture and Genetically Modified Crops	71
Acad. Emil Q. Javier	

TECHNICAL PAPERS

Mathematical, Physical and Engineering Sciences

Henstock Integration in a Hilbertian Countably Normed Space with Nuclearity	83
Sergio R. Canoy Jr.	

Measurement of Cosmic Ray Flux in Iligan City	91
Rosemarie R. Terio, Eusebio Ninofranco, Roelison Solidum and Angellias M. Bacala	

Biological Sciences

Influence of Elevated CO ₂ and Photon Flux Density of Growth Carbohydrate Content, and Survival of <i>Pinus radiata</i> Shoot Cultures Supplied with Varying Sucrose Levels	105
Merab A. Chan and Jann Conroy	
Cytogenetic Effects of Aqueous Bark Extract of Duhat (<i>Syzygium Cumini</i> L.) and Leaf Extract of Periwinkle	119
Merlyn S. Mendiola, Jonathan S. Cu, Krishlex Anthony G. Gruezo, Marites C. Palma, Luisa N. Villanuel, and Rosalina T. Tandang	

Agricultural Sciences

Antagonistic Plants for the Management of the Rice Root-Knot Nematode <i>Meloidogyne graminicola</i> in a Rice-Onion System	131
Ruben M. Gapasin, Sally A. Miller and C.V. Sanchez	

Health Sciences

A Simple Model for School-Based Intestinal Helminths Control Using Mass Treatment: Initial Results	137
VY Belizario, Jr., Maria Lourdes E. Amarillo, Ana Belinda E. de los Reyes, Winifreda U. de Leon, Antonio D. de Guzman, jr. and Mark Philip G. Bugayong	
Detection of Chikungunya Virus from Sera of Dengue-Suspected Patients in the Philippines	157
S. Inoue, Ronald R. Mañas, Futoshi Hasebe, Kouichi Morita, Kazunori Oishi, Jhoel A. R. Alfon, Jingle R. Candelario, Deu J.M. Cruz, Giselle Espiritu, Alma Gonzales, Mary A. Pancho, Cyathia Mapua, Efren M. Dimasano, Celia Carlos, Akira Igarashi, and Filipinas F. Nativiland	

Social Sciences

Human Resettlement as an Intervention in Community Development	167
Fe L. Porciuncula and Puta Depositario	

Partnering in Agricultural Extension	187
Lutgarda L. Tolentino, Imelda M. Gesmundo, Apolinario L. Zara, Florescia P. Elliot, Juanito B. Reyes, Pedrito R. Banallao, Melicio J. Maghanoy and Moises L. Sardido	

The Social Reproduction of the Medical Profession: The Case of the University of the Philippines-Philippine General Hospital Medical Center	197
Erlyn A. Sana	

Abstracts of Poster Papers Presented during the NAST 22nd Annual Scientific Meeting, 5-6 July, 2000, Manila

Mathematical, Physical and Engineering Sciences

1. Nonsingularity Conditions for Two Classes of Circulant Graphs	211
Leonor Aquino-Ruivivar and Edgardo S. Cureg	
2. Pulsed 1064 nm Nd:YAG Laser Deposition of Titanium on Silicon	212
in an Ambient Nitrogen Environment Jose Omar Amisano, Edgardo Pabit, Marilyn Hui, and Wilson Garcia	
3. Tournaments that are not Residually Graceful	212
Severino V. Gervacio	
4. Assessment of Influential Observations in Principal Factor Analysis	213
Zenaida F. Mateo and Yutaka Tanaka	
5. Introduction to the JLC Study Framework	214
Akiya Miyamoto, Keisuke Fujii, Allister Levi C. Sanchez, and Angelina M. Bacala	
6. Computing in a Beowulf Class Computer System	215
Allen S. Dahili	
7. The Full Pinch Technique Gauge Invariant Higgs Bosson Self-Energy	215
Cesar P. Palisoc	
8. New Measurement of Mean Lifetime of Atmospheric Muons	217
Rosario L. Reserva, Ruelson S. Solidum and Angelina M. Bacala	
9. MBE Growth of ZnTe/Zn (S, Te) Short-Period Superlattices	217
Shirley Tiong-Palisoc, Mathis Korn and Wolfgang Fashinger	
10. Energy Analysis of a "Dragon Kiln" for Firing "Vigan Jars"	219
Samuel S. Franco and James R. Nalandanan	
11. An Investigation of the Potential of Highly Absorbent Material Obtained from Surplus Diapers and Sanitary Napkins as Lahar Conditioner	219
Emilyn Q. Espiritu, Khervin Cheng Chua, and Abigail Torres	
12. Generation of NaCl Loops of Small Order	220
Raoul E. Cawagas	

13.	Immobilization of Toxic Heavy Metals from Academic Waste	221
	Noel T. De Ocampo	
14.	Caustic Soda Recovery in a Bottle Washing Plant Using Membrane Technology	222
	C. Visvanathan and Anna Marie M. Hufemia	
15.	A Composite Indicator for Lake Water Quality Monitoring and Assessment	223
	Noel L. Gauran and Felino P. Lansigan	
16.	Radiological Assessment of Former US Bases: I. Clark Air Base	224
	Emerenciana B. Duran, Teresa Y. Nazarea, Teofilo Y. Garcia, Cecile M. De Vera, Rolando Reyes, Alejandro Q. Nato, Jr. and Antonio A. Asada, Jr.	
17.	Viscosity Sensor Based on a Piezoelectric Quartz Crystal	225
	Dominic S. Cuadra and Fortunato S. Sevilla III	
18.	Processable pH Sensor Based on Conducting Polyaniline	226
	Karen S. Santiago and Christina A. Binag	
19.	Fabrication and Characterization of Conducting Polythiophene and Poly (3-Methylthiophene) Modified Sensors	227
	Christina A. Binag, Jelyn J. Jahon, Glenn Wesley S. Cu and Karina Milagros R. Cui	
20.	Comparison – Continuous Process of Lysine Production Using Immobilized and Free Cells of <i>Coryne-bacterium glutamicum</i>	228
	Chay Binh Pham and Reynaldo V. Odiamar	
21.	Determination of Anionic Surfactants in Fresh Water Bodies in the Philippines by Negative Ion Electrospray Ionization-Mass Spectrometry	228
	Jewel Racquel S. Unson, Cristina Dancel and Fabian M. Dayrit	
22.	Studies on a Lectin Isolated from the Seeds of <i>Dolichos lablab</i> L. (Batao)	228
	Sonia D. Jacinto, Roberto Elias M. Caganda, Luarni Paz Amorico, Erika Joy Flores, Ma. Loreta V. Quitoriano, Marnie Grace I. Sonico, Hans-Joachim Gabius	
23.	Production of Protein-Enriched Banana Peelings for Animal Feed Ingredient	230
	Chay Binh Pham and Jennifer A. Dante	

Biological Sciences

24.	Ecological Risk Assessment for High Environmental Quality	233
	Josefino M. Magallanes	
25.	Multiple-Shooting in Cotyledonary Nodes and <i>Agrobacterium</i> -Mediated Transformation in <i>Pterocarpus indicus</i> Willd. (Fabaceae)	235
	Minda P. Follosco and Katsuki Ishii	
26.	Embryonic Development of "Hanga"	236
	Vivian Tokentino and Presciliano M. Zamora	

41.	The Effect of <i>Tinospora rumphii</i> Boerl on Ovarian 3 β -Hydroxysteroid Dehydrogenase Activate in Rats	249
	Else G. Dapal and Glorina N. Pocsidio	
42.	Design of a Quantitative Behavioral Test for Hyperactivity in Mutant Mice	249
	Francis Isidore Totanes and Cynthia Palmes-Salom	
43.	Hepatotoxicity of Metaaldehyde	250
	Arsemia A. Casauay and Felizardo N. Pulumbani, Jr.	
44.	Assessment of the Acute Toxicity of Surfactants (LAS and CFAS) Using Selected Species of Fish	250
	Emilyn Q. Espiritu, Enrico Cruz, Cristin Dancel and Fabian M. Dayrit	
45.	General Protein Banding Patterns of the Freshwater Prawn, <i>Macrobrachium juncifrons</i> , Collected from Various Sites in Laguna Lake	251
	Ivan-Marcelo A. Duka, Marni E. Cuenco, and Victor Jun M. Ulat	
46.	Purification, Characterization, and Antimicrobial Spectrum of a Bacteriocin from Fermented Sauce Isolate	252
	Evelyn B. Delgado and Ida F. Dalmacio	
47.	DNA Fingerprinting of Cobalt-60 Gamma Radiation-Induced Variants of Foliage Plants Using AFLP-PCR	253
	Teresa Yulo-Nazarea, Alejandro Q. Nato, Jr., and Carol Baldovino-Coloma	
48.	Mitochondrial DNA (mtDNA) Polymorphism in the Asian Honeybee, <i>Apis cerana</i> F. in the Philippines	254
	Lynn S. Villafuerte, Rita P. Laude, and Deborah R. Smith	
49.	Mechanism of DNA Immunization: How DNA Vaccines Initiate Immune Responses	255
	Celia T. Torres-Villanueva, Akiko Iwazaki, Brian H. Barber, and Harriel L. Robinson	
50.	Sequence Analysis of DNA Vaccine Constructs: Determining Potential Risk for Homologous Recombination with the Human Genome and Optimizing Codon Usage	256
	Celia T. Torres-Villanueva and Godwin Vivar	
51.	Use of Ribotyping and Random Amplified Polymorphic DNA (RAPD) to Differentiate Strains of <i>Burkholderia</i> and <i>ropogonis</i>	257
	Rina D. Bagsic-Opulencia, Allen Chris Hayward, and Mark Fegan	
52.	Cloning and Characterization of the Acyl Carrier Protein (ACP) Gene of the Coconut (<i>Cocos nucifera</i> L.) Endosperm	258
	Victor Jun M. Ulat, Merlyn S. Mendioro, Ivan-Marcelo A. Duka, Marni E. Cuenco, Ma. Genaleen Q. Diaz, Rita P. Laude, Antonio Laurena, and Evelyn Mae T. Mendoza	
53.	The Coconut Gene Project: Primer Design	259
	Ivan-Marcelo A. Duka, Marni E. Cuenco, Victor Jun M. Ulat, Merlyn S. Mendioro, Ma. Genaleen Q. Diaz, Rita P. Laude, Antonio C. Laurena, and Evelyn Mae T. Mendoza	

27.	The Culture of <i>Kappaphycus alvarezii</i> (Doty) at the Three Different Water Levels in the Murine Waters of Northern Poblacion, San Francisco, Cebu	237
	Serapion N. Tanduyan	
28.	Variation in Ginger (<i>Zingiber officinale</i> Rosc.) and Related Taxa Using Isozyme Patterns	238
	Remedios R. Roderos and Erna Louise L. Elias	
29.	Correlation of <i>Pandanus</i> Alkaloids to the Taxonomy of <i>Pandanaceae</i>	239
	Maribel G. Nonato	
30.	Littoral Fishes from a Seagrass Area in Samal Island, Davao del Norte, Philippines	240
	Porfirio A. Caronan and Nellie C. Lopez	
31.	The Effects of Salinity on Survival and Growth of Tilapia <i>Oreochromis niloticus</i> Exposed at Various Ages	240
	Arsenia C. Casauay and Christopher P. Gamboa	
32.	Reproductive Development of the Supermale (YY) Tilapia (<i>Oreochromis niloticus</i>)	241
	Annabelle A. Herrera, Rinella Cruz, and The Fish Genetics Breeding Program-Genetic Breeding Program-Genetically Male Tilapia Project	
33.	Morphoanatomy of <i>Vivipara costata</i> Quoy and Gaimard (<i>Mollusca</i> : Viviparidae) During Early Development	242
	Zenaida G. Bacunan, Imelda F. Pagulayan, and Roberto G. Pagulayan	
34.	Diversity of the Molluscan Gastropods, <i>Terebralia sulcata</i> and <i>Cerithidea cingulata</i> in Two Mangrove Areas in Cutandunes, Bicol Region	243
	Jimmy T. Masagca	
35.	Electron Microscope Analysis of Sexual Induction in a Fissiparous Planarian, <i>Dugesia ryukyuensis</i>	244
	Jocelyn Cabrera, Annabelle A. Herrera, Kuzuya Kobayashi, and Motonori Hoshi	
36.	Gill Parasites of <i>Terapon jarbua</i> Forskal from Lingayen Gulf	244
	Nellie C. Lopez	
37.	Isolation of Indigenous Bacteria for the Development of Probiotics in the Biocontrol of Clinically Important Pathogens	245
	Doralyn S. Dalisay, Megan Grace Ursua, Gladys Mae Vasquez, Chiara Villaruz, and Sheila Ureta	
38.	Δ^4 -3-Ketosteroids from <i>Morinda citrifolia</i> L. as Potential Inhibitors of <i>Mycobacterium tuberculosis</i> H37Rv	246
	Jonel P. Saludes, Mary J. Garson, Scott G. Franzblau, and Alicia M. Aguinaldo	
39.	Pigmented Offspring of Albino Mice: Screening for Gain-of-Function Mutations in Five Exons of their Tyrosinase Gene by Multiplex PCR-SSCP Analysis	247
	Paolo Migul F. Mangahas and Cynthia Palmes-Saloma	
40.	<i>Schefflera odorata</i> Inhibitors of Mark Activation in Cultured Airway Smooth Muscle Cells	248
	Maria Cristina R. Ramos, Gloria D. Castro Bernas and Nigel J. Pyne	

Agricultural Sciences

54.	Polymerase Chain Reaction (PCR) Amplification of DNA Genome Segments of Banana Bunchy Top Virus (BBTV)	261
	Teresa B. De Leon and Vermando M. Aquino	
55.	Cloning, Characterization, and Sequencing of Maturation-Related cDNAs from Sugarcane (<i>Saccharum Officinarum</i> L.)	262
	Eliel B. Tumimbang, Antonio C. Laurena, and Evelyn Mae Tecson-Mendoza	
56.	Molecular Cloning of DNA Segments of Abaca Bunchy top Virus (ABTV)	262
	Medino Gedeon N. Yebson, Jr. and Vermando M. Aquino	
57.	Gene Introgression in the Non-Tuber Forming <i>Solanum</i>	263
	Abella C. dela Vina and Dolores A. Ramirez	
58.	Segregation Analysis in Coconut Using Molecular Markers	264
	Shirley J.E. Segovia, Hayde F. Galvez, Ronnel Joey U. Carcallas, Consorcia E. Reaño, Ramon Rivera, Gerardo A. Santos, and Desiree M. Hautea	
59.	Molecular Tagging of Bruchid and Cercospora Leaf Spot Resistance Genes in Mungbean Using AFLP and RGA Markers	264
	Alma O. Canama, Hayde F. Galvez, Desiree M. Hautea, and Conrado H. Balatero	
60.	Regeneration of Transgenic New Plant Type Lines from <i>A. Tumefaciens</i> -Infected Immature Inflorescence	265
	Glen H. Har, Eleanor S. Avellanosa, and Rhodora R. Aldemita	
61.	Molecular Analysis of On-Farm Biodiversity of Rice in the Philippines	266
	Leocadio S. Sebastian, Lorna R. Hipolito, Guada O. Redondo, Alice N. Briones, Girtie Nora A. Abrigo, Cheryl B. Casiwan, and Sergio R. Francisco	
62.	Identification and Characterization of ACC Synthase cDNAs Expressed During Sinta Papaya (<i>Carica papaya</i> L.) Fruit Ripening	267
	Marie-Sol P. Hidalgo, Antonio C. Laurena, and Evelyn Mae Tecson-Mendoza	
63.	Molecular Cloning of the Coat Protein Gene of Papaya Ringspot Virus (Philippine Isolate)	267
	Pieriden A. Perez and Vermando M. Aquino	
64.	Control of Ripening in Papaya by Genetic Engineering	268
	Antonio C. Laurena, Pablito M. Magdalita, Bessie Y. Perez, Evelyn Mae T. Mendoza, Violeta N. Villegas and Jose Ramon Botella	
65.	Development of Vegetable IPM Program in Rice-Based Cropping System	269
	Teotimo M. Aganon, Marilyn G. Patricio, L. Jose I. Calderon, and Jose S. Soriano, Jr.	
66.	Transplanted Irrigated Lowland Rice Program at Philrice	270
	Rodante E. Tabien and Leocadio S. Sebastian	
67.	Improvement of IR64, C4-63G, PSB Rc 4, and BPL-RI-10 for Transplanted Irrigated Lowlands	271
	Rodante E. Tabien, Marlou C. Abalos, Johna R. Casayuran, Yolanda A. Dimaano, Leocadio S. Sebastian	

68.	Analysis of Technical Efficiency of Rice Production in Camarines Norte and Nueva Ecija in the Presence of the El Niño Phenomenon	272
	Flordeliza C. Hidalgo, Christianne Emmanuelle V. Flores and Alejandro H. Herrin	
69.	Rapid Development of Improved Indica Rice Variety Through Anther Culture Technology	273
	Nenita V. Desamero, Yolanda Dimaano, Celia L. Diaz, Leonilo G. Domingo, Emily R. Corpuz, and Rodante E. Tambien	
70.	Comparative Anther Culture Response of Genetically Diverse and Highly Heterozygous Indica Rice to 2,4-D and PAA-Enriched medium	274
	Wilhelmina E. Villalba, Ma. Corazon N. Julaton, Laila B. Sta. Maria, Lenie P. Romano, Martha V. Chico, Sharon S. Macabale, Leonilo G. Domingo and Nenita V. Desamero	
71.	In Vitro Response of Anther Culture-Derived IR64 Breeding Lines	275
	Martha V. Chico, Sharon S. Macabale, and Nenita V. Desamero	
72.	Rice Varieties and Grain Quality Attributes Preferred in Adverse Environment	275
	Marissa V. Romero, Juma Novie B. Ayap, Alice M. Briones, Jesusa M. Cabling, Irene R. Tanzo, and Renee E. Valdez	
73.	Mass Screening for Rice Seedling Salt Tolerance at PhilRice	276
	Noemi S. Dones, Jonathan M. Niones, and Philbert S. Bonilla	
74.	Hunting the Rice Tungro Resistance Gene Using Bacterial Artificial Chromosomes and Resistance Gene Analogues	277
	Gabriel O. Romero, Vivian A. Panes, Leocadio S. Sebastian, and Violeta Tolentino	
75.	PCR-Based DNA Fingerprinting of Anther-Culture Derived Indica Rice Breeding Lines	278
	Sharon S. Macabale, Martha V. Chico, and Nenita V. Desamero	
76.	Genetic Transformation of Rice (<i>Oryza sativa</i> L) Using Pin2 and GNA Genes for Insect Resistance	279
	Reynaldo B. Evara, Violeta N. Villegas, Maria Teresa B. Peralta, Victoria P. Chavez Lapitan, Leocadio S. Sebastian, Ma. Regina C. Garcia, Julieta U. Sajise, Marilou R. Calapardo, Paul Christou, and Rey Wu	
77.	Effect of Mist-Polishing on the Physicochemical and Sensory Properties of Rice	280
	Juman Novie B. Ayap, Nanette V. Zulueta, Evelyn M. Herrera, Rene E. Valdez, Estrella G. Antolin, Tessie Q. Ramirez, and Danilo G. Natividad	
78.	Physicochemical Properties of Iron Fortified Rice Flour	281
	Nanette V. Zulueta and Ma. Patricia V. Azanza	
79.	Rice Noodle Characteristics as Affected by Iron	281
	Nanette V. Zulueta and Ma. Patricia V. Azanza	
80.	Variability in Rice Stem Borer Populations and Its Implications in the Development and Possible Release of BT Engineered Rice	282
	Cesar G. Demayo	

81.	Resistance Screening of Farmer's and Commercial Varieties of Eggplant Against The Leafhopper, <i>Amrasca biguttula</i> (ISHIDA) and the Eggplant Borer, <i>Leucinodes orbonalis</i> Buenece	284
	Merdelyn Caasi-Lit, Victor P. Gapud, Clarinda Pil, Belen Santiago Gina Balagot, N.S. Talekar, and Edwin Rajotte	
82.	Effects of Temperature, pH, and Nitrogen Sources on the Growth and Sporulation of <i>Fusarium</i> Causing Wilt and Root Rot of Garden Pea (<i>Pisum sativum</i> L.)	285
	Asuncion L. Nagpala and Lina L. Ilag	
83.	Management Strategies for Cotton Flower Weevil, <i>Amorphaidea lata</i> Motschulsky	286
	Teodoro S. Solsoloy, Ma. Magdalena C. Damo, Rustico G. David, Estrelita O. Domingo, Fredolin P. Julian, Nenita D. Cacayorin and Benita U. Bilgera	
84.	Rice Hull Burning: A Farmer's Technology for Management of Rice Root-Knot Nematode in a Rice-Onion Cropping System	287
	Evelyn B. Gergon, Oscar Opina, and Santiago R. Obien	
85.	Arthropod Pests of Bamboos: Taxonomy, Biology, Natural Enemies, and Host Plant Resistance	287
	Ireneo L. Lit, Jr., Merdelyn Caasi-Lit, and Ma. Amabel A. Capricho	
86.	Critical Pest Level for Boll Worm, <i>Helicoverpa armigera</i> (HUBN.)	288
	Benita U. Bilgera	
87.	Insecticide and Fungicide Effects of Betel, Piper betle, Volatile Oil on Selected Cotton Pests	289
	Aida D. Solsoloy, Estrelita O. Domingo, Nenita D. Cacayorin and Ma. Magdalena C. Damo	
88.	<i>Argemone mexicana</i> L. (Papaveraceae) Prickly Poppy: A New Non-Quarantine Pest Record in the Philippines	290
	Bonifacio F. Cayabyab, Emma M. Alforja, Carlos L. Padilla, Juanita Bariuan, Rolando G. Bayot, Melvin D. Ebuenga, and Alicia G. Aquino	
89.	Damage Assessment of Lepidopterous Pests of Onion in Nueva Ecija	291
	Bonifacio F. Cayabyab, Carlos L. Padilla, Melvin A. Ebuenga, Rolando G. Bayot, Jeannym R. Adorada, Fredelino Peñalba, Emma P. Perez, and Alicia G. Aquino	
90.	Insect Pests and Natural Enemies from Stored Products in Japan	292
	Marissa V. Romero and Keiichi Takahashi	
91.	A Survey of Butterflies and Skippers (Lepidoptera: Rhopalocera) from Mt. Banahao de Luchan, Quezon Province, Philippines	293
	Ireneo L. Lit, Jr. Orlando L. Eusebio, and Ariel R. Larona	
92.	Growth and Development of Rice Stemborer in an Artificial Diet	293
	Amelia T. Angeles and Genaro S. Rillon	
93.	Butterfly Manure: A Novel Source of Bio-Organic Fertilizer	294
	Bonifacio F. Cayabyab, Rolando B. Bayot, Fredilino P. Peñalba, Alicia G. Aquino, and Florante F. Cayabyab	
94.	Grafted Tomato for Off-Season Production	294
	Lun G. Mateo, Teotimo M. Aganon, James R. Burtleigh, Dennis R. Cacho, and Alex S. Caspilla	

95.	Bamboo Shoots as Substitute Vegetable During La Niña	295
	Merdelyn Caasi-Lit, Roy B. Candelaria, Linda B. Mabesa and Rowena P. Urriza	
96.	Freeze-Drying Characteristics of Mango (<i>Mangifera indica</i> L.) Puree at Three Levels of Pre-Freezing Temperature	296
	Ofero A. Caparino	
97.	Analyses of the Shelf Life of Commercial Tempura and Sauce in Cebu City	297
	Renissa B. Sario and Corazon P. Macachor	
98.	Changes in Soil Properties Associated with Application and Buring of Rice Hulls in Peri-Urban Vegetable Production Area in Central Luzon	298
	Clarita P. Aganon and Orlando Ramos	
99.	Regional Assessment and Collection of Available Dye-Yielding Plants in the Philippines	299
100.	<i>Limncharis flava</i> L. Buch., and <i>Salvinia molesta</i> Mitchell: Potential Threats to Aquatic Ecosystem in Luzon	300
	Emma M. Alforja, Emma A. Perez, and Juanito V. Barican	
101.	Mangrove Community Structure in Carmen, Cebu	301
	Corazon P. Macachor and Severino R. Romano	
102.	The Influence of Body Weight and Diet on the Ammonia Excretion of the African Catfish, <i>Clarias gariepinus</i>	302
	Ronelic Chato-Salvador and Liberato V. Laureta	
103.	Seed Quality Response to Fungicide Treatment of a Line and F1, Hybrid Seeds	303
	Susan R. Brena, Frisco M. Malabanan, and Bonnie M. Valiente	
104.	Pedological Characterization and Agronomic Potential of Soils Associated with Kennon Limestone	303
	Carlito P. Laurean and Rodrigo B. Badayos	
105.	Genetic Diversity Analysis of Philippine Maize Inbred Lines Using Microsatellite Markers	305
	Nancy B. Coronado, Alexander David L. Josue, Peter S. Guzman and Desiree M. Hautea	
106.	Mineral Concentration in the Blood of Grazing Goats and Some Forages in Halar-Laden Ares of Tarlac, Central Luzon	306
	Edgar A. Orden, Alexander B. Serra, Clarita P. Aganon, Emilio M. Cruz, Ma. Excelsis M. Orden, Libertado C. Cruz, and Tsutomu Fujihara	
107.	Selenium Supplementation in Grazing Goats: Effects on Blood and Milk Selenium and Growth Performance of Kids Born to Does Receiving Selenium-Soluble Glass-Bolus	307

Health Sciences

108.	Cyclosporin Analysis in Blood by Automated Reserved-Phase High Performance Liquid Chromatographic Method Coupled With Solid Phase Extraction and Speed Vacuum Evaporation	309
	Ma. Cristina B. Portilla, Melchor V. Cantorias, and Cherrie B. Pascual	
109.	Growth Patterns and Infectivity of of A Dengue-2 Virus Strain Propagated in the Human Myelomonocytic Cell Line K562	310
	Corazon C. Buerano, Kouichi Morita, Futoshi Hasebe, Shingo Inoue, Ronald R. Matias, Filipinas F. Natividad, and Akira Igarashi	

110. The Analgesic Activity of the Alkaloids of (<i>Ipomea Muricata</i>) Jacq. Fam. Convolvaceae: A Correlation of in Vivo and in Vitro Studies	311
Christine De Vera, Charles Felix Simbillo, Marrisa F. Valencia, and Mafel C. Ysrael	
111. Cervical Adenocarcinoma in Filipinos	311
Arsenia A. Casauay, Edna A. Amparado, Sonia D. Jacinto, Annabelle A. Herrera, and Ryan C. Fonanilla	
112. Cytogenetic Abnormalities in Filipino Colon and Colorectal Carcinoma Patients	312
Ma. Luisa D. Enriquez, Irving Tan, Ricardo W. Lo, Pia Donna N. Lorena, Filipinas F. Natividad, and the SLMC Colon Cancer Group	
113. The Indole Alkaloids from the Leaves of <i>Alstonia scholaris</i> (L.) Don Apocynaceae) – Comparative Antimycobacterial Activity and Anticarcinogenicity Against Human Oral Epidermoid Carcinoma Cell-Lines	313
Allan Patrick G. Macabeo, Scott G. Franzblau, Geoffrey A. Cordell, and Ma. Alicia M. Aguinaldo	
114. Molecular Detection of Enteroviruses Associated with Dilated Cardiomyopathy	314
Joyce D. Reyes, Fabio Enrique B. Posas, Ronald R. Matias, and Filipinas F. Natividad	
115. Detection of <i>Helicobacter pylori</i> From Formalin-Fixed, Paraffin-Embedded Gastric Biopsy Specimens; a Strategy for vacA Genotyping	315
Blanquita B. De Guzman, Liza P. Faustino, Ma. Corazon B. Paredes, Francisco V. Narciso, and Filipinas F. Natividad	
116. Primers for Cysteine Proteinase Gene Could Distinguish Pathogenic <i>Entamoeba histolytica</i> from Non-Pathogenic E. dispar	316
Ronald R. Matias, Pia Donna N. Lorena, Roche De Guzman, Filipinas F. Natividad, and Gloria L. Enriquez	
117. Polymerase Chain Reaction-Sequence Specific Primer (PCR-SSP) System for Bone Marrow Typing	317
Leonora T. DL Salda, Jingle R. Candelario, Ludovico Tonoletto, Filipinas F. Natividad, and Raymundo W. LO	

Social Sciences

118. An Analysis of the Seasonal Movements of Palay Prices and Intertemporal Price Efficiency	319
Alice M. Briones	
119. Urban Impact of the Quality of Life of Metro Manila Residents	320
Daniel S. Santos	

Author Index	321
Subject Index	325

Addressing Challenges in the New Millennium for Science and Technology

WELCOME REMARKS

ACD. PERLA D. SANTOS OCAMPO

President, National Academy of Science and Technology

Honored guests, distinguished National Scientists, our beloved Secretary Dr. Filemon A. Uriarte, Jr. of the Department of Science and Technology, eminent scientist and fellow Academician Dr. Onofre D. Corpuz, the honorable Undersecretaries and Assistant Secretaries of the Department of Science and Technology, fellow Academicians, esteemed co-workers in science, friends, ladies, and gentlemen:

The modern English term "science" is derived from the Latin word for knowledge, *scientia* and in the early days referred to knowledge possessed by anyone, be he peasant or king, unschooled or learned man. Nowadays, science has come to be recognized as a special kind of knowledge belonging to a special group of individuals, obtained by strictly defined methods. The word science has therefore become self-limiting. It is the goal of the National Academy of Science and Technology to expand the meaning of the word in order to better serve humanity.

The new millennium brings before us many challenges. Thus, the Steering Committee of this Annual Scientific Meeting led by Academicians Jose O. Juliano and Apolinario D. Nuzarea broadened the theme and addressed several pressing issues in science and technology. Five general concerns were identified: (1) environment and health; (2) climatic and environmental changes; (3) transportation and traffic management; (4) information and communications technology; and (5) modern biotechnology in agriculture. With these concerns in mind, NAST conceived the theme *Challenges in the New Millennium for Science and Technology*.

For the five concerns identified, we invited authoritative experts to speak. As the program unfolds, each of them will be introduced to you and they will share with us their insights and recommendations.

The emerging and re-emerging problems and concerns on health, environment, and climatic changes require immediate and appropriate policies.

The traffic and transportation situations in the country, especially in Metro Manila, are becoming increasingly serious and need special attention and implementable solutions.

With the advancement in accessibility to information and communications technology (ICT), a new kind of economy surfaces in which knowledge plays a vital role in the creation of wealth of the country. We should urgently address the issues and concerns in ICT if we are to survive and prosper in the highly competitive twenty-first century.

Important concerns in biotechnology and genetic engineering leading to sustainably productive agriculture have to be approached vigorously.

As the government's highest advisory body on science and technology, the Academy has the responsibility of advising our policy makers and civil society on all vital concerns. I am delighted that the public and many experts in different scientific fields are present and interested to participate in this very important event, I hope we can put all our bright ideas together to come up at the end of the meeting with resolutions and recommendations that would best serve our country and the whole of humanity.

It is also during the Annual Scientific Meetings that NAST gives its awards. Tomorrow you will witness the winners of science on stage and know more about their contributions to science and technology, including the investiture of an Academician.

I bid everyone in this eventful occasion, warmest welcome, and may this be a fruitful, insightful, and successful meeting. Congratulations on your quest for knowledge so that the blessings of science may be enjoyed by all.

Thank you and good day.

HUMAN REASON, SCIENCE, AND PHILOSOPHY¹

ACD. ONOFRE D. CORPUZ

Member, National Academy of Science and Technology, Philippines

I begin by noting a basic element of scientific thinking. In 1916 Albert Einstein completed his General Theory of Relativity, with conclusions and predictions on space, time, gravitational fields, that modified/corrected Newton's theories. The latter had governed as accepted principles for the previous two centuries. A measure of Einstein as a scientist is that he expressly required that his conclusions and predictions be validated, and he himself stipulated three empirical tests for the purpose. The tests by other scientists confirmed the General Theory, the third test being completed in 1919. And yet, through it all, Einstein retained the openness of mind that is essential to the scientific attitude: he felt that the validation did not yet categorically prove his theories failed any of the tests, then it was clear that they were untenable.

Not all intellectual propositions and writings on life, matter, and the universe are scientific in the Einstein sense. This is when they are not susceptible of validation or empirical verification. In the scientific world the leading examples of non-empirically verifiable thinking are those of Marxist ideology and Freudian teachings. This class of non-empirically verifiable thinking may be termed a *dogma*. A related type of thinking is recognizable as *doctrinaire*, which is characteristic of some religious thinking.

I contrast scientific thinking on the one hand, and dogmatic thinking on the other. The former proceeds from the attitude "I must know, so that I can believe"; while the latter starts with the attitude "I must believe, so that I can know and understand".

But not all human thinking is neatly comprehended in the two classes of the dichotomy. Human beings are rightly said to be endowed with reason and some

¹Keynote Address, 22nd Annual Scientific Meeting, National Academy of Science and Technology, 5-6 July 2000, Manila.

think rationally without being scientists, while others think according to belief without being dogmatic.

This is because human life is larger than either or both science and dogma. The members of the Academy, reputed to be scientists, are not scientists twenty-four hours a day. They have to cope with life and its wondrous complexities of emotion, bias and conviction, ambition, and chance for most of their waking hours. That they make time to think scientifically attests to the fact that they are a rare breed.

Indeed, there is more rational thinking by ordinary individuals than we normally credit them with. An individual, as family head, for instance, whether deprived or rich, schooled or unschooled, makes decisions that cover the moment or look to the future; the decisions are shaped by limited options and knowledge of facts, by resource constraints, and changing life goals. But if, in analyzing the decisions we input the contextual and operating factors, we will have to concede more than a slight degree of rationality in the decisions. They are not scientific, but they are products of reason.

What I am leading up to is the point that when human thinking is freed from constraints, it can become fully rational- and scientific. Scientific thinking, in other words, requires independence of mind and *freedom*.

I return to my introductory note on empirical testing or validation as a basic element in scientific thinking. The scientific mind autonomously incorporated a built-in corrective against error or ambiguity. This corrective is logic in scientific methodology, the principle of *order*. It is this order that disciplines and strengthens scientific theories.

And so we come to two principles that are inherent in scientific thinking: Freedom and Order. Freedom and Order liberate and discipline the scientific mind. Freedom makes thinking a human adventure; Order imposes objectivity and stability of thought.

Next, because human beings are endowed with reason, the principles of freedom and order operate *pari passu* in human communities. The best human communities guarantee freedom to their citizens; but unrestricted freedom leads to anarchy; human communities therefore maintain order among their citizens; but total order leads to totalitarian control not only of thinking but also of behavior. In the end, the truly best communities maintain a delicate balance of freedom amidst order, and order amidst freedom. It may be concluded that science flourishes most in communities where there is this balance between freedom and order.

* * *

We turn, finally, to philosophy and science. The word "philosophy" is derived from a compound of the Greek "philo", love; and "sophia", wisdom. Philosophy is the love of wisdom, and a philosopher is a lover of wisdom.

The word "science" is derived from the Latin root and means the acquisition of knowledge through study. Scientific thinking focuses on the knowable, beginning with empirical data, then analyses of their interrelationships, theoretically formulated in equations of conceptual variables and arriving at logical conclusions. The power of scientific thinking and the knowledge it has generated are awesome. Einstein's theories alone led to the making of the atomic bomb, space travel, electronics, and quantum physics; works by a host of other scientists made possible impressive increases in food production and still continuing advances in medicine, to refer to only a few cases.

So - science, in generating knowledge, expands the options of human beings and communities. But it is not scientists who decide what use is to be made of scientific knowledge. It is human communities, through political leadership, that decide on the ends and purposes they aim to attain with scientific knowledge. In sum, science *per se* is descriptive, not prescriptive.

The acknowledged early masters of philosophy are the Greeks Socrates who lived ca. 469-399 B.C. and Platon, ca. 427-347 B.C. Their thinking was as rational and logical, they were as inquisitive, as modern scientists. But their concern was different. Their concern was based on the proposition that it is the nature of the human being to strive to perfect himself or herself, and that the human community must seek the perfection of the individual. In short, Socrates and Platon were concerned with the end, the good, the WHY? of human life. Their thinking was aimed at philosophical *wisdom*, the ability to judge on right and wrong in life.

If scientific knowledge explains the HOW? of phenomena and matter in the universe, philosophical wisdom defines the WHY? in human life and conduct. The wisdom of philosophy is prescriptive.

The disciple of Socrates and Platon, Aristoteles (384-322 B.C.), faithfully continued the concern of his masters with philosophy. But he extended his own labors into physics, biology, logic, metaphysics, and ethics. In a sense, he married philosophy with science. Aristoteles's influence is still felt in modern times. St. Tomasso Aquino, the official philosopher of the Roman Catholic Church, was a avowed Aristotelian.

* * *

You will have observed that I had recourse to some truisms in my presentation. I asserted that human beings are endowed with reason. It is the intrinsic tendency to reason that distinguishes human life on the one hand, from non-human life and inanimate physical matter on the other. It is reason in human society that makes both science and philosophy possible. In return, society's options are broadened by scientific knowledge, and society's choices among its options are directed to what is right and beneficent by philosophical wisdom.

I cannot close without saying how privileged and honored I am to have been asked to speak before this distinguished audience.

Republic of the Philippines
NATIONAL ACADEMY OF SCIENCE AND TECHNOLOGY
DOST Complex, Bicutan, Taguig, Metro Manila

RESOLUTIONS

WHEREAS, issues on health care, environment, climate change, transportation and traffic management, information and communications technology, and modern biotechnology are vital and pressing concerns that continue to affect the daily lives of Filipinos;

WHEREAS, cognizant of the paramount importance, multidimensions, and complex interrelationships of these factors, the NAST adopted "Challenges in the New Millennium for Science and Technology" as its theme during its 22nd Annual Scientific Meeting (ASM) held on 5-6 July 2000 at the Manila Hotel;

WHEREAS, the ASM provided a venue for the generation, flow, and exchange of ideas on the issues from invited experts, speakers, and participants;

WHEREAS, the Government and society need to address the issues and concerns on health care, environment, climate change, transportation and traffic management, information and communications technology, and modern biotechnology in the dawn of the New Millennium;

HEALTH

WHEREAS, the prices of drugs in the Philippines are much higher than those in neighboring Asian countries and way beyond the reach of the majority of Filipinos;

NOW, THEREFORE, the NAST, as the Government's highest advisory body on science and technology, hereby resolves that the following proposals be immediately acted upon by the proper authorities:

Support transformation of products of research on bio-active components into commercially viable local medical products;

Support the development of support systems and incentives for the development of drugs from locally available bio-resources.

CLIMATIC AND ENVIRONMENTAL CHANGES

WHEREAS, historical surface and satellite data show that global warming is enhanced by greenhouse gases such as carbon dioxide that is emitted through the human use of fossil fuels;

WHEREAS, satellite technology is a powerful tool in detecting climate and environmental changes;

NOW, THEREFORE, the NAST, as the Government's highest advisory body on science and technology, hereby resolves that the following proposals be immediately acted upon by the proper authorities:

Support the establishment of improved database network to monitor climate and environmental changes that are of local interest. This will include the use of satellite data and the establishment of more meteorological stations around the country;

Support the development of a sophisticated numerical model to simulate local climate conditions and be able to forecast future conditions;

Support the establishment of a program that will generate greater awareness of human impacts on climate and environment. More specific examples are the use of less fossil fuel, proper disposal of harmful chemicals, less deforestation, and encouragement of curtailed population growth.

TRANSPORTATION AND TRAFFIC MANAGEMENT

WHEREAS, there is a real and pressing traffic congestion problem in Metro Manila;

WHEREAS, there is a need to improve existing solutions to the traffic congestion problem;

WHEREAS, conditions of traffic facilities in Metro Manila are fast deteriorating;

WHEREAS, there is a need to improve coordination among government agencies involved in transportation and traffic management;

NOW, THEREFORE, the NAST, as the Government's highest advisory body on science and technology, hereby resolves that the following proposals be immediately acted upon by the proper authorities:

Support the construction of new roads over highways in Metro Manila;

Support the rehabilitation of existing road networks;

Support the introduction of mass transport systems;

Support the application of short-term travel demand management schemes, e.g., one-way scheme, car-pooling, bus lanes, etc.

Support programs to retain, retool, and educate traffic enforcement agencies and the general public.

INFORMATION AND COMMUNICATIONS TECHNOLOGY

WHEREAS, the Philippines needs to transform its economy into a knowledge-economy;

WHEREAS, e-commerce and e-business are now possible;

NOW, THEREFORE, the NAST, as the Government's highest advisory body on science and technology, hereby resolves that the following proposals be immediately acted upon by the proper authorities:

Support the initiation of incentives for setting up of e-business in cyberparks which are situated nationwide.

BIOTECHNOLOGY

NOW, THEREFORE, the NAST, as the Government's highest advisory body on science and technology, hereby resolves that the following proposals be immediately acted upon by the proper authorities:

Support safe and responsible applications of modern biotechnology to agriculture and food production, health and medicine, environmental protection, and trade and industry;

Support the information and educational campaigns on modern biotechnology to enlighten the public about the benefits and risks of the technology;

Support government efforts in biotechnology; and

Support regulatory bodies for biotech activities and products, and biotech research institutions in the universities in their efforts to develop biotechnology.

RESOLVE AS IT IS HEREBY RESOLVED THAT THE NAST (PHILIPPINES) ADOPTS THIS SET OF RESOLUTIONS FOR ENDORSEMENT TO THE DEPARTMENT OF SCIENCE AND TECHNOLOGY (DOST), DEPARTMENT OF AGRICULTURE (DA), DEPARTMENT OF HEALTH (DOH), DEPARTMENT OF ENVIRONMENT AND NATURAL RESOURCES (DENR), DEPARTMENT OF TRADE AND INDUSTRY (DTI), DEPARTMENT OF TRANSPORTATION AND COMMUNICATIONS (DOTC), AND OTHER RELEVANT AGENCIES FOR CONSIDERATION AND ACTION.

DONE this 6th day of July 2000 in Manila, Philippines.

THE DETECTION OF CLIMATE AND ENVIRONMENTAL CHANGES FROM SATELLITE AND IN SITU OBSERVATIONS

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ABSTRACT

The observed positive trends of anthropogenic CO₂ and other greenhouse gases in the atmosphere has been linked to human activities and may cause a profound influence on climate. Global surface temperatures, as observed by meteorological stations, have been increasing at the rate of 0.4 K per decade during the last century. Surface and satellite data show that warming actually accelerated during the last century. Surface and satellite data show that warming actually accelerated during the recent decade providing evidence that the greenhouse induced change may already be occurring. This phenomenon is studied in detail in the polar regions where climate change signal is expected to be amplified due to feedback effects associated with the high albedo of ice and snow. The 50- to 100-year data records from polar stations show consistently higher warming trends in both regions than global trends. The 20 year satellite record and corresponding in situ data, however, show symmetric effects: a warming and a sea ice retreat in the Arctic but a slight cooling and a sea ice cover advance in the Antarctic. This counter-intuitive observations are actually supported by a previous simulation study of greenhouse warming effects using a global circulation model. Satellite data also reveal large regional variability countries. Ensuing changes in the environment and associated impacts on society may be drastic and costly depending on location. The strategy to mitigate the impacts must include a good understanding of the Earth's climate system.

Key words: climate change, environment, warming, trends, sea ice,

1. INTRODUCTION

Throughout history, the way of life of human societies has been profoundly influenced by climatic events. Some civilizations have greatly benefited from occurrences of favorable climates while some others have even disappeared because

of failure to adjust to adverse climatic conditions. There have been major ones such as the ice ages, that kept the northern hemisphere in deep freeze for a long time, and relatively minor ones, such as the El Nino, that have relatively short durations but can be difficult to cope with. The El Nino of 1997 to 1998, for example, came out as a very damaging climatic event since it caused the lost of many lives and billions of dollars in property damages.

Climatic events have been regarded as part of natural cycles that are driven by external forces such as changes in the luminosity of the sun and the orbital parameters of the Earth. The discovery of increasing levels of CO_2 in the atmosphere at Mauna Loa, Hawaii in 1980, however, started a big debate on the possible influence of human activities on climate. CO_2 is on the greenhouse gases in the atmosphere that keeps radiation from escaping the Earth and thereby causes surface warming (Daly, 1989). It has been postulated that the observed acceleration in the rate of increase in temperature in recent years is caused by such greenhouse warming (Hansen et al., 1984). Recent reports about the retreat and thinning of sea ice, the disappearance of glaciers, the rising sea level, and the calving of big icebergs (Parkinson et al., 1999 Rothrock et al., 1999; Williams et al., 1993) only serve to reinforce the credibility of this hypothesis. The unusual intensity of the 1997 to 1998 El Nino has also been cited by some as a consequence of such an anthropogenic driven climatic influence.

The industrial revolution and the energy needs of an ever increasing global population have been regarded as the culprit for the observed increases in CO_2 and other greenhouse gases in the atmosphere. Although the burning of fossil fuels has been identified as the key reason for the increase, there has been a strong resistance against cutting the practice because it has become an integral part of modern society and is currently the most viable source of energy for our factories, motor vehicles, home heating and air conditioning. To compound the problem, global population has been increasing at a high rate and in addition to the demand for more energy, there is the demand for more residential and agriculture lands that may involve the clearing and/or burning of forests and the elimination of wetlands. The accounting of all the sources and sinks of greenhouses and the study of the global carbon cycle have become a serious endeavor for both scientists and policy makers who are anxious to find an amenable resolution to the problem.

The atmospheric increase in CO_2 is not the only issue, however, in terms of human impact. The same factories, facilities, and motor vehicles also cause the introduction of pollutants to the atmosphere and the environment and substantially degrade the overall quality of the latter. Pollution also threatens the health and well being not only of human beings but also of plants and animals that we all depend on. Some of our lakes and rivers have become toxic on account of improper disposal of waste, fertilizers and pesticides. The elimination of swamps and wetlands have also altered the ecology and caused devastating impacts on birds and marine life. Air pollution, a big health threat, has also grown to be a huge problem in big cities where traffic jam is part of daily activities. Moreover, the innocent release

of some chemicals unsuspectingly led to the depletion of the ozone layer in the stratosphere (Schoeberl, 1993) and caused what is now popularly known as the ozone hole, the consequences of which are yet to be completely understood.

The direct impact of human activities on climate and environment has been studied but results have been controversial and subject to intense scrutiny because the solutions to the problem are expensive and unpopular. While there are observed indicators of a global warming that are convincing, the link of such warming to human activities has not been established beyond reasonable doubt. Sorting out the anthropogenic effects from those of natural cycles has been a problem because the Earth system is such a complex system the study of which requires the availability of a comprehensive global data set and a sophisticated model of the interactions and feedback effects of so many variables. In this paper, we will review the issues, present new insights on the issues, and discuss short as well as long term impacts of a climate change on the environment and society.

2. NATURAL CLIMATE CYCLES AND CHANGES

The task of making climate predictions has been difficult and could benefit enormously from accurate identification and characterization of the natural cycles of climate. A dependable prediction capability would enable society to be better prepared for changes and to implement an affective utilization of climate as a natural resource. The biblical account on "seven years of plenty and seven years of famine," as cited in the Book of Genesis, is a good example of how knowledge of climate cycles can be utilized for the good of mankind. However, historical records have indicated that man rarely utilized climate in this same way because generally, climate has not been so predictable.

Since the sun is the primary source of energy that drives the Earth's climate, studies of the cycles have been focused on changes in the net input of solar energy on the Earth. Such changes in solar energy input can come from changes in orbital parameters of the Earth with respect to the Sun and/or changes in the luminosity of the Sun itself. While in search for explanation for ice ages, Milankovitch postulated in the early 19th century three climate cycles related to changes in the Earth's orbital parameter: (a) 100,000 to 413,000 years associated with changes in the shape of the elliptical orbit (eccentricity) of the Earth; (b) 19,000 to 23,000 years due to the wobble (precession) of the Earth's axis; (c) 41,000 years, associated with changes in the tilt of the Earth's axis. The concept for these three cycles is illustrated schematically in the top three panels of Figure 1. Since the Sun is such a powerful source of energy, even the small change in orbital parameter can make a big difference. Historical records of the Earth's climate over the past hundreds of thousand of years have been preserved in ice sheets, glaciers and bedrock. Studies of cores from these places have confirmed that such cycles in the climate indeed existed. The last panel in Figure 1 shows a comparison of modeled overall effect of the three cycles (black line) and inferred temperature data indicating

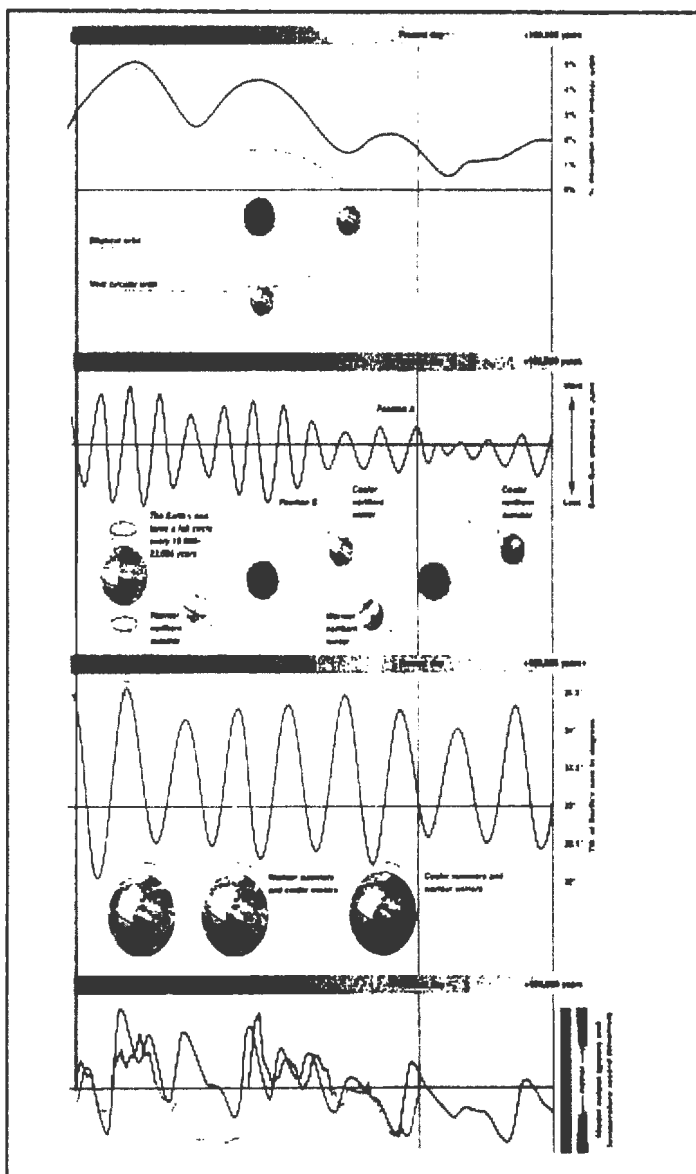


Figure 1. Schematic of the Milankovitch cycles for (a) orbital eccentricity, (b) wobble on the axis, (c) tilt on the axis (that rocks back and forth), and (d) the temperature predictions with the bold line being the model output and the gray being the observed temperatures. (from Burroughs, 1999)

credibility of the hypothesis. The schematics also show where we stand today in terms of these cycles and what to expect in the next 100,000 years. Because of the long term periodicity, the impacts within a human lifetime are usually minimal.

Another possible source of a cycle would be a changing Sun which incidentally has been observed to be indeed changing. The periodicity of these changes are more short term and are therefore more relevant to present day problems. The discovery of the presence of sunspots came as a big surprise when Galileo turned his telescope towards the Sun in the 17th century. It turns out that the Sun gets its energy from nuclear fusion and that the sunspots are areas on the surface that are active with flares, coronal mass ejections, and other forms of activity. It was also discovered that the number of sunspots changes in a cyclic manner with a period of about 11.2 years (Figure 2). Such periodicity were soon observed in many proxy records including those of tree rings and oxygen isotopes from ice sheet cores. The separation of the tree rings provide a means to interpret growth rates which changes from one year to another because of changes in annual temperature and amount of rain. Tree rings record goes back 9000 years and yield periodic fluctuations similar to solar activity. The isotopic ratio of O^{18} to O^{16} from ice cores also provides a means to obtain proxy temperatures and studies of this ratio have revealed that the Earth's surface temperature has been changing with the same periodicity as the sunspots over time. Using data from space probes that started in 1980, there is now a direct confirmation of solar energy variability and the existence of an 11.2 year cycle.

Along with the 11.2-year cycle associated with the Sun is the "Gleissberg" cycle which has a time scale variation of approximately 90 to 100 years (Gleissberg, 1966). This variation would cause longer term climatic changes on the surface of the Earth than the sunspot cycle. Evidence of this cycle was also found in oxygen isotopes (Danggaard et al., 1971) and in tree rings (Moseley, 1940).

There are many other cycles that have been discovered over the years. Examples are the North Atlantic Oscillation and the Arctic Oscillation that have decadal variability (Maysak et al., 1999) and hence have been associated with the sunspot cycle. There is also the Southern Oscillation with a period of about 5 years, associated with the periodic occurrences of the El Niño. Furthermore, there is the quasi-biennial oscillation which occurs every 27 months or so associated with the periodic reversal of winds in the lower stratosphere over the equatorial region. We should also mention the seasonal and diurnal cycles that we are all familiar with.

The cycles are sometimes not so easy to recognize because there are so many of them and the net effect of some cycles is suppressed by other cycles. There are also other effects, like volcanic eruptions, that affect the overall influence of the natural cycles. During volcanic eruptions, tons of sulfur dioxide and dust are emitted to the mid-atmosphere where they stay for a few years and are spread out around the globe. The sulfur dioxide turns to tiny sulfuric acid droplets which together with the dust particles form a veil that absorb sunlight in the stratosphere thereby reducing the amount of solar radiation reaching the Earth's surface. It was Benjamin Franklin who first recognized the impact of volcanic eruption to weather

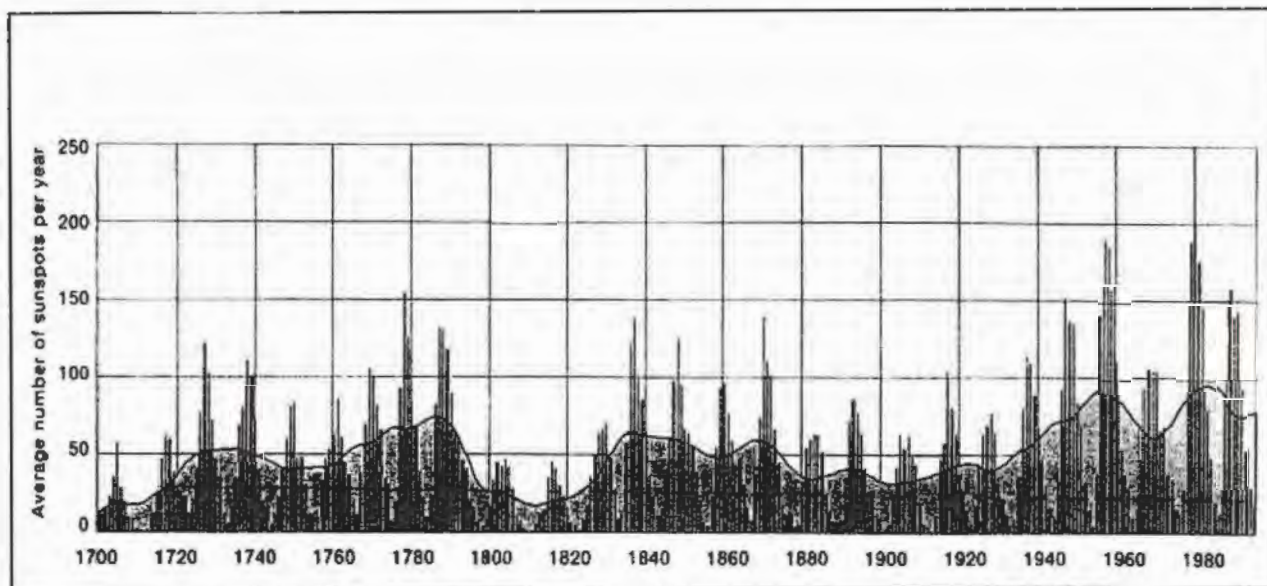


Figure 2. Number of sunspots per year from 1700 to 1990.

when he accurately interpreted the cooling in northern Europe in the winter of 1783 to 1784 as due to dust clouds from the eruption of Laki in Iceland in July 1783. The eruption of Mount Pinatubo in 1991 provided the clearest evidence yet of how volcanoes affect the climate since the impact was well documented and monitored by sophisticated satellite systems.

3. ANTHROPOGENIC IMPACTS AND GLOBAL MODELING

The possible impact of atmospheric CO_2 on the climate of the Earth due to greenhouse effects was first postulated in 1896 by Arrhenius. When radiation from the Sun hits the surface of the Earth, longwave radiation is emitted back to the atmosphere where it is absorbed and re-emitted back to Earth. Much of the radiation is thus trapped between the Earth and the atmosphere by these so-called greenhouse gases. The time series of CO_2 concentrations in the atmosphere at Mauna Loa, Hawaii (Figure 3), shows a 30% increase since the industrial revolution and started the big concern that greenhouse gases might cause significant warming effect on the planet Earth.

Other trace gases are also known to cause greenhouse warming as well, including methane, ozone, CFCs (chlorofluorocarbons) and nitrous oxide (released by nitrogen-based fertilizers). Of these, methane is the most important since its concentration in the atmosphere has more than doubled since the pre-industrial period. The sources for the latter include the biological activity of bacteria in paddy fields and the guts of cattle, as well as the release of natural gas from commercial oil and gas fields and landfills. While not as concentrated in the atmosphere as CO_2 , the effectiveness of these gases as greenhouse gases on molecule by molecule basis can be much more potent. For example, a molecule from the two most common CFCs has the same greenhouse warming effect as 10,000 molecules of CO_2 .

The best way to understand the impact of greenhouse gases is through the use of climate simulation numerical models that is usually referred to as Global Circulation Models (GCM). The GCMs incorporate the basic conservation equations and their external factors, namely radiation and other interactive processes, such as the transfer of momentum, heat, and water substance across the Earth's surface as illustrated in Figure 4. These models started basically as global atmospheric models but they have been enhanced to account for interactions with the ocean, land, cryosphere and biosphere. At present, there are around 16 GCMs put together by scientists in climate centers around the globe. It has been predicted by some of these models that a doubling in CO_2 in the atmosphere would cause the global surface temperature to increase by 2°C (e.g., Manabe et al., 1992). The different models, however, do not always produce consistent results revealing the complexity of the problem and the need to evaluate the different techniques to verify that they formulate the physics of the Earth system in the same manner. Currently, none of them could simulate the occurrence of some distinct climate phenomena, like the

El Niño and cannot reproduce the spatial extent of the sea ice cover as observed by satellites. However, they are the only means to evaluate the sensitivity of each geophysical parameter to global change and they are also useful in the interpretation and extrapolation of observational data in time and space. With the advent of more powerful computers and more comprehensive global data sets provided by satellites, these models will only get better.

4. WARMING SIGNALS: IN SITU AND SATELLITE DATA

The temperature of the Earth as observed by meteorological stations around the globe (Jones et al., 1999) has been increasing at the rate of 0.04 K per decade (Figure 5a). More importantly, the record shows accelerated warming during the last few decades. It is such changes in the rate of increase that has been postulated as the potential warming effect of greenhouse gases. If such a rate of warming is sustained during the next few decades, the climate of the Earth would become very different from what we are accustomed with. However, the temperatures go through some cycles as the running average (in bold) indicates and the result from Fourier analyses of the data reveals some periodicities at different periods, one of which is around 12 years that may be associated with the solar (sunspot) cycle (Figure 5b).

The use of long term meteorological station data for climate change studies has been criticized in the past as lacking adequate quality control. It was discovered that there are mismatches of some records from different stations but close to each other. Also, the temperature record from some stations that are located in what used to be outside urban areas did not take into account additional surface temperature changes due to the expansion of the urban area. But even after these temperature data have been enhanced to take care of these problems, the results still yield positive trends in temperatures. It should be noted that when we think of global trends, we usually refer to global averages and because of the paucity of stations, the global station data set does not really provide global average values. The only way that we could come out with real global averages is through the use of satellite data which became available in the 1970s. However, because of the relatively short record length of satellite data, the latter have to be analyzed concurrently with the longer term station data.

To study the warming phenomenon, it is best to start with the polar regions because the latter are expected to provide significantly enhanced signal of a climate change. The high reflectivity of ice and snow, which are dominant in the polar regions, triggers a feedback effect that causes an amplification of a warming signal in the region. The amplification factor has been predicted by some models to be as high as five. This is an important consideration since the average temperature of the West Antarctic Ice Sheet is about -6°C and a 2°C rise globally produced by a doubling in CO_2 would bring the average temperature of the West Antarctic Ice Sheet to melt temperatures. The immediate impact of the melting of the West Antarctic Ice Sheet would be a rise in sea level of about 5 meters.

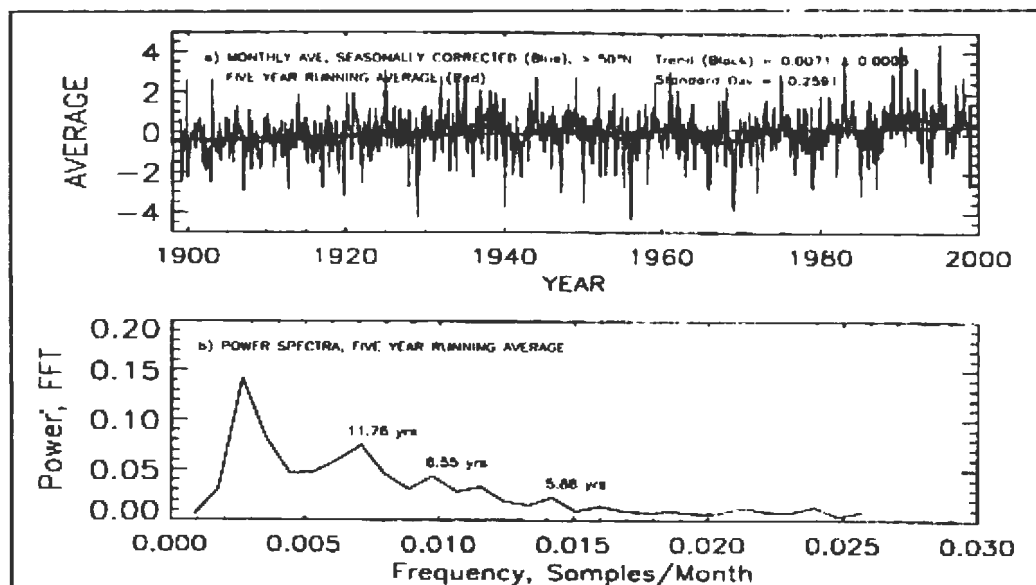


Figure 5. (a) Time series of Arctic temperatures with 5 year running average; and (b) power spectral plot of the 5 year running average data.

Using the same data set used for global studies by Jones et al. (1999), the temperature anomalies derived from the stations north of 50°N for different time periods are shown in Figure 6. The trends as inferred from these anomalies during the last 20, 50, and 100 years (Figures 6a, 6b, and 6c) are 0.0384 ± 0.0119 K, 0.0219 ± 0.0036 , and 0.0076 ± 0.0010 per year, respectively. These results show higher trends than the global data for the polar region and that the 20-year trend is about 5 times more positive than the 100 year trend indicating an accelerating rate of temperature increase. The stations from 50°N to the North Pole, especially those with the record lengths of about 50 years or more are located mainly in the Eurasian continent and are only few in numbers.

Yearly averages in surface temperature as inferred from satellite AVHRR data from 1981 through 2000 are shown in Figure 7. The yearly averages, which represent the mean of the ice season that starts in August each year, provide a means to compare unique spatial features of temperature distributions from one year to another. The last image in Figure 7 is the average of all available data and is used in calculating the yearly anomalies. The Greenland ice sheet appears to be consistently the coldest region in the yearly maps although this is not always the case in the monthly maps. Also, large interannual variations in the temperature distributions are apparent and for different regions, the year of coldest temperatures is usually different. For example, the coldest year in the Central Arctic appears to be 1987 while that in Greenland appears to be 1992, which was the year right after the Pinatubo eruption and represents a general cooling period that started in 1991 and ended in 1993. Regions of temperature anomalies are better depicted in the anomaly maps for each year as shown in Figure 8. The anomaly maps show many striking features in the temperature distributions, especially when the first half of the record is compared with the second half. The coldest temperature in the Central Arctic and Eurasia occurred in 1987 but for the same year, the temperatures in North America were warmer than the average. In 1992, the temperatures in practically the entire Arctic region was colder than the average. During the period 1995-1998, the temperatures became warmer than usual in most regions. The warmest two years appear to be 1995 and 1998, with the rate of warming being the highest in the Eurasian side in 1995, whereas it was highest in the North American side in 1998. To date 1998 is the warmest year in this century.

Trends in surface temperature over the last 18 years for each satellite pixel are depicted in spatial detail for the entire Arctic in Figure 9a. The trends were inferred using monthly anomalies from 1981 through 1999. Except in Mongolia (top right portion), the rate of warming is shown to be generally positive with the highest in the Central Arctic, Greenland, and Northern Canada. The uncertainties and standard deviations in these trends are shown in Figure 9b and 9c, respectively. It is apparent that there are regional variations in the trends, especially inside the 60 degree latitude.

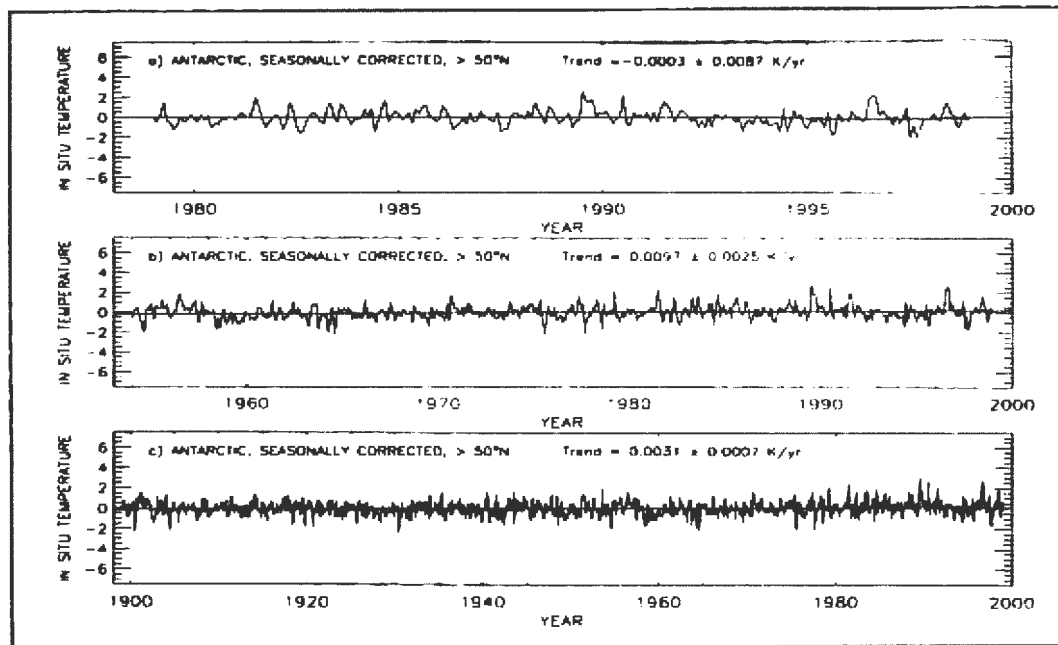


Figure 6. Arctic temperature anomalies and trend lines for (a) 1979 to 1998; (b) to 1955 to 1998; and (c) 1900 to 1998 using meteorological station data.

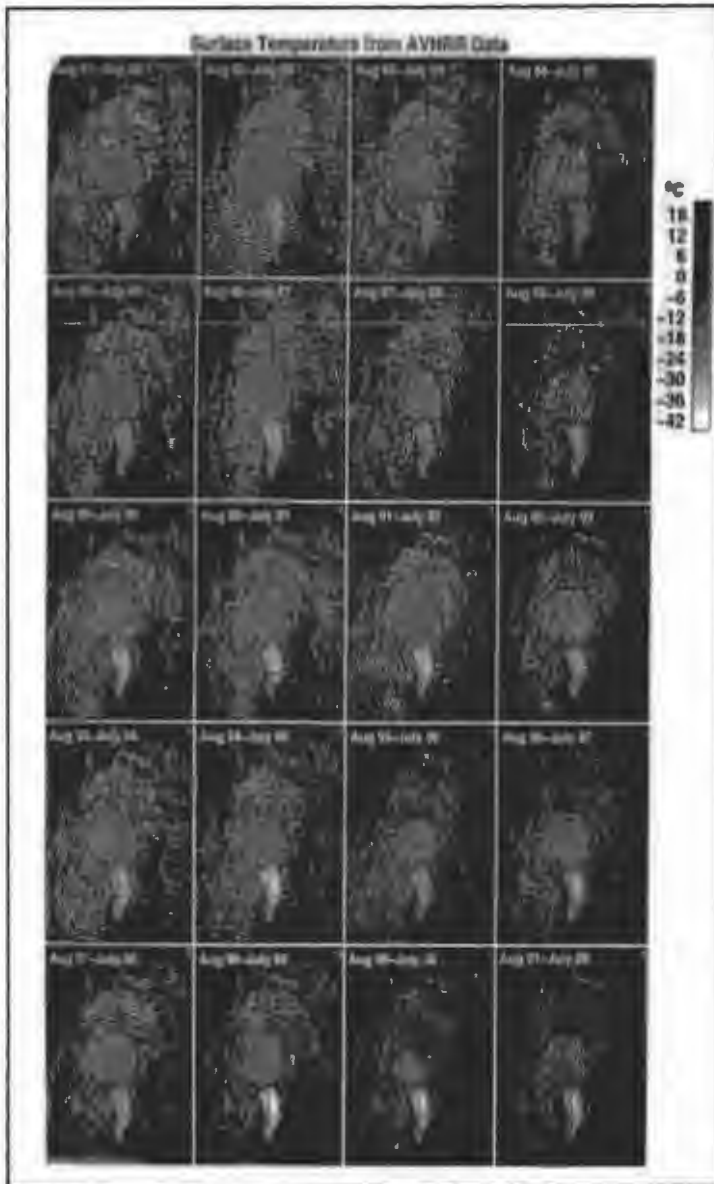


Figure 7. Yearly average of surface temperature data in the Arctic from 1981 to 2000. The averages are from August of one year to July the following year and reflect the average over an ice season. The last image is the climatological yearly average or the average of all data from August 1981 to July 2000.

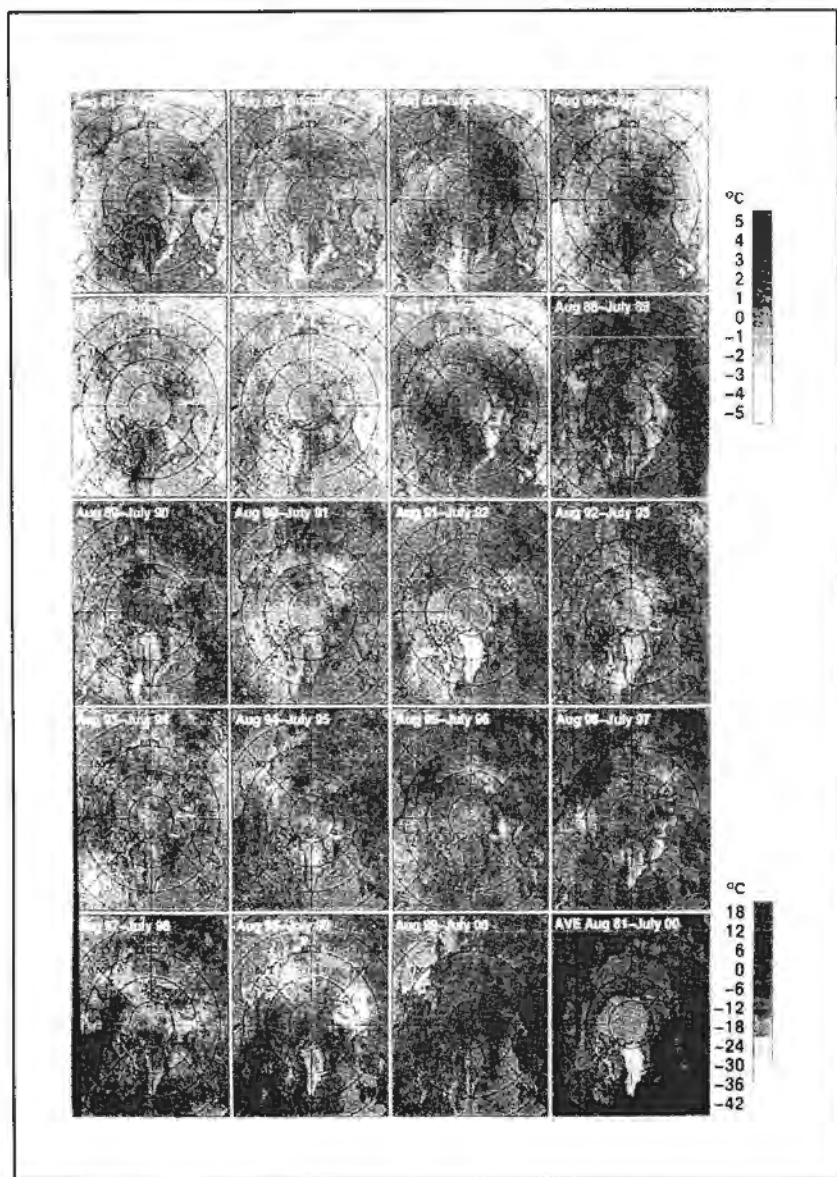


Figure 8. Yearly anomalies of Arctic surface temperatures from 1981 to 2000. The last image is the climatological yearly average used in calculating the anomalies

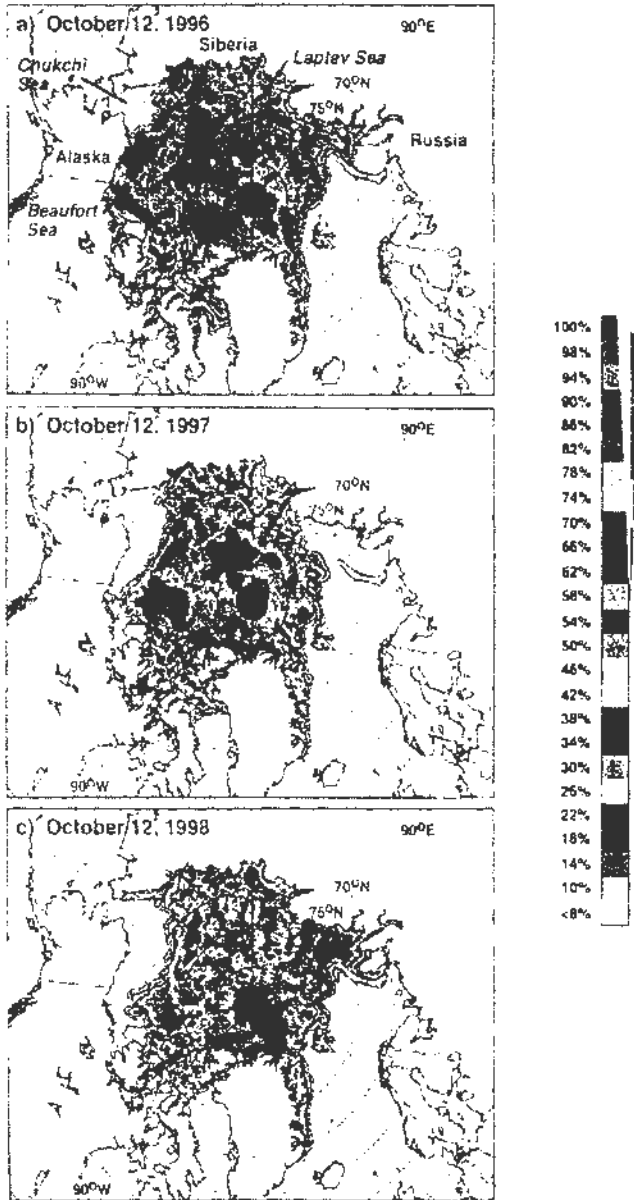


Figure 9. Trends in surface temperature from 1981 to 2000 on a pixel by pixel basis.

The warming scenario is further supported by sea ice cover data. There have been recent reports about thinning of sea ice as observed by submarines (Wadhams et al., 1989, Rothrock et al., 1999). Passive microwave satellite data also show that the ice have been retreating as illustrated by a sequence of images on October 12 during the years 1996 to 1998 (Figure 10). The coded ice concentration images, correspond to the time when the Arctic basin is almost all frozen up. During this time, the area not covered by ice in the Beaufort Sea Region (left side) has an areal extent of 300,000, 700,000, and 970,000 km², in 1996, 1997, and 1998, respectively. This reduction in the ice cover is highly correlated with the warming anomalies shown in Figure 8. Such a reduction can also impact the circulation of the underlying ocean which is a big component of the climate system. Analysis of 18 to 20 years of ice concentration data also reveals a trend in ice extent of about 3% per decade (Bjorgo et al., 1997; Parkinson et al., 1999). Also, a 7% per decade decrease in the multiyear ice cover has been reported (Johannessen et al., 1999).

In the Antarctic region, the eighteen to twenty year record shows either no trend or a slight positive trend for the entire region (Bjorgo et al., 1997; Cavalieri et al., 1997). Regionally, it turns out that while the Bellingshausen and Amundsen Seas have been losing sea ice cover as reported by Jacobs and Comiso (1997), the Ross Sea ice cover has been increasing and those from other regions show practically no change. These regions have been the site of big iceberg calving in recent years and the Antarctic Peninsula has been regarded as climatologically changing region (King, 1994). An updated version of the ice extent and ice area anomalies with trend lines superimposed are shown in Figure 11. The results show a trend of 0.4 ± 0.2 %/decade for the entire hemisphere and general consistency with previous results.

Surface temperature data derived from satellite AVHRR infrared data actually provided useful insights into this Antarctic phenomenon. The observed slightly positive trend in ice extent during the last 20 years is coherent with a slight negative trend in surface temperature in the continental region as reported by Comiso (2000). The 20-, 50-, and 100-year records are shown in Figures 12a, 12b and 12c, respectively, and the only negative trend is the first case. The longer term records show significant warming which is also consistent with the observed decline in the ice cover from the 1940s to the 1980s, as reported using whaling data by de la Mare (1997).

Evidences of warming are also observed in other parts of the globe. The Greenland ice sheet has recently been observed to be thinning in some areas by Krabill et al. (1999), using an aircraft laser mapping instrument. Permafrost in the northern hemisphere has also been observed to be thawing and decreasing in area. In the high mountains, the glaciers are generally retreating. Figure 13 shows images of the Horn and Waxeggkees glaciers in the Austrian Tyrol for 1921 and 1994 and depicts huge recession of the glaciers. It should be pointed out that while a direct relationship to global warming is evident in the observables, the interpretation of data may not be straightforward. For example, in some areas

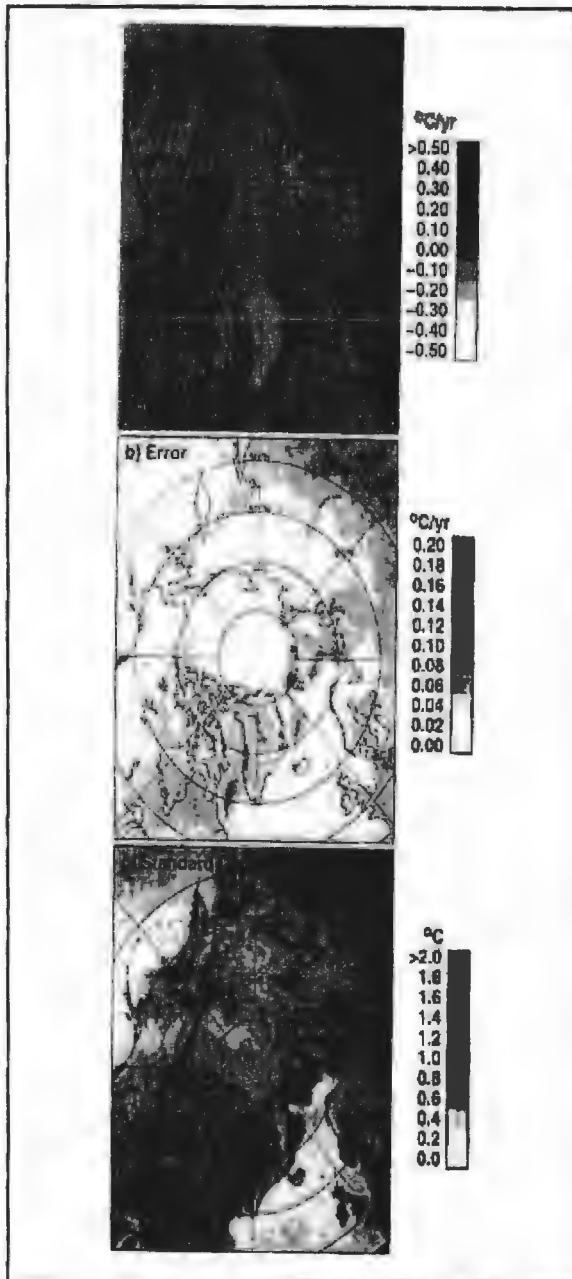


Figure 10. Coded ice-concentration maps in the Arctic for (a) October 15, 1996; (b) October 15, 1997; and (c) October 15, 1998, using the Bootstrap Algorithm.

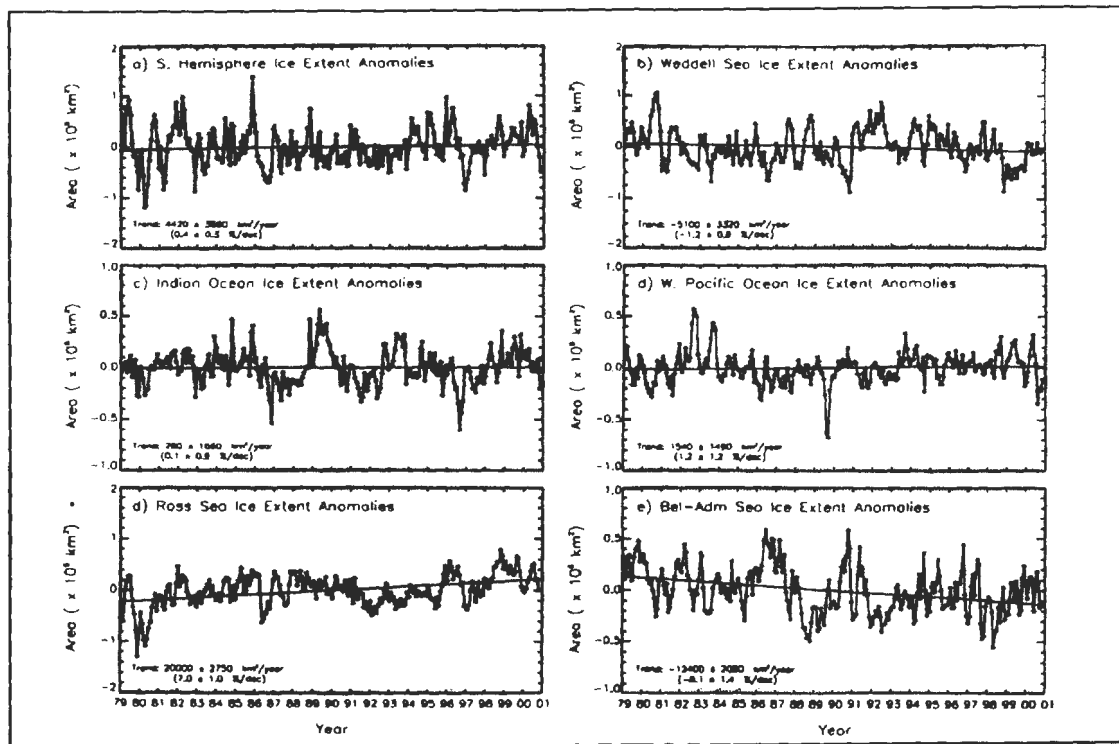


Figure 11. Monthly average ice extent anomalies from satellite data for 1979-2000 in (a) the entire Antarctic region; (b) Weddell Sea Region; (c) Indian Ocean; (d) Western Pacific; (e) Ross Sea; and (f) Bellingshausen-Amundsen Seas.

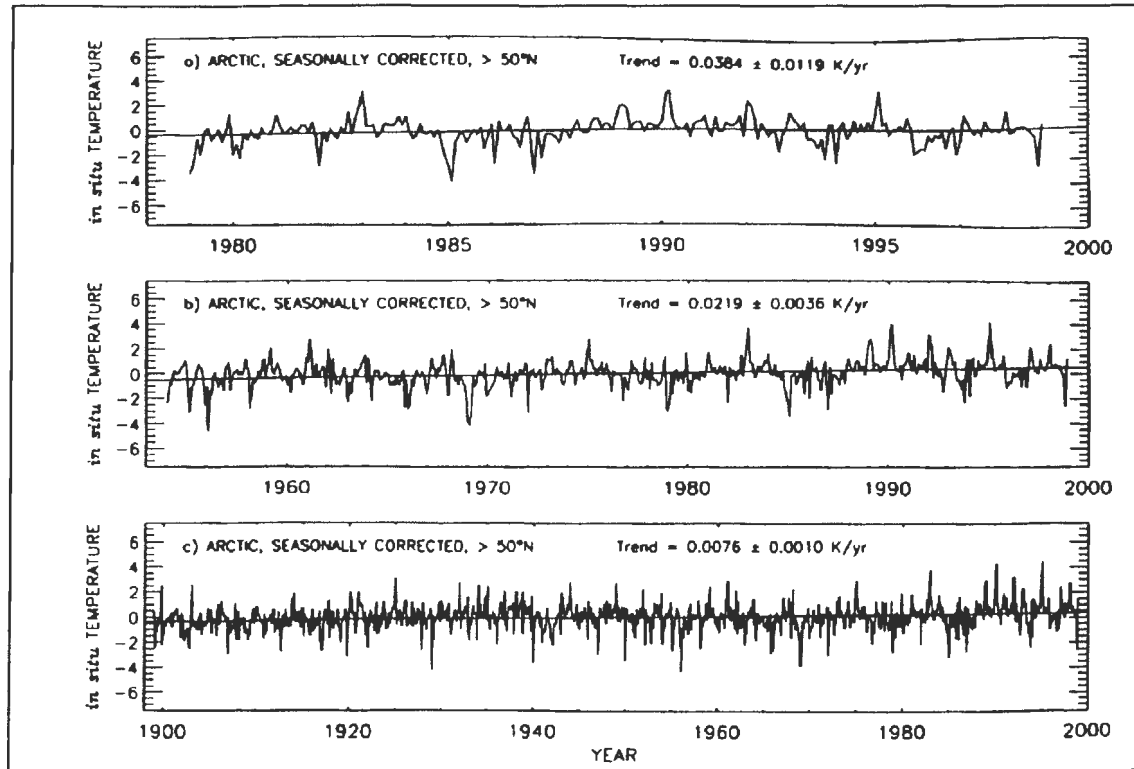


Figure 12. Trends in surface temperature in the Antarctic using meteorological station data for the periods (a) 1979 to 1998; (b) 1955 to 1998; and *c) 1900 to 1998.



Figure 13. Images glacial retreat at the Horn and Waxeggkees glaciers in the Austrian Tyrol for 1921 and 1994 period. (from Burroughs, 1999).

of Europe, glaciers have been advancing but this has been interpreted as caused by increased precipitation due to more evaporation in the ocean caused by warming during the same period.

5. ENVIRONMENTAL CHANGES AND IMPACTS ON HUMANS

Although it is not yet certain that the observed global warming is associated with the influence of greenhouse gases, modeling results point to the need for an aggressive plan in response to what may happen in the foreseeable future. The Arctic sea ice has been retreating significantly and may be thinning, glaciers are receding and the rise in surface temperatures has been accelerating. Pending sudden changes in these trends, which are not likely, it would not be a good strategy to just wait and do nothing until all the problems of sorting out the different effects from a complex climate system are all resolved.

A panel of experts was assembled through a mandate by the US congress in 1990 to study what the climate will be like in the 21st Century. The key projections by the panel have just been released and summarized as follows: (a) The average global temperature will rise by about 3-6°C prompting more summer heat waves and gentler winter; (b) Agricultural production will likely surge and forest will likely flourish because of the fertilizing effect of more carbon dioxide in air. (c) Snowpack will probably diminish by 50% on the average, while winter rains are likely to increase, bringing 60 to 100 percent more showers to much of Southern California and the parched southwest; and (d) Total precipitation nationwide which rose to 5 to 10 percent during the 20th century, will probably increase another 10 percent by 2100, chiefly in the form of extreme storms, exacerbating runoff pollution in places like the Chesapeake Bay and other sensitive areas. The study also points out that many long-suffering ecosystems, such as alpine meadows, coral reefs, coastal wetlands and Alaskan permafrost, will likely deteriorate further or disappear altogether. Also, the threat of drought will rise and the water levels could drop by as much as five feet in the Great Lakes because hotter conditions will enhance evaporation. As for health effects, a doubling or tripling of heat-related deaths in big cities are expected while there would be substantial shift in the habitat of disease-bearing mosquitoes and other animals.

Although the report is meant for the United States, much of it is likely relevant worldwide. Some of these projections are familiar and have been published as a scientific report. Many of them are based on sensitivity studies using two of the most established GCMs. Where the two GCMs disagree the average of the results is taken. But the two models have significant disagreements in some derived parameters and this may be an indication that they are not mature enough to be reliable. Assuming that they are correct, it appears that human beings will be able to adjust and adapt to most of the impacts.

It should be pointed out that the economic impact will vary regionally and should be assessed on a case by case basis. For example, a 50 cm rise in sea level

is projected for the next century on the assumption that the trends from the last century will continue. Such a rise in sea level is primarily from thermal expansion of the ocean and is readily considered to cause a very serious impact on many countries (Schneider, 1997). However, a rise of 3°C to 6°C in surface temperature would cause the disappearance of the West Antarctic Ice Sheet which in turn would cause the sea level to rise by about 5 m. A comparison of current shoreline with that corresponding to a 5 m sea level rise is shown in Figure 14. It is apparent that such change in sea level would cause a large fractions of Florida and Vietnam to be underwater. Large amount of occupied lands worldwide, including big cities, are expected to be suffer the same fate as well.

The consequence of the projected change in climate is not all negative and can positive in some areas. Higher levels of CO₂ is expected to cause an enhanced forest and an increase timber production by 8 to 25%. Also, agriculture yields for most commercial crops are expected to increase 15 to 50%. Moreover, a warmer and accelerated growing season may reduce the need for crop irrigation by 30 to 40% which will be a big relief to water resources. But even such positives could lead to negatives. For example, higher agricultural yields would require the use of 5 to 20% more pesticides that would raise the threat of more nitrogen fertilizer runoff into bays and estuaries.

It should also be pointed out that man has enormous capacity to overcome some of the problem. For example, the rapid expansion of the Sahara desert (50 km per year) became such a big concern that in 1977 the United Nations decided to launched on a \$6 billion project to prevent desertification over the next 15 years. The result is favorable as indicated in two images of the same region in 1984 and 1991 in Figure 15. During the period, the northern border of the desert retreated and the area of the desert declined by about 695,000 km². The successful implementation of such a huge activity is promising and is a strong manifestation of the ability of man to counteract adverse trends.

6. DISCUSSION AND CONCLUSIONS

Changes in climate and environment have been occurring throughout history but it is only in recent times that human activities may be causing an alternation in the climate. The advent of the industrial revolution had caused a substantial increase in the emission of greenhouse gases into the atmosphere and a likely consequence is an enhanced warming of the Earth's surface. Historical records from a global network of meteorological stations reveal that surface temperatures have indeed been on the rise during the last century and that the rate of increase has been accelerating during the last few decades. Meteorological station data do not provide the long term record needed to analyze trends and periodic cycles and where there is overlap, they are generally consistent with satellite data. The latter on the other hand show for the first time spatially detailed observations that reveal locations and persistence of anomalies in both temperature and sea ice cover. The

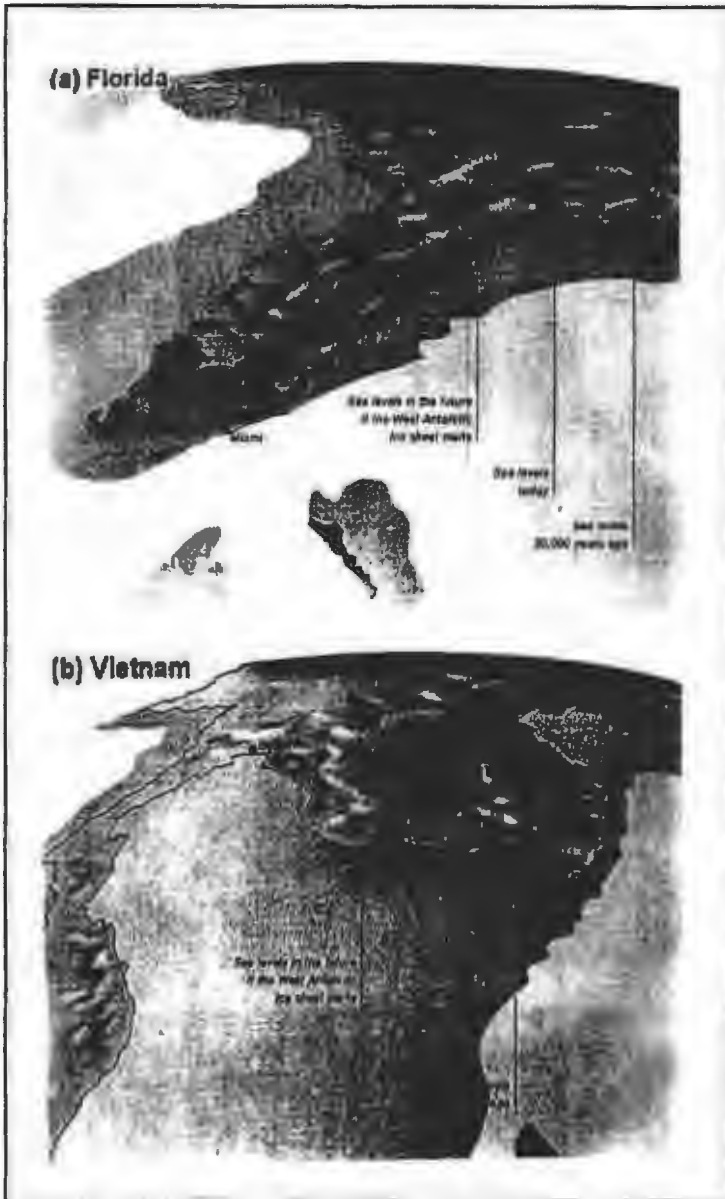


Figure 14. Sea level rise if the west Antarctic ice sheet collapse in (a) Florida and (b) Vietnam. (from Burroughs, 1999).

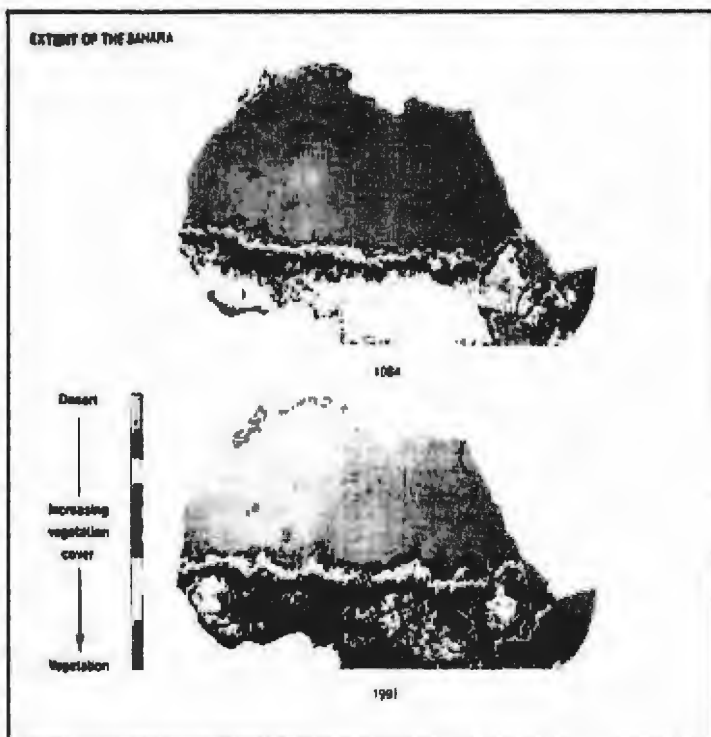


Figure 15. Change in the Sahara desert from 1984 to 1991 after intervention by the United Nations to restore the desert. During this period, the desert has declined by about 695,000 km². (from Burroughs, 1999)

satellite data also indicate that the influence of climate change can be very regional, e.g., anomalously warm in some areas and cold in other areas during the same year. Trends in temperature also varies from one region to another but a predominance of a warming trend is observed. The warming trend is reflected in other areas of the globe and rare manifested in terms of retreating glaciers, thawing permafrosts, expanding deserts, more stormy weather and increasing sea level. The consequence of a climate change can be far reaching and should be studied in detail. The key question is whether currently observed changes are indeed caused by greenhouse warming and if so whether there is anything that man can do minimize the change. The answer to the question requires a good strategy.

The strategy should begin with a careful analysis of the problem and an evaluation of the steps needed to mitigate or minimize the problem. Since it is a global problem, a global solution is required, which means that the participation of all countries around the world is needed. It is important that a good understanding of the Earth climate system is acquired before policy decisions are made. This

includes the acquisition of a comprehensive global data sets of geophysical parameters with the proper temporal and spatial coverage. Such venture involves the design, and implementation of expensive satellite and field programs that may take years to execute. Such data are already collected but more are needed to improve our understanding of the climate system and to be able to confidently predict changes in the climate through the use of sophisticated and reliable models. The regional and global consequences of such predictions have to be analyzed and evaluated and key information have to be managed and reduced into a format that is useful for policy makers. The solutions to the problem may require big sacrifices and enormous efforts. This makes it imperative that mitigation policies are based on well founded and carefully executed studies. There should also be a strategy that addresses short term changes observed in recent years. A warming trend could cause a demise of the Arctic perennial ice cover in the next century whether or not the warming is associated with greenhouse gases.

REFERENCES:

- Bjorgo E, Johannessen, OM, and Miles MW. 1997. Analysis of merged SSMR-SSM/I time series of Arctic and Antarctic sea ice parameters 1978-1995. *Geophys. Res. Lett.* 24(4):413-416.
- Burnoughs, WJ. 1999. *The Climate Revealed*. Cambridge: Cambridge University Press. 192 p.
- Cavalieri DJ, Gloersen P, Parkinson CL, Comiso JC, and Zwally HJ. 1997. Observed hemispheric asymmetry in global sea ice changes. *Science* 287: 1104-1106.
- Comiso JC. 2000. Variability and trends in Antarctic surface temperatures from in situ and satellite infrared measurements. *J. Climate*, 13(10): 1674-1696.
- Daly, JL. 1989. *The Greenhouse Trap*. Sydney: Bantam. 192p.
- Dansgaard W, Johnsen SJ, Clausen HB, and Langway, CC Jr. 1971. Climate record revealed the Camp Century Core. In: Turekian KK, editor. *The Late Cenozoic Glacial Ages*. New Haven: Yale University Press. p37-56.
- de la Mare WK. 1998. Abrupt mid-twentieth-century decline in Antarctic sea ice extent from whaling records. *Nature* 389 57-60.
- Gleissberg W. 1966. Ascent and descent in the eighty-year cycles of solar activity. *J. British Astron. Soc.* 76: 265-270.
- Hansen JA, Lacis, Rind D, Russel G, Stone, P, Fung I, Ruedy R, and Lerner J. 1984. Climate sensitivity: Analysis of feedback mechanisms. In: *Climate Processes and Climate Sensitivity: Geophysical Monograph 29*, Washington DC: American Geophysical Union. p130-163.
- Jacobs SS., and Comiso JC. 1997. A climate anomaly in the Amundsen and Bellingshausen Seas. *J. Climate*, 10(4):697-709. Johannessen, O.M., E.V. Shalina, and M.W. Miles, 1999. Satellite evidence for an Arctic sea ice cover in transformation. *Science*, 286: 1937-1939.
- Jones PD, New M, Parker DE, Martin, and Rigor IG. 1999. Surface air temperature and its changes over the past 150 years. *Rev. Geophys.*, 37:173-199.
- King JC. 1994. Recent climate variability in vicinity of the Antarctic peninsula. *Int. J. Climatol.* 14:357-369.
- Krabill WC, Frederick E, Manizade S, Martin C, Sonntag J, Swift R, Thomas R, Writhe W, Yungel J. 1999. Rapid thinning of parts of the Southern Greenland Ice Sheet. *Science*. 283:1522-1524.
- Manabe S, Spelman MJ, and Stouffer RJ. 1992. Transient responses of a coupled ocean-atmosphere model to gradual changes of atmospheric CO₂. Part II: Seasonal response. *J. Climate*. 5:105-126.
- Moseley EL. 1944. Recurrence of floods and droughts after intervals of about 90.4 years. *Popular Astronomy*. 52:73-84.

- Mysak LA. 1999. Interdecadal variability at northern high latitudes. 1999. In: *Beyond El Nino: Decadal and interdecadal climate variability*, A. Navarra, editor. Berlin: Springer-Verlag. p 1-24.
- Parkinson CL., Cavalieri DJ, Gloersen P, Zwally HJ, and Comiso JC. 1999. Arctic Sea ice extents, areas, and trends, 1978-1996. *J. Geophys. Res.* 104(C9): 20837-20856.
- Peixoto JP., and Oort AH 1992. *Physics of the Climate*: American Institute of Physics. New York, N.Y., 520 p.
- Rothrock DA, Yu Y, and Maykut GA. 1999. Thinning of the Arctic sea-ice cover. *Geophys. Res. Letters*. 26 (23):3469-3472.
- Schoeberl MR. 1993. Stratospheric Ozone Depletion. In: *Atlas of Satellite Observations related to Global Change*. Gurney RJ, Foster JL, Parkinson CL, editors. Cambridge: Cambridge University Press. p 59-66.
- Schneider, D. 1997: The rising seas, *Scientific American*, 112-117.
- Wadhams P. 1988. Evidence of thinning of the Arctic ice cover north of Greenland. *Nature*, 345(6279), 795-797.
- Williams RS Jr. and Hall DK, *Glaciers*, 1993: In: *Atlas of Satellite Observations related to Global Change*. Gurney RJ, Foster JL, and Parkinson CL, editors. Cambridge: Cambridge University Press. p401-424.

A Look at the Transportation Situation in Metro Manila and the Mitigating Measures to Alleviate the Impacts of Traffic

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ABSTRACT

Metro Manila continuously experiences traffic congestion similar to growing cities in Asia. Traffic congestion is noted to be a manifestation of inter-related urban problems such as the influx of migrants from the provinces and local areas, over-concentration of economic, cultural and social activities in major urban areas, rapid development and uncontrolled urbanization of urban areas, lack of public transportation system, increasing growth in car ownership and usage, poor traffic management and control schemes and lack of coordination among government agencies involved in transportation and traffic and lack of strict enforcement.

This paper presents the current transportation and traffic situation in Metro Manila and mitigating measures or schemes that are being implemented, as well as those proposed to alleviate the impacts of traffic congestion. Among these are the Do-Fixed Project, Do-Existing Project and Do-Maximum Project which cover existing transport network, completing expressways, skyway and LRT/MRT lines, which should be incorporated with city plans of the LGUs.

Key words: traffic congestions, sociological and economic impacts, mitigating measures

INTRODUCTION

Like its neighboring growing metropolis in Asia, notably in the Southeast Asian region, Metro Manila is continuously experiencing traffic congestion and deteriorating transportation and traffic infrastructure facilities. It has been accepted that traffic congestion and deterioration of the conditions of traffic facili-

ties are directly interrelated. Albeit this observation, it could also be noted that traffic congestion is considered more as a manifestation problem.

Traffic congestion is a manifestation of the inter-related urban problems. Notable of these urban issues are the following:

- The influx of migrants from the provinces and local areas;
- Over-concentration of economic, commercial, cultural and social activities in major urban areas;
- Rapid development and uncontrolled urbanization of urban areas;
- Lack of public transportation system;
- Increasing growth in car ownership and usage;
- Poor traffic management and control schemes; and
- Lack of coordination among government agencies involved in transportation and traffic and lack of strict enforcement;

The above issues are considered as among the root causes of traffic congestion in an urban area. Thus, the mitigating measures to alleviate traffic congestion require the understanding of the root problems. Technical solutions alone may not alleviate traffic congestion. Given this, it should be important to understand the root problems of traffic congestion as technical and institutional.

The gravity of the impacts of traffic congestion on urban areas is becoming a serious concern not only to the government but also to the business sector and the people. An unpublished report stated that the annual cost attributed to traffic congestion is roughly 100 Billion Pesos¹. The report also stated this may still be undervalued due to averaging. Apart from this economic impact, traffic congestion also has social and environmental impacts.

Given the above background on traffic congestion, this paper will present the current transportation and traffic situation in Metro Manila and the mitigating measures or schemes being implemented or proposed to alleviate the impacts of traffic congestion. The paper is organized as follows: a) brief profile of Metro Manila and its existing transportation system, b) pressing transportation and traffic concerns, c) mitigating schemes being implemented or proposed, and d) concluding remarks. Most of the materials used for this paper come from the Metro Manila Urban Transportation Integration Study (MMUTIS) of DOTC and a paper prepared on traffic for NAST.

PROFILE OF METRO MANILA

Socioeconomic Status and Transportation System

Population Trends. Metro Manila, the national capital region of the Philippines, has an area of roughly 636 sq. kms. Being the national capital region of the country, it is the center of major socioeconomic, cultural and political activities of the country. Its population of less than 2 million in 1950 has increased to 5.9 million in 1980 and 9.5 million in 1995² (as shown in Figure 1). Since its conception, Metro Manila has been constantly growing at a rapid rate. Its urban and economic influence has spread to adjoining municipalities and cities – with an area of about 3,670 sq. km.

Considering its close links with its neighboring areas, the total population of the Metro Manila is said to be 14.4 million as of 1995. It is likely that the urban population will continue to increase and reach 25 million by year 2015, Table 1). As such, Metro Manila will grow to become a mega city with a population size that has been hardly experienced in the world.³ The sprawling of Metro Manila is perceived to be a result of the absence of proper planning. The trend in urbanization as experienced by Metro Manila poses a pitfall as well as a chance for the transportation system in a growing metropolitan region. It is therefore apparent that present Metro Manila is not a lone urban area but rather a core of the expanded metropolitan capital region. This definitely necessitates that a new policy issue has to be desired and met by the Government and the people of the region.

Employment and School Population. Closely related to the growth in population is the increase in employment and school population in Metro Manila and its influenced areas. These two demographic factors affect the spatial separation of residences from work and educational places. It is becoming evident that more households are opting to reside outside Metro Manila and places of work and schools are moving farther from the metropolis. This trend definitely will increase the number of trips and distances traveled. Table 2 summarizes the trend in employment and school enrolment from 1980 to 2015.

¹ NCTS. Economic Impact of Traffic Congestion in Metro Manila, April 2000, unpublished report for NEDA-LEDAC.

² A Factbook on Metro Manila's Traffic and Transportation Situation (MMUTIS Factbook), Prepared by MMUTIS Study Team, JICA, 1998.

³ MMUTIS Factbook

Table 1. Metro Manila's Population Trend

Area	Population ('000)			Growth			
				1980-1995		1995-2015	
	1980	1995	2015	95/80	%/Yr.	2015/95	%/Yr.
Metro Manila	5,926	9,454	13,157	1.6	3.2	1.4	1.7
Adjoining Areas	2,434	4,914	12,563	2.0	4.8	2.6	4.8
TOTAL	8,360	14,368	25,720	1.7	3.7	1.8	3.0

Area	Population Density* (persons/hectare)		
	1980	1995	2015
Metro Manila	99	158	220
Adjoining Areas	8	15	38
TOTAL	22	38	68

*per gross including all uninhabitable areas such as waterways

Source: MMUTIS Factbook, 1998

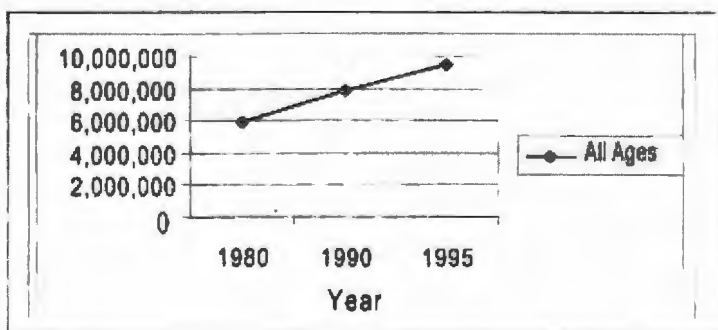


Figure 1. Population Trend (1980-1995)

Table 2. Trend of Employment and School Enrolment

Area	('000)			Growth			
				1980-1995		1995-2015	
	1980	1995	2015	95/80	%/Yr.	2015:95	% Yr.
Employment							
Metro Manila	1,784	3,709	5,815	2.1	5.0	1.6	2.3
Adjoining Areas	n.a.	1,440	3,628	n.a.	n.a.	2.5	4.7
TOTAL	n.a.	5,149	9,443	n.a.	n.a.	1.8	3.1
School Enrolment							
Metro Manila	1,547	2,966	4,167	2.0	4.7	1.4	1.5
Adjoining Areas	n.a.	1,624	4,227	n.a.	n.a.	2.9	5.5
TOTAL	n.a.	4,580	8,394	n.a.	n.a.	1.8	3.1

Source: MMUTIS Factbook, 1998

Using the database from the Metro Manila Urban Transportation Integration Study (MMUTIS) Person Trip Survey conducted in 1996 with a sample household size of 50,500 and additional 8,000 households in the adjoining areas, some socioeconomic characteristics of the region can be presented. The sample households roughly represent 2.5% of Metro Manila's household population and 0.8% of the adjoining areas.

Socioeconomic Profile. The socioeconomic profile of Metro Manila is considerably different to that of the adjoining areas. There are more opportunities for employment in the tertiary sector in Metro Manila than the adjoining areas. On the other hand, the adjoining areas have higher employment in the secondary sector. Likewise, Metro Manila has more students enrolled in the higher level compared to the adjoining areas. Similarly, Metro Manila residents have higher car ownership and average household income – 20% and 30% respectively. These are shown in Table 3. These observations validate the notion that Metro Manila still has the concentration of economic activities in the country. Furthermore, it is becoming apparent that population grows faster in the adjoining areas while higher level of education enrolment and employment opportunities still concentrate in Metro Manila.

Vehicle Ownership Profile. From Table 3 above, it can be observed that Metro Manila has the higher concentration of vehicles and car-owning households. This is clearly an indication of the increasing motorization of the region. Looking at Table 4, the number of registered vehicles had increased at an average rate of roughly 6% annually.

Table 3. Socioeconomic Profile, 1996

	Metro Manila		Adjoining Areas		MMUTIS PT Survey	
Population ('000)	9,454		4,914		14,368	
No. of HHs ('000)	1,988		1,002		2,990	
Ave. HH Size	4.8		4.9		4.8	
Employment ('000)	3,708	100.0%	1,440	100.0%	5,146	100.0%
Primary	39	1.1%	118	8.2	156	3.0
Secondary	851	22.9%	440	30.6	1,291	25.1
Tertiary	2,818	76.0%	882	61.3	3,699	71.9
Enrolment ('000)	2,966	100.0%	1,624	100.0%	4,589	100.0%
Pupil	1,696	100.0%	1,624	100.0%	4,589	100.0%
Student	1,270	42.8%	634	39.0	1,903	41.5%
Car Ownership						
No. of 4-wheel ('000)	527		212		739	
Car Owning HHs (%)	19.7		16.9		18.7	
Ownership*	59		45		54	
Household Income						
Ave. (Peso/month)	11,760		9,740		11,090	
% HHs below Poverty Line	6.5		12.8		8.7	

*no./000 pop'n.

Note: Employment and enrolment are at workplace and school place respectively

Source: MMUTIS Factbook, 1998

Table 4 No. of Registered Vehicles in Metro Manila

YEAR	1980	1990	1990	1980-1995	
				1995/19980	%/Yr.
Private	391,178	623,498	928,381	2.0	4.7
For Hire	55,964	61,280	127,331	2.3	5.6
TOTAL	446,142	684,778	1,055,692	2.4	5.9

Source: MMUTIS Factbook, 1998

The structure of car ownership is given in Table 5 and it shows the significant increase in car ownership in Metro Manila from 9.5% in 1980 to 19.7% in 1996. However, there was no notable increase in the average number of cars per car owning household and the percentage of multiple car owning households in Metro Manila. This would imply that there was no change in the structure of car ownership in Metro Manila.

Table 5. Car Ownership Structure

YEAR	Metro Manila		Adjoining Areas
	1980	1996	1996
% of Car Owning Households	9.5	19.7	16.9
Ave. No. of Cars Per Car Owning Households	1.4	1.3	1.2
% of Multiple Car Owning Households	19.0	20.1	13.3

Source: MMUTIS Factbook, 1998

Predicting the car ownership for year 2015, it is expected that there will be a sharp and significant increase (Table 6). This is perceived to be attributed to the rise in income level and population. The MMUTIS Study estimated that the number of cars in year 2015 is slightly more than 2,12 million. This is almost tripled the current number. Likewise, the future growth of car ownership in the adjoining areas is significant. This is an indication of the movement of people from the metropolis to the adjoining areas.

Table 6. Car Ownership Forecast in Metro Manila

Area		1996	2015	2015/1996
No. of Vehicles ('000)	Metro Manila	527	1,047	2.0
	Adjoining Areas	212	1,072	5.1
	TOTAL	739	2,119	2.9
No. Per 1000 Population	Metro Manila	56	80	1.4
	Adjoining Areas	43	85	2.0
	TOTAL	51	82	1.6

Source: MMUTIS Factbook, 1998

Table 7 Vehicles Per Kilometer in ASEAN Cities

City	vehicles/km
Jakarta*	840
Bangkok*	695
Kuala Lumpur*	620
Hong Kong*	266
Singapore*	214
Metro Manila**	352

Source: *Asiaweek, February 21, 1997

**Philippine Statistical Yearbook, 1995

Table 7 compares the vehicles per kilometer of Metro Manila to its neighboring ASEAN cities. The table indicates that Metro Manila ranks fourth among the ASEAN cities. This implies that the number of motorized vehicles in Metro Manila's road network is indeed among the highest in Southeast Asia.

Transportation System

The present land transportation system of the metropolis is supported by major infrastructure systems of roads and railways. The major roads are characterized mainly as circumferential (presently at 5) and radial (10). The total length of roads is at 3,425.3 kms. Of these, 47% are city roads, 28% national roads, 17% municipal roads, 8% barangay roads and a very small 0.1% private roads.

The only two railway lines of the country are found in Metro Manila: the Philippine National Railways (PNR) and the Light Rail Transit (LRT) system. The former consists of lines servicing not only the metropolis but also connects the area with the adjacent provinces on the north and on the south. On the other hand, the LRT system, with 18 stations has been operating on a single corridor since 1985. The LRT system has been significantly contributing to the improvement of the traffic situation in that corridor and has an estimated share of about 3% of total trips by public transport.

As for the dynamic component of land transportation, the following statistics show the existing number of vehicles serving both private and public transport users, Table 8.

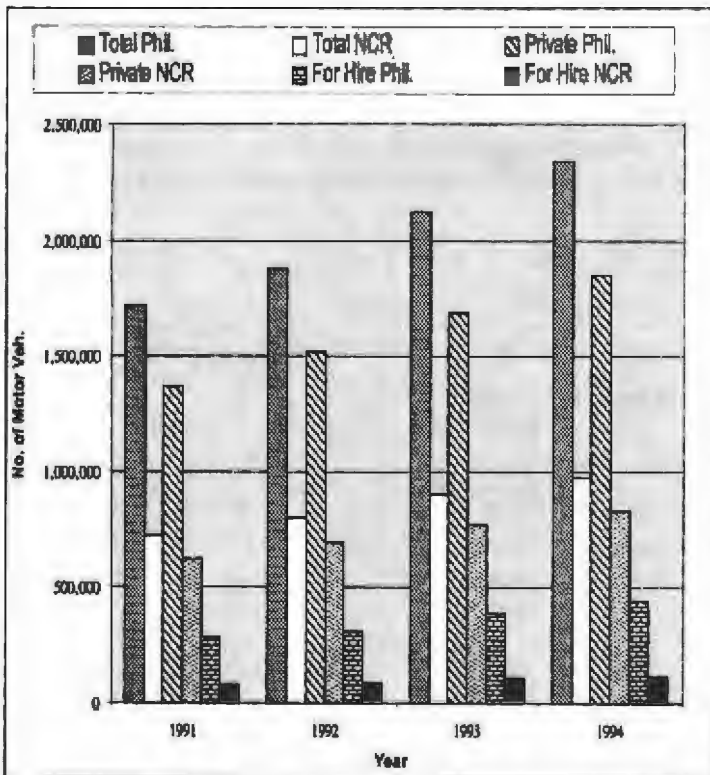
In 1995, registered motor vehicles reached 1.06 million. Of this total, the share of the private vehicles is overwhelmingly at 86.14% followed far behind by vehicles for hire at 12.06%, and government vehicles at 1.22%. To illustrate the aggregation of private cars in the metropolis, Figure 2 shows the share of the metropolis in national total.

Utility vehicles are locally referred to as "jeepneys". These are the most popular mode of public transport in Metro Manila as they are cheap in fare and provide access to any part of the metropolis. They can only seat approximately 16

Table 8. Registered Motor Vehicles by Major Classification, 1990-1995

Year	Total	Private	Govt.	For Hire	Others ^{1/}
1990	684,778	596,769	19,469	61,280	7,260
1991	721,776	620,060	16,620	16,620	78,203
1992	796,719	694,705	15,447	82,865	3,702
1993	901,312	772,074	17,594	105,590	6,054
1994	973,550	833,611	15,883	117,088	6,968
1995	1,055,692	909,411	12,873	127,311	6,097

Source: Land Transportation Office



Source: Philippine Statistical Yearbook, 1996

Figure 2. Changes in the Number of Registered Vehicles.

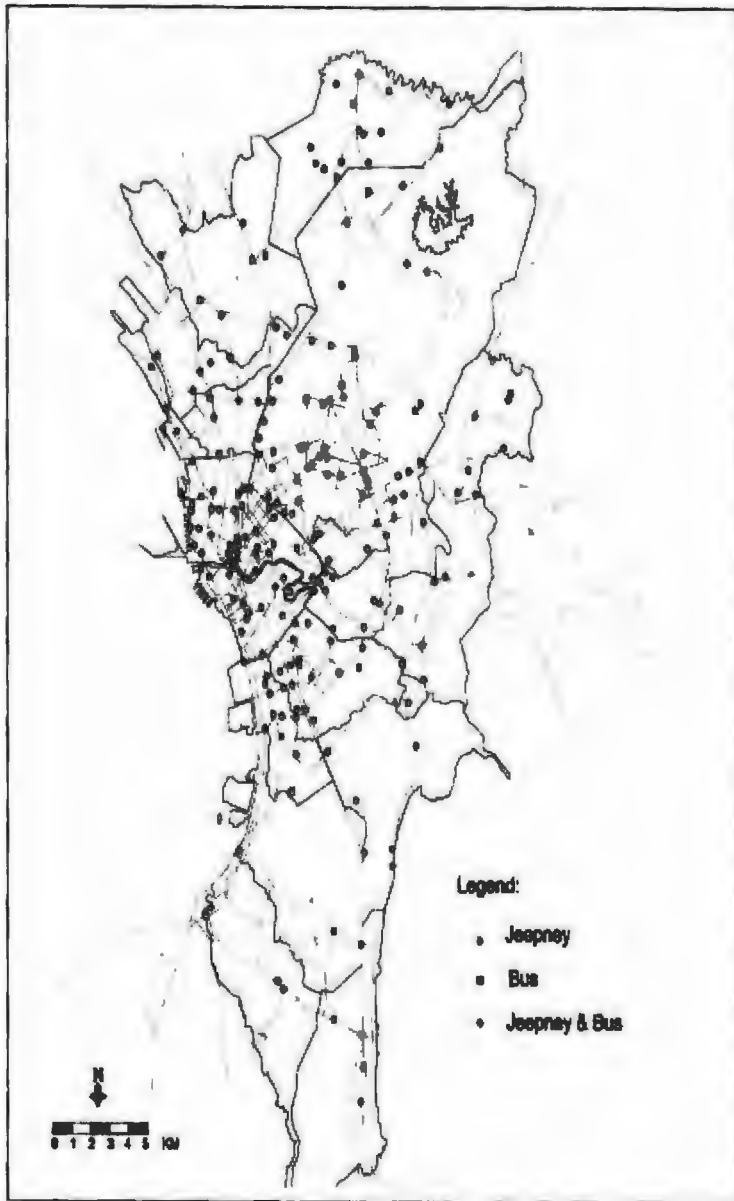


Figure 3. Service Coverage and Location of Terminals.

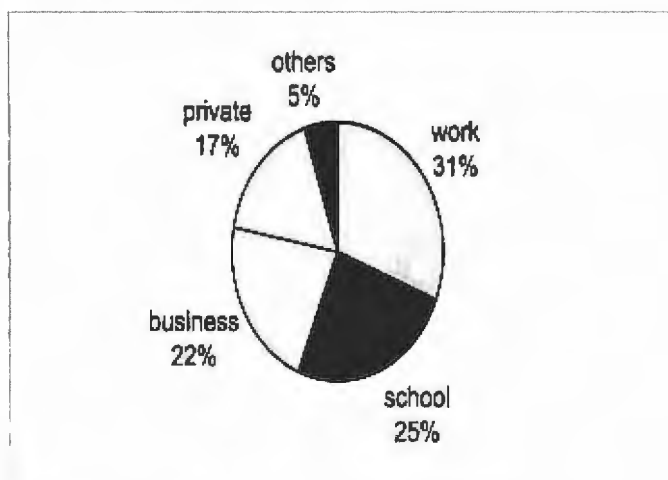


Figure 4. Trip Purpose Composition (Excl. "to home" and walk trips)

Table 10 below shows the modal share by trip purpose. It is still evident that majority of the people are still dependent on the public modes of transportation. However, still from the table below it is also noticeable that there is a significant percentage of business trips using private modes of transportation.

Table 10 Modal Share by Trip Purpose (%)

Trip Purpose	Private		Public				
	Car	Others	LRT/PNR	Jeepney	Tricycle	Bus	Taxi
To Home	16 22%	6	3 78	39	14	16	6
To Work	20 28%	8	3 72%	34	6	24	5
Business	25 43%	18 57%	1	24	13	5	14
To School	10 20%	10	3 80%	46	21	8	2
Private	21 24	3	2 76	42	12	13	7

Source: MMUTIS Factbook, 1998

Looking at the average trip length by mode, as given in Table 11, it can be observed that there was an increase in the trips made in Metro Manila from 1983 to 1996. The increase in trip lengths could be attributed to the spread in residential locations, work places and various shopping and commercial centers. Another factor is the worsening traffic conditions in the metropolis. The increase in the number of shopping and commercial centers is perceived to be related to the worsening traffic conditions.

Table 11. Average Trip Length, for all trip purposes (in minutes)

Mode	Year	
	1983	1996
Car	42.8	53.0
Jeepney	34.7	43.4
Bus	56.3	77.9
Taxi	34.4	55.5
Tricycle	13.6	18.1

Source: MMUTIS Factbook, 1998

The travel demand is distributed unevenly throughout the day. Concentration of trips is significant in the morning between 6 to 9 A.M. and in the afternoon between 4 to 7 P.M. Third peak is also seen during lunchtime. The morning peak hours are usually dominated by to school trips (between 6 and 7 A.M. and to work trips (between 7 and 9 A.M.). (MMUTIS Factbook, 1998)

In terms of travel demand by type of facilities (Table 12), residential and educational facilities have the bulk of trips generated and attracted. They are followed by wholesale and office facilities.

The MMUTIS Study has shown that there has been an intensified movement of people when it compared the distribution of travel demand between 1980 and 1996. Most notable is the increase in trips outwards to the south, north and east. The Study further noted that what used to be suburban areas outside of EDSA (the major circumferential road traversing Metro Manila) in 1980 are already urbanized. This observation further validated the increase in travel distance and travel time in the metropolis.

Similarly, the commercial and business centers in Metro Manila vary in character. The cities of Manila and Makati attract traffic from all over the metropolis. On the other hand, the EDSA area in Quezon City attracts mostly those residing in the northern half of Metro Manila. These observations indicate that analysis of area-wide traffic characteristics will yield relevant information in formulating traffic and urban plan of municipalities. This is one of the major recommendations of the MMUTIS Study. By establishing area-wide database on transportation and land use will strongly put emphasis on an integrated transportation-land use planning approach in alleviating transportation and traffic problems in Metro Manila.

Table 12. Trip Generation/Attraction by Type of Facility (1996)

Types of Facility	Generation		Attraction	
	'000 Trips	%	'000 Trips	%
Residential	14,223	46.6	14,238	46.7
Commercial	651	2.1	642	2.1
Office	2,289	7.5	2,260	7.4
Factory	1,239	4.1	1,231	4.0
Educational	5,612	18.4	5,670	18.6
Recreational	112	0.4	112	0.4
Medical	320	1.1	314	1.0
Social	406	1.3	408	1.3
Wholesale	2,660	8.7	2,655	8.7
Restaurant	527	1.7	513	1.7
Others	2,453	8.0	2,447	8.0
TOTAL	30,491	100.0	30,491	100.0

Source: MMUTIS Factbook

Traffic Condition in Metro Manila

Like its counterpart in Southeast Asia, Metro Manila has already experienced worsening traffic congestion in its road network system. Traffic volume on the roads and streets has aggravated in the past 20 years. The increase in traffic volume is not at the central portion of Metro Manila since the roads there have already been saturated. The growth of the traffic volume has been in EDSA, South Superhighway and other radial corridors outside EDSA. This is attributed to the expansion of urban development in those corridors especially at those areas considered as suburban before 1980.

The significant increase in traffic volume on all major corridors of Metro Manila is attributed mainly on the rise in volume of private vehicles. EDSA, which is bus-dominated corridor, and Shaw Boulevard, jeepney-dominated, have shown notable increase in private car volumes (as reflected in Table 13). From Table 5 in the previous section, the share of private modes in 1995 is 53.2% of the total vehicular traffic demand though it only accounts to 21.6% of the person trips due to its low occupancy.

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Table 13 . Traffic Volume on Major Corridors, 1980 & 1995

Corridor	1980	1995		1995/1980	
		Total	Car/ Truck		Jeepney/ Bus
EDSA (bet. Guadalupe & Buendia	99,900	172,500	154,700	17,800	1.7
SSH (intersecting Pres. Quirino Ave.)	72,900	113,700	111,300	2,400	1.6
Quezon Ave. (near España Rotonda)	53,300	102,500	68,400	34,100	2.0
Roxas Blvd. (intersecting P. Burgos)	67,800	82,300	74,100	8,200	1.2
Ortigas Ave. (intersecting Santolan)	51,500	76,300	72,900	3,400	1.5
Shaw Blvd. (intersecting Acacia)	37,300	43,500	27,800	15,700	1.2

Source: MMUTIS Factbook, 1998

The immediate outcome of the increase in traffic volume already mentioned above is the deterioration of the traffic condition in Metro Manila. Traffic congestion has become chronic and people tend to associate it with their daily lives. Almost all sections of the road network are congested in the region. It is perceived that the major reason for congestion is the lack of road infrastructure. Albeit this perception, as shown in Table 7, the vehicular density per kilometer is not as much compared to the other cities. If traffic management is improved and traffic rules and regulations are properly enforced, traffic congestion can be alleviated at some degrees since the road infrastructure could be utilized efficiently.

The extent of the impacts of traffic congestion has already affected significantly the levels of service of the public transportation system. There has been a sharp decline in travel speeds and tremendous increase in travel time not only of private vehicles but also of the public transportation modes. Likewise, vehicles have become crowded with average passenger occupancies increased by almost 1.3 times for buses, 1.5 for jeepneys and double for tricycles as reflected in Table 14.

The preceding section presented the present profile of Metro Manila and its transportation system. It could be noted that in a matter of several years the region will eventually become a megacity. Metro Manila is rapidly expanding its development and urbanization to its adjoining areas. The continuous growth and expansion of the metropolis will definitely affect its transportation system.

From the unpublished report prepared by the NCTS for NEDA-LEDAC, Economic Impact of Traffic Congestion in Metro Manila (April 2000), it is estimated that the average annual cost of traffic congestion is about 100 Billion Pesos for people working in Metro Manila. Table 15 below summarizes the calculation of traffic congestion cost by type of occupation.

Table 14. Average Travel Time and Occupancy of Public Transport.

Mode	Ave. Travel Time (in min.)			Ave. Occupancy		
	1983	1996	1996/1983	1983	1996	1996/1983
Bus	56.3	77.9	1.38	39.7	50.0	1.26
Jeepney	34.7	43.4	1.25	10.3	15.0	1.46
Tricycle	13.6	18.1	1.33	1.3	2.6	2.00
Taxi	34.4	55.5	1.61	2.1	2.2	1.05

Source: MMUTIS Factbook

Table 15. Calculation of Congestion Cost.

	Average Delay (hr.)	Ave. Hourly Income (Peso)	Ave. Congestion Cost (Peso)	Total Number of Trips	Total Cost (Peso)
Gov't Officials	0.63	93.75	29.38	3,427,860	100,693,388
Professionals	0.69	187.50	65	1,460,326	94,921,190
Technicians	0.73	62.50	22.86	853,063	19,504,930
Clerical Workers	0.72	50.00	17.88	1,134,083	20,271,734
Service Workers	0.61	62.50	19.05	2,194,432	41,793,262

Source: Economic Impact of Traffic Congestion in Metro Manila, April 2000 (an unpublished report)

Traffic congestion has worsened as shown by some indicators above; notably its economic impact. However, traffic congestion is just a manifestation of the transportation problems of the metropolis. Unless something has to be done, the urban transportation problems will further aggravate. As such, proper and integrated transportation and urban planning should be done to alleviate the situation. The succeeding sections will further elaborate on the pressing urban transportation problems faced by Metro Manila. Similarly, the courses of actions being formulated and implemented to alleviate the issues will be discussed.

TRANSPORTATION AND TRAFFIC CONCERNS

The following discussions on the pressing transportation and traffic issues are culled from an unpublished paper of the same author, *Present Urban Transport Problems and Its Countermeasures – the Case of the Philippines, 1999*.

As already elaborated in the previous section, the traffic congestion problem in Metro Manila has become a very serious concern not only of the government but also of the community and the industry. The quality of life in the metropolis has deteriorated and in fact affected those in the adjoining areas. Almost all industrial and business sectors have incurred considerable due to longer travel times and lower productivity.

Traffic congestion has reached the level of becoming a social problem that affects, the motorists, commuters, and pedestrians, but also people from all walks of life. This problem has brought about yet other serious problems such as:

- a. Wastage of time and energy due to delays incurred in congested roadways;
- b. Environmental degradation caused by increased vehicle exhausts in traffic jams;
- c. Damage to and/or loss of life and property due to traffic accidents.¹

People tend to only perceive traffic congestion as the main cause of the urban transportation issue and problems in the metropolis. However, as already mentioned earlier, traffic congestion is not the root but rather a manifestation of the intertwining and related effects of urban transportation problems in the region.

The root causes of traffic congestion may be classified into two: technical or planning and institutional. The technical aspect concerns the absence of an integrated master plan agreed upon by the cities and municipalities comprising Metro Manila. Most projects undertaken in the region do not follow any plan, which could be identified with any particular land use or zoning policy. The conveyance of people from one place to another is hindered by deficiencies in a transportation system, which cannot cope with the sudden and undirected growth of certain areas. A good example of such is the continued sprouting of huge shopping malls, condominiums and the like in places where they critically contribute to the worsening state of traffic flow. Due to such undirected growth, adequate infrastructure cannot be provided to address the growing demand. Note, for example, the lack of parking spaces, the narrow roads, the incomplete road network, the lack of an efficient mass transit system, and inadequate traffic signal control system.²

The institutional aspect can be easily observed in the poor coordination among government agencies, and the absence of an integrated and determined

¹ Alleviating Traffic Congestion in Metro Manila, a white paper drafted by UP and NAST, 1997

² *Ibid.*

among government agencies, and the absence of an integrated and determined effort to straighten out the traffic mess. Such is due in part to the creation and presence of numerous agencies (e.g., DOTC, DPWH, MMDA, TEC, PNP-TMC, LTO, etc.) tasked with overlapping responsibilities concerning the alleviation of the traffic problem. Policy-making and implementation/ enforcement are assigned to specific agencies. However, these organizations usually disregard or bypass one another in the performance of their functions. Moreover, there seems to be a number of conflicts among the personalities heading the said agencies regarding priorities and interests. Finally, it may be pointed out that although there are already a great number of policies, rules and regulations, there is an evident shortcoming when it comes to implementation and enforcement. This laxity has encouraged the lack of discipline among drivers and pedestrians - something that would take a long time to correct. There is also a lack or absence of effort to coordinate with other agencies/companies involved in providing utility services such as the MWSS, MERALCO, PLDT, etc., in order to avoid this perennial diggings and/or repairs on the road.³

Apart from the technical and institutional aspects, another factor that contributed to the growing urban transportation problems is the increasing rate of in-migration to Metro Manila from the other regions of the country. The continuous movement of people toward the metropolis also brought with it other inter-related urban problems, such as lack of housing, lack of employment opportunities, environmental problems, etc. The transportation system could not cope with the growing population in the region.⁴

Specifically related to transportation planning, the lack of reliable databases on all aspects of transportation and personnel with expertise in transportation planning and engineering likewise aggravated the issues. There are measures and schemes implemented that were not supported by technical studies and surveys. There were instances where the implementation of some transportation and traffic measures that contributed to the transportation problems rather than alleviating them.

Given the above predicaments on the root causes of the urban transportation problems, especially traffic congestion, the courses of actions to alleviate, if not minimize, the gravity of these problems should focused or addressed directly these issues. These courses of actions should be looked at a macro level and over time. The solutions to these problems are not just limited to technical and institutional strategies but should also social and cultural concerns. The succeeding section shall outline the courses of actions formulated and implemented to respond to the urban transportation problems of Metro Manila.

³Ibid.

⁴Ibid.

MITIGATING MEASURES IMPLEMENTED OR PROPOSED TO ADDRESS THE CONCERNS ON TRAFFIC CONGESTION

A white paper prepared for the University of the Philippines (UP) and the National Academy for Science and Technology (NAST), of which the author of this paper was one of the persons involved, has summarized the measures formulated and implemented. The measures taken and to be taken by the government in alleviating transportation and traffic problems in Metro Manila, based on existing plans and projects, can be categorized as follows:⁵

- a) Construction of new roads or highways, specifically completion of Metro Manila's road network;
- b) Rehabilitation of existing road network;
- c) Introduction of mass transportation system (e.g., LRT);
- d) Application of short-term travel demand management (TDM) schemes.

Aside from the PNR commuter rail, the introduction of a rail-based mass transportation system in Metro Manila commenced with the construction of LRT Line I. Now the construction of a network of LRTs becomes one of the priorities of the government due to the entry of the private sector through the build-operate-transfer (BOT) scheme or its variants.⁶

The following are some of the TDM and TSM measures that have been applied or suggested in Metro Manila to help alleviate traffic congestion.⁷

- a) One-way Scheme
- b) Car-pooling /Van-pooling
- c) Reversible Lane
- d) Truck Routes
- e) Staggered and Flexible Work Hours
- f) Flexible Work/School Days
- g) Fare Surcharge
- h) Improvement to Public Transport Services
- i) Bus Lanes
- j) Odd-Even Scheme

Looking back at the clusters of recommendations based on plans and projects for Metro Manila, one could notice that they were not comprehensive enough. They seem to be adaptable only on the time or era they were formulated. It would seem that a number of the measures formulated were not properly coordinated among the transportation agencies concerned. Likewise, some measures were

⁵Ibid.

⁶Ibid.

⁷Ibid.

adopted without studies to support them. For this end, the next section presents alternative recommendations that could be more appropriate

Transportation System Integration

The white paper mentioned in the previous section provided several alternative courses of actions in alleviating urban transportation problems in Metro Manila. These will be presented again in this section. The existing transportation modes in Metro-Manila lack the essential features of a fully integrated transportation system. This is due mainly to the ineffective coordination and project-oriented planning of various agencies of government dealing with different, and in some cases, common aspects of the transportation system. And with the government's recent effort to attract private sector investments in infrastructure development, coordination of transport development has become even more difficult to manage because of the need to consider private sector interests, which sometimes are in conflict with public interests.

This lack of integration results in wasted transport capacities, poor quality of transport services, and low system-wide transport productivity. Hence, it is not sufficient to simply build new roads and put up new mass transit lines without formulating a comprehensive master plan for integrating these various road and rail projects. The key elements of an integrated transportation system should have a hierarchical road and public transport networks, and strategically planned station and terminal facilities.⁸

Hierarchical Road and Public Transport Networks

The design of road and rail networks should follow the basic principle of functional integration. In the case of roads, there should be a fully connected hierarchical set of expressways, arterial, and local roads, which provide mobility and access to private and public vehicles. Presently, Metro-Manila does not have a sufficient expressway network, which can relieve the arterial roads of through vehicular traffic. Furthermore, many arterial roads are disconnected and thus altogether do not perform well their function of distributing traffic between the expressways and local roads. On local roads, traffic flow and access to land and property are also impeded by ineffective parking controls and unregulated use of road space. While the government's projects to construct flyovers at key intersections have improved traffic flow, their effects are localized in nature. Greater focus should be placed in enhancing the capacity of expressways and connectivity of the entire road network.

In the case of public transportation, the task of integrating the various modes is extremely difficult but most important. The table below shows the various

⁸Ibid.

modes in Metro-Manila together with their basic characteristics (i.e., capacity and service area).

MODE	CAPACITY	SERVICE AREA
LRT	350 persons/train	Intra-urban
Bus	47-64 seats	Intra and inter-urban
Jeepney	14-20 seats	Intra and inter-urban
Taxi	4 seats	Intra-urban
Tricycle	2 seats	local
PNR Commuter	500-1000 persons/train	Inter and intra-urban

Given their respective characteristics, each mode is naturally well suited to operate under very specific market demand and operating conditions. For example, smaller vehicles such as tricycles and jeepneys are ideal in performing collection and distribution functions within low-density residential areas. On the other hand, larger vehicles such as the LRT and Buses are ideal in performing a line-haul function connecting the residential areas with central business districts. In terms of trip lengths, smaller vehicles are ideal for shorter trips, while larger vehicles are ideal for longer trips. Therefore, in order to optimize the performance of the entire public transport network system, it is necessary to operate each mode within their ideal operating environment. This concept of hierarchical structuring of the functions of each mode is illustrated in Figure 5. The main features of this concept are described as follows:⁹

- A heavy rail transit system will become the main line-haul or trunk line serving regional passenger and freight transport demand to and from Metro-Manila.
- A light rail transit system will become the main line-haul network serving commuter travel demand within Metro-Manila. Buses may serve as main line-haul in certain areas.
- Within Metro-Manila, buses and jeepneys will serve as feeder routes to the heavy and light rail transit systems. In certain areas, jeepneys may also serve as feeder routes to bus stations and stops.
- Outside Metro-Manila, jeepneys and provincial buses will serve as feeder routes to the regular heavy rail transit system.
- Provincial buses terminate at the heavy rail transit stations outside of Metro Manila. This strategy of consolidating provincial passenger trips outside the metropolitan area would improve the turnaround time and

⁹Ibid.

productivity of provincial buses, increase ridership of the heavy rail transit system, reduce bus traffic volume inside Metro-Manila, and reduce the need for bus terminal facilities within Metro-Manila.

Strategically Planned Station and Terminal Facilities

The public transport station and terminal facilities comprise the most important element of an integrated transportation system. Since passengers and freight enter and leave the rail system through the stations, it is very important that adequate facilities and amenities are provided. Each station should be so designed as to give the passengers a feeling that it is a safe, comfortable, and convenient place to board, alight, wait, and transfer to and from various transportation modes.¹⁰

Currently, the station and terminal facilities are among the most neglected aspects of Metro-Manila's transportation system. Several LRT and other rail development projects are being planned and implemented without a strategic master plan for integrating these facilities. It is necessary for the government to carefully plan the location and design of these facilities.¹¹

These facilities may feature retail/commercial spaces, park-and-ride facility, kiss-and-ride facility, and bus/jeepney terminal areas. The park-and-ride facility should be well secured so those auto trip makers would be encouraged to leave their cars behind. This facility will also serve to reduce the need for more parking spaces in the key urban centers within Metro-Manila.¹²

The kiss-and-ride facility should be well-designed as to provide easy access for private vehicles to drop-off their passengers at the train station; enough holding area for cars waiting to pick-up their passengers; and, comfortable and safe area for passengers waiting to be picked-up.¹³

The bus and jeepney terminal areas should be an integral part of the railway stations. This complex will be designed to provide public transport users with convenient transfer facilities and bus/jeepney operators with terminal areas for their fleet.¹⁴

Demand Control Measures

Another way of comprehensively addressing the present traffic problem is by looking at the demand-side of the transportation system. The guiding principles in formulating the schemes or measures to curb travel demand are: a) economic concepts on how the road users shall be priced for the use of the transportation

¹⁰Ibid.

¹¹Ibid.

¹²Ibid.

¹³Ibid.

¹⁴Ibid.

system - employing the beneficiary-pay-principle or polluter-pay-principle concepts, b) discouraging the use of or demand for single-occupancy vehicles, and c) volume-reduction. These measures are based on the premise that the use of the road and the transportation system is not a right but rather a privilege. Furthermore, the above three guiding concepts should be viewed as inter-related and should complement the measures formulated in approaching the supply-side of the system.¹⁵

Ideally, the most economic measure is to raise the cost of gasoline; however, the repercussions of such a measure are considered to be serious in the context of Metro Manila. As such, this approach needs further studies and therefore has to be considered rather as a long-term measure. Other means such as raising the fees related to acquisition and usage of vehicles (license, price of vehicles, etc.), impose high parking fees, congestion pricing and toll fees in the present and future expressways can be applicable in Metro Manila and the country in general.¹⁶ The kiss-and-ride facility should be well-designed as to provide easy access for private vehicles to drop-off their passengers at the train station; enough holding area for cars waiting to pick-up their passengers; and, comfortable and safe area for passengers waiting to be picked-up.¹⁷

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There are several viable ways in discouraging the demand for single-occupancy vehicles (estimated to be 50% of the total private users). The present plan to construct several mass transit systems in Metro Manila can be useful in this aspect. Likewise, promoting high occupancy vehicles such as car or van-pooling can also reduce the demand for single-occupancy vehicles. Volume reduction scheme (such as the odd-even scheme) is in a way a concept to curb travel demand and at the same time a goal to alleviate traffic congestion.²¹

Some TDM actions have already been experimented on in Metro Manila. However, they have not been appropriately implemented. There is a need to further study or evaluate the effectiveness and appropriateness of these schemes. Among the TDM measures for the demand-side applied are: a) Car-pooling Van-pooling, b) Staggering Work Hours and Flexible Work Hours, c) Flexible work/school days, and d) Odd-even scheme.²²

Aside from the promising TDM measures mentioned above, the following are hereby proposed:²³

- a. Land Use Control - Administrative approval for:
 - Location of trip generators like big malls and other commercial establishments;
 - Building permits with imposition of parking requirements; and
 - Location of bus/truck terminals.
- b. Discouragement of private car ownership or usage
 - Stricter implementation of driver licensing policy; and
 - Requirement/proof of garage/parking facility.
 - Congestion pricing/road user taxation

Linking Land Use and Transport Development

One of the major reasons for the perennial traffic congestion problem is the absence of sufficient controls for managing the development of land and corresponding provision of adequate transport infrastructure. It is well known that

²⁰Ibid.

²¹Ibid.

²²Ibid.

²³Ibid.

traffic is a function of land use and yet the current zoning ordinances and building code do not provide enough safeguards to ensure that land and transportation developments are synchronized.²⁴

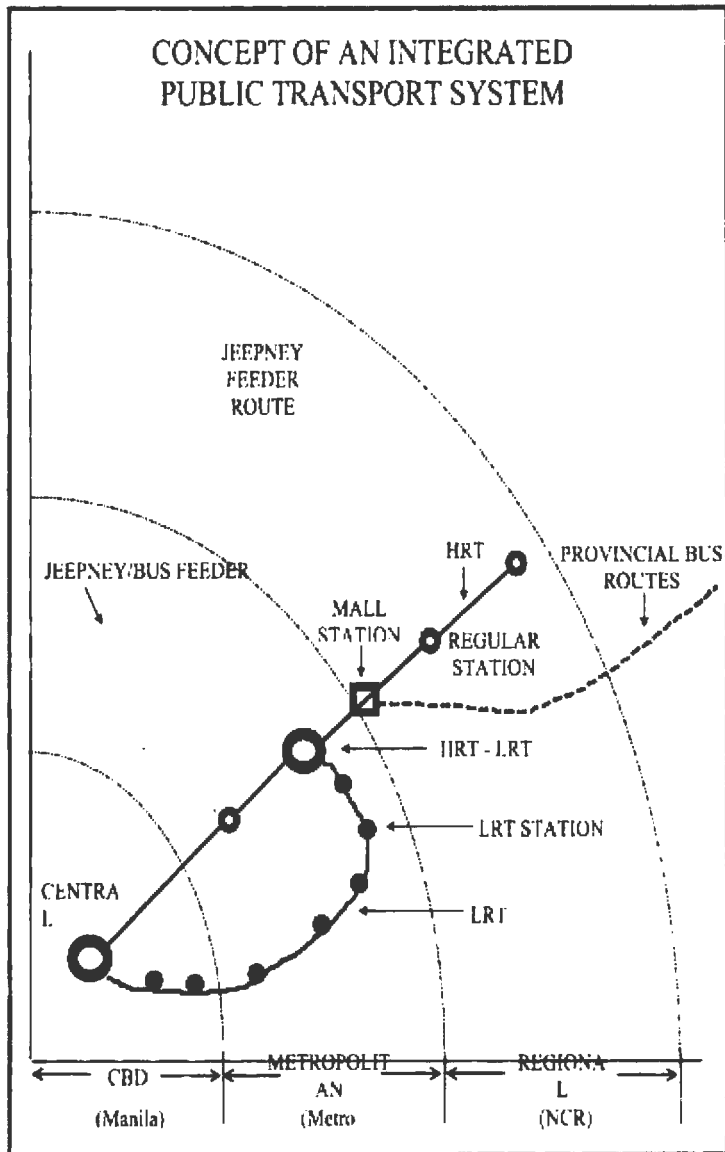


Figure 5. Concept of an integrated public transport system.

Presently, land developers do not fully shoulder the cost of traffic congestion they induce on the road and public transport system. A system for estimating such cost and a mechanism charging it as part of land development cost charged to the developers should be implemented. A traffic impact assessment (TIA) must be a requirement for any land development. As a matter of principle, land developers must shoulder the cost of providing additional transport infrastructure necessary to maintain the same transport level of service in the areas affected by its development.²⁵

Traffic education is three-fold: a) educating personnel in the transport and traffic sectors including the traffic enforcers, b) educating the road users and the public in general on proper values, and c) education through research.²⁶

The number of local experts in the field of transportation is still very limited. Key personnel of various government agencies related to traffic need intensive training, both here and abroad.²⁷

One contributing factor in the aggravation of traffic congestion in the streets of Metro Manila is road users' poor driving behavior or attitude. Educating (or re-educating) people on their respective roles in traffic can contribute in alleviating the worsening traffic congestion. The present system of seminars for persons seeking driver's license and violators needs improvements. It must include value formation for both enforcers and followers. Traffic safety education must be integrated in the curriculum of schools especially from the primary levels. This has been found to be effective in other countries.²⁸

Finally, transportation research (both basic and applied) must be promoted. A better understanding of the transportation and traffic problems in Metro Manila is necessary in finding appropriate solutions. One of the major factors contributing to wrong forecasts and modeling in the country especially in Metro Manila is the lack of accurate data. In addition, most of the standards and modeling techniques employed in many transport studies are patterned after other countries' standards, notably from the developed ones. These resulted to misspecifications of the models thereby resulting to inaccurate and/or inappropriate recommendations. Hence, it is essential that the establishment of a transportation database and improvement of data collection be promoted and encouraged. There is also a need to establish standards appropriate for Metro Manila and the country in general. Moreover, transport and traffic models need to be specified and calibrated with the local conditions. It is only through these that transportation research can be used as a powerful tool in solving traffic problems in Metro Manila.²⁹

²⁵Ibid.

²⁶Ibid.

²⁷Ibid.

²⁸Ibid.

²⁹Ibid.

The above recommendations of the white paper indicated that the courses of actions in alleviating urban transportation problems should be holistic and focus on the root causes of these problems. Not only the technical aspects should be addressed, but also the institutional and academic or research aspects of transportation. Furthermore, it was mentioned elsewhere in this paper that social and cultural aspects should also be included in the formulation of the measures. These aspects are relevant in considering the human factor of the urban transportation problems.

Finally, the social acceptance of any courses of actions has to be considered for these actions to be effective and viable.

As already mentioned elsewhere in this paper, the database of the Metro Manila Urban Transportation Integration Study (MMUTIS) was used in the presentation of the current situation of Metro Manila's transportation and traffic system. The major output of the MMUTIS study is a transportation master plan for Metro Manila. Highlights of the MMUTIS transportation master plan is summarized below.

The MMUTIS Transportation Master Plan

The Department of Transportation and Communications (DOTC) was tasked to prepare a transportation master plan for Metro Manila (and its adjacent areas) with technical assistance from the Japan International Cooperation Agency (JICA). The three-year Metro Manila Urban Transportation Integration Study (MMUTIS) started in 1996 and was finished in 1999; however, the final report was officially transmitted to the Philippine Government in May 2000.

The Metro Manila transportation master plan is believed to formulate a "good" future transport network plan, which is efficient in terms of meeting future demand and affordable in terms of public sectors funding.³⁰ The MMUTIS study indicated four plan implementation options - "*Do-Nothing Project*", "*Do-Fixed Project*", "*Do-Existing Project*", and "*Do-Maximum Project*". "*Do-Nothing Project*" would assume the transport network as of 1996. "*Do-Fixed Project*" includes committed projects such as LRT 2, Line 3, Skyway Phase 1 and Phase 2, Manila-Cavite Expressways and unfinished sections of C-5. "*Do-Existing Project*" further include other existing plans of the government for Metro Manila and BOT proposals. Finally, the "*Do-Maximum*" was prepared to satisfy the gap and to provide the existing and anticipated urban areas with reasonable transport network which comprises a combination of primary and secondary roads, expressways, MRT/LRT and busways (Figures 6 and 7). The "*Do-Maximum*" network gives a structure of future transport system, which is to be incorporated with the city plans of the LGUs.

³⁰Iwala, S., MMUTIS Master Plan Highlights, 12th MMUTIS Seminar on Strategic Management of Urban Transportation, Manila, Sept. 1998.

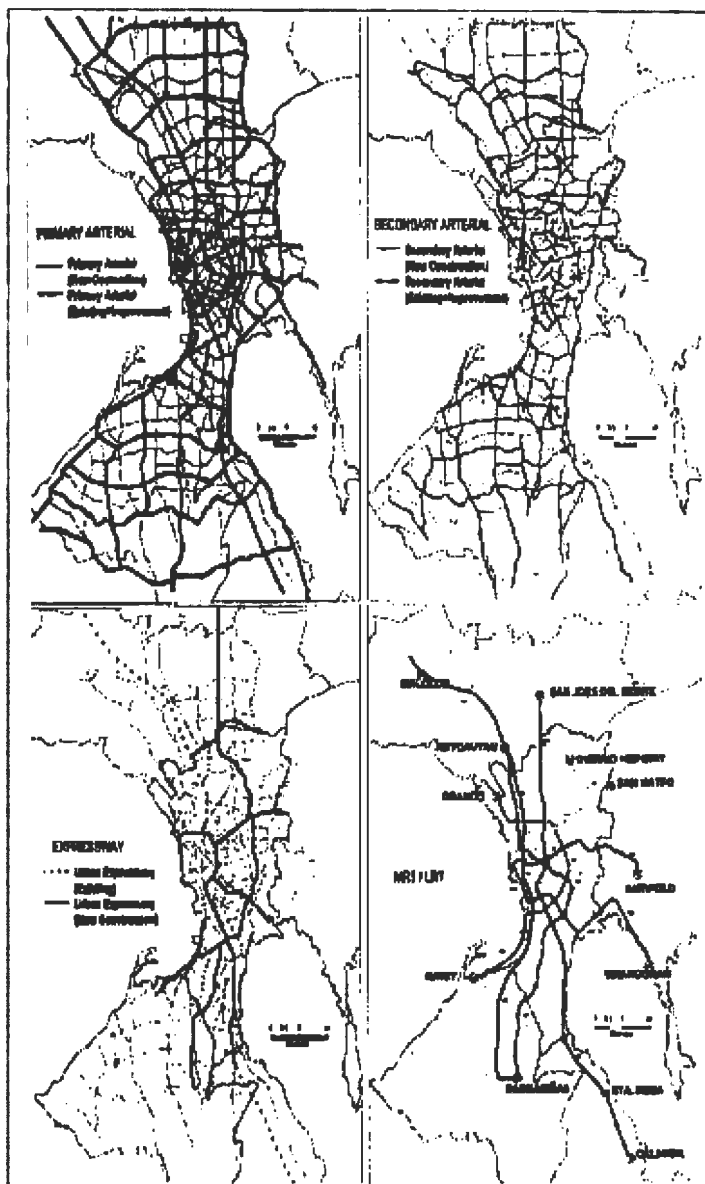


Figure 6. Future Transport Network (do maximum) in Metro Manila.

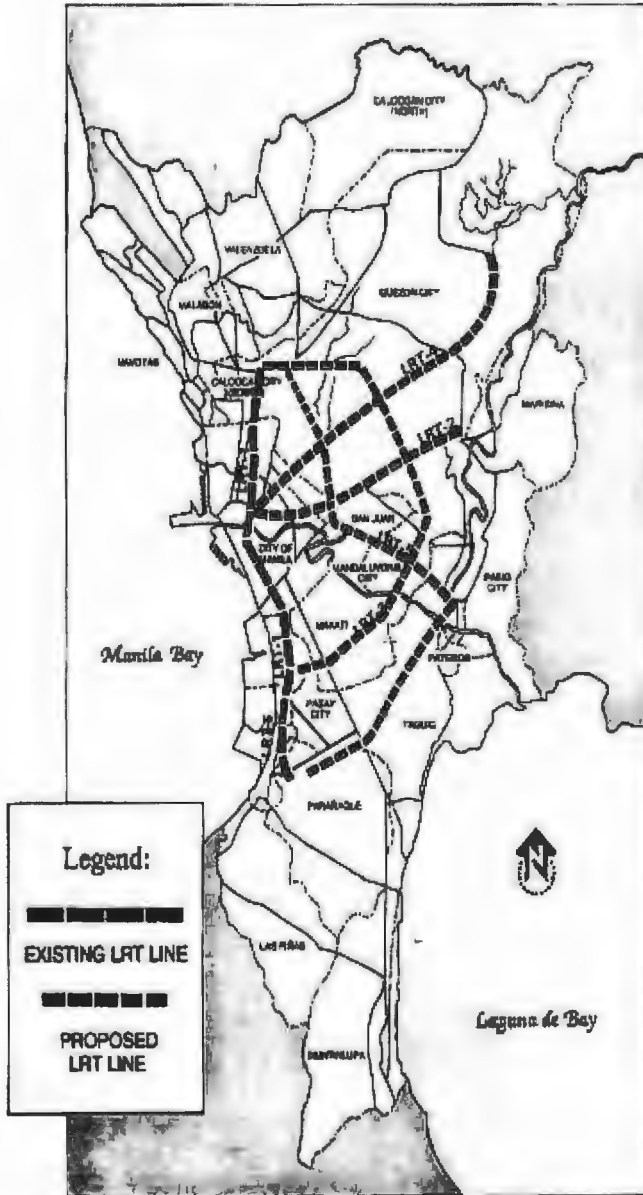


Figure 7. Proposed MRT Line Projects.

The above alternative options recommended by the MMUTIS Study based on a policy of combining road and rail transit development. Apart from the road and rail infrastructure projects the MMUTIS Master Plan has also recommended responsive TDM schemes to support the infrastructure development. It is expected that the combined road and rail transit development will be able to meet the future socioeconomic needs of the region. It is further hoped that when Metro Manila will become a megacity by year 2015 it will be ready to address the transportation requirements of the megacity residents.

The alternative options proposed by the MMUTIS Study obviously entails enormous capital investments. The estimated investment cost for the future transportation network range from US \$ 4 Billion for "Do-Nothing Project" to US \$ 10 Billion for Do-Maximum". Tapping public investment funds to implement any of the four options would be very difficult considering the limited budget of the government. The government has other regions to take care too. Likewise, increasing taxes, such as fuel tax, registration fees, etc., would also be possible source of funding. This could be justified through the concept of road pricing. However, it is expected that there would be a strong opposition from the people.³¹

Thus, the above suggested that there is a need to look for other sources of investment funds to implement the transportation master plan for Metro Manila. The government could still rely on foreign assistance or loan from major international institutions, such as World Bank, ADB, OECF, or from country donors like Japan, US, Germany. These would still not be sufficient to realize the master. Furthermore, regional economic crises would aggravate the situation if relying mainly on foreign loans.³²

The MMUTIS project has identified PFI as one of the major institutional mechanisms to materialize the Metro Manila transportation master plan. Albeit this suggestion, the limitations and constraints of PFI have to be carefully addressed. There is a need to look at the opacity in project planning/implementation procedure for those projects under PFI. Integration among projects has to be in place.³³

³¹ Lidasan, HS, Private Sector Investment in Infrastructure Planning The Concept of PFI, a paper presented in the SPRING Workshop, Dortmund University, November 1998.

³² Ibid.

³³ Ibid.

Master Plan Investment Summary

Investment	Amount (US \$ billions)	Remarks
1. BASIC PROGRAM		
a) Low Cost Management		
b) Maintenance/Rehabilitation	1.50	
c) Existing Network Improvements		
2. ON-GOING MAJOR PROJECTS ^a	0.50 (0.72) (1.84)	Skyway (17.0 kms.) LRT 2,3 (30.8 kms.)
3. NEW MAJOR PROJECTS		
a) Primary Roads	3.70	310 kms. (80%) ^b
b) Secondary Roads	1.40	265 kms. (50%) ^b
c) Expressways	0.40	100 kms. (10%) ^b
d) MRT/LRT Systems	3.30	200 kms. (54%) ^b
e) Busways		

^aUS\$ 0.5 billion (85% of OECF loan portion) will be included.

^bPercentage of public sector share in total investment.

Source: MMUTIS Master Plan Highlights, 13th MMUTIS Seminar.

The recommendations of MMUTIS rely heavily on infrastructure developments in alleviating traffic congestion and other urban transportation problems. Albeit these physical courses of actions, MMUTIS likewise proposes a menu of TDM measures in support of the infrastructure developments. The huge investment necessary for the infrastructure developments necessitates the need to formulate TDM measures. Furthermore, the concept of PFI is strongly suggested as an institutional mechanism for the implementation of the infrastructure developments.

CONCLUDING REMARKS

This paper, culled from various studies and similar papers (of which the author is involved) has provided an overview of the present transportation system in Metro Manila, in particular the pressing concerns on its growing traffic problems, notably congestion. It is expected that Metro Manila will become one of the megacities in this part of the world. As such, it is anticipated also that the current

transportation and traffic problems will aggravate unless responsive courses of actions will be formulated and implemented.

A white paper on the issues of traffic congestion in Metro Manila proposed several measures and schemes. These were presented again in this paper. The measures and schemes proposed focused on the technical and institutional aspects of the solutions to alleviate traffic congestion by looking deeper on the roots of traffic congestion.

The Department of Transportation and Communications (DOTC), with technical assistance from JICA, has conducted a three-year study in Metro Manila to develop a transportation master plan principally to meet the future transportation needs of the metropolis. The MMUTIS Study has recommended several road and rail developments and TDM measures to alleviate transportation and traffic problems in the metropolis in the next twenty years.

The infrastructure developments require huge investments. The MMUTIS Study has suggested the concept of PFI as among the potential source of investments in implementing the infrastructure projects. However, there are some cautions on adopting the concept of PFI. There is a need for the government to institute certain measures so that PFI can be for the benefit of the country. It is therefore important that the responsibilities of the government be defined in the project preparations, setting of standards on facilities and services and financing schemes to be instituted. Moreover, the government must conduct critical reviews of unsolicited proposals and preparatory studies on possible PFI projects ahead of private sector involvement.³⁴

Thus, in closing, it is hoped that with the various courses of actions being proposed or formulated urban transportation problems in Metro Manila can be alleviated especially in the future when it will become a megacity.

REFERENCES

1. *Alleviating Traffic Congestion in Metro Manila*, a white paper prepared for the University of the Philippines (UP) and the National Academy of Science and Technology (NAST), 1997
2. DPWH, *Road Handbook in the Philippines*, 1994.
3. Iwata, S., *MMUTIS Master Plan Highlights*, 12th MMUTIS Seminar on Strategic Management of Urban Transportation, Manila, September 1998.
4. JICA, *Metro Manila Urban Transportation Integration Study (MMUTIS)*, 1998.
5. Lidasan, H.S., *Present Urban Transport Problems and Its Countermeasures – The Case of the Philippines*, an unpublished paper presented at the Symposium on “Growth of Mega-Cities and Road Transport Policy in Southeast Asia”, under the sponsorship of the Institute of Highway Economics of Japan, Tokyo Mercantile Marine University, Tokyo, Japan, March 8 – 13, 1999.
6. Lidasan, H.S., *Present Status and Existing Problems of Asian Logistics – The Case of the Philippines*, an unpublished paper presented in the OECD TRILOG Seminar Workshop on Logistics, Institute of Highway Economics, Tokyo, June 1998.
7. Lidasan, HS, *Private Sector Investment in Infrastructure Planning – The Concept of PFI*, an unpublished paper presented in the SPR(NG Workshop, Dortmund University, November 1998.
8. Lidasan, H.S., Tamura, T. and Sison, V.L., *A Study on the Perception on Unified Vehicular Volume Reduction Program (UVVRP)*, Vol. 2, Proceedings of the 1st Asia Pacific Conference on Transportation and the Environment, Singapore, 1998.
9. National Statistical Coordination Board, *Philippine Statistical Year*, 1996.
10. NEDA, *Philippine Transport Strategy Study (PTSS)*, 1996.
11. Nishioka, S., *Opportunities and Constraints of Private Finance Initiative (PFI)*, a paper presented in the 12th MMUTIS Seminar on Strategic Management of Urban Transportation, Manila, September 1998.
12. Trinidad-Lichauco, J., DOTC Under-Secretary, “*Public-Private Partnership in Transportation and Communications Development: The Legacy of the Ramos Administration*”. March 16, 1998.

SUSTAINABLY PRODUCTIVE AGRICULTURE AND GENETICALLY MODIFIED CROPS

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ABSTRACT

Sustainable agriculture is defined by FAO as the management and conservation of the natural resources base, and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations. The author emphasizes the need for sustainably productive agriculture in the 21st century because of increasing world population for which food production must be improved by from 40 to 80% for cereals. Such crop yield increases must also come primarily from higher biological yields and not from area expansion and more irrigation.

The author proposes that through modern biotechnology, crops may be genetically modified (GM) to raise yield ceilings, improve resistance to pests and diseases, develop tolerance to drought, excessive temperatures, soil acidity and salinity and other abiotic stresses and improve the nutritional, processing and keeping quality of produce. While applications of modern biotechnology in health and industry are widely accepted, there are objections to and unease in uses of GM crops in food and agriculture. The paper discusses the risks, both technological and technology-transcendent, associated with biotechnology, and proposes.

To address the question of unequal access to modern biotechnology by developing countries such as the Philippines, the author further proposes that (1) strengthening of national capacity to conduct agricultural biotechnology R&D, (2) put in place the proper intellectual property rights (IPR) to encourage private sector to invest on the problems of Philippine agriculture and (3) provide appropriate incentives so that the new technologies can be accessed by poor farmers.

Key Words: sustainable agriculture, biotechnology, genetically modified (GM) crops

I. INTRODUCTION

Sustainable agriculture and rural development has been defined by FAO as the management and conservation of the natural resources base, and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations.

The need for sustainably productive agriculture looms larger and larger in the horizon as we begin the 21st century. Between the years 2000 and 2025 the world population will increase by almost two billion people. To feed this additional population it has been calculated that the average yields of cereals must be 80% higher than the average yields in 1990.

In the Philippines, our population has been projected to increase from 77 million in 2000 to 108 million in 2020. For rice alone our requirement will escalate from 12.8 million tons to 17.9 million tons, an increase of 40% (Hossain and Sombilla, 1999).

However, because land and water are becoming increasingly scarce, these increases must come primarily from increasing biological yields, not from area expansion and more irrigation (Serageldin, 1999).

The Convention on Biological Diversity (CBD) defines biotechnology as any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific uses. It is the new label for a process that humans have used for thousands of years to ferment foods such as beer, wine, bread and cheese (Vogt and Parish, 1999).

Modern biotechnology, in the narrow sense, refers to applications based on the new science of molecular biology. With the new knowledge in the molecular sciences, it is now possible to identify specific genes in the genomes of organisms; understand their functions in the whole organisms; modify, clone and transfer the genes across natural species barriers, and make the genes express their products in specific tissues, at specific growth stages at specific dosages in the recipient organisms.

In conventional plant breeding which is one form of biotechnology widely applied in agriculture, gene transfers are limited to between varieties of the same species; occasionally between species of the same genus, and rarely between species belonging to different genera. Transferring genes between plant families, much less from bacteria or animals to plants was impossible. But now with the capability of modern biotechnology to precisely manipulate, transfer and control gene expression, these very wide genetic introgressions are possible.

With modern biotechnology, man has at his disposal a new tool for dramatically increasing and stabilizing biological yields while protecting the natural resources base. Crops can be genetically modified to raise yield ceilings, improve resistance to pest and diseases, develop tolerance to drought, excessive temperatures, soil acidity and salinity and other abiotic stresses and to improve the nutritional, processing and keeping quality of farm produce.

The positive impact on the environment from modern biotechnology will come from (1) the more efficient use of land, mineral nutrients and water, (2) the less need for pesticides as more durable genetic resistances are built into crops, (3) the less need for cultivation with herbicide tolerant crops and more robust seedlings thus protecting the soil from erosion and (4) from the better conservation and management of biodiversity.

II. OBJECTIONS TO MODERN BIOTECHNOLOGY

In one sense, modern biotechnology is simply a logical continuation of the old. The essential unity of the genetics of all living organisms had been there all along. We simply discovered the secrets of what the discrete units of inheritance are made of, how they function, and how we can manipulate them with more precision compared with the random statistical methods we have employed in the past.

Apart from agriculture, modern biotechnology has many other potential beneficial applications in health, industry and environment. It is used in producing recombinant DNA vaccines and in gene therapy to treat debilitating human diseases and genetic disorders. Microbial, animal and plant cells are now being genetically modified to produce enzymes, fine chemicals and biodegradable polymers to replace traditional agricultural and chemical factory processes. Microbial cells and genetically modified plants which have unique capability to selectively accumulate heavy metals are now being used to clean up the environment. DNA techniques are being employed to precisely characterize biodiversity to facilitate conservation.

Except for the small minority of people who object to all modern science, the health, industrial and environment applications of modern biotechnology are acceptable to most people. Most of the objections are directed to its applications to food and agriculture, particularly to genetically modified crops.

These detractors see peril in possible introduction of allergens and anti-nutrition factors in foods, in the accidental release of new but harmful organisms into the environment, the hegemony by a few multinational corporations who control the new technology over the world economy, and the replacement of traditional agriculture and the rural way of life by modern, corporate agriculture.

They perceive modern biotechnology as ethically objectionable as it is akin to playing God with nature. It is unnatural and therefore undesirable. They preach the virtues of organic farming (as opposed to modern chemical-based agriculture) to produce safe, healthy food and to conserve the environment.

They attack the Green Revolution as anti-poor unmindful of the fact that if you promote organic farming of the major food crops in the developing countries, this will result in low yields and therefore inadequate food supplies and ultimately high prices. Since food constitute the bulk of the family expenses of the poor, high food prices will hurt the poor more than the rich who could always purchase their food from the market.

The yield inefficiency of organic farming has another very profound negative consequence to the environment of which people are generally unaware. To produce the amount of cereals the world consumes today with the average yields before the Green Revolution, Evenson (private communication), estimated that the world needs to put 200 million more hectares of land under the plow. Since practically all the arable lands are now under cultivation, those additional farmlands will have to come from cutting down tropical rainforests and plowing marginal, environmentally-vulnerable grazing lands.

III. COMMERCIAL RELEASE OF GENETICALLY MODIFIED CROPS

Modern biotechnology in agriculture consists of at least six components (Persley and Doyle, 1999):

- genomics: the molecular characterization of species;
- bioinformatics: the assembly of data from genomic analysis into accessible forms;
- transformation: the introduction of novel genes into crops, forest, livestock and fish species;
- molecular breeding: identification and evaluation of desirable traits in breeding programs with the aid of molecular genetic markers;
- diagnostics: the use of molecular characterization to provide more accurate and quicker identification of pathogens; and
- vaccine technology: development of recombinant DNA vaccines for control of diseases.

Rapid scientific progress is being made on all these fronts. The mapping of the entire genome of the experimental plant *Arabidopsis thaliana* has been completed. The genomic characterization of the major crop commodities are underway. The first that should be completely mapped will be rice, which has a relatively small-sized genome. A Japanese-led consortium is expected to complete the rice genomic map in a couple of years. This process has been greatly facilitated by the private sector initiatives using massive computing and high throughput DNA sequencing machines, in the characterization of the human genome. However to be useful, these genomic maps have to be accompanied by information indicating gene function (functional genomics) which will still take some time to complete.

Marker-assisted breeding is in progress in many countries. Bacterial blight is a devastating disease in rice which had been nearly impossible to control because of the occurrence of many races of the pathogen. Using molecular genetic markers, rice breeders have succeeded in pyramiding bacterial blight genes to develop much more durable resistance to the disease.

Among the modern biotechnology components applied in agriculture, the development of genetically modified crops with specific desirable traits (transgenic

crops) had been the most commercially advanced. The first GM crop was the Flavr Savr tomato with long shelf life released in 1994. Since then commercial release and adoption of transgenic crops has dramatically increased. Between 1996 and 1999, the global area planted to transgenic crops increased from 1.7 million hectares to 39.9 million hectares (James, 1999). Sales are estimated to have risen from \$75 million in 1995 to \$2.1-\$2.3 billion in 1999.

The following major observations characterize this initial phase of commercialization of biotechnology-derived crop varieties:

- a) Most of the early technology adopters were commercial farms in developed countries with the USA and Canada accounting for 72% and 10% respectively of the area planted.
- b) All the subject crops are crops widely grown in developed countries i.e., soybean, corn, cotton and canola.
- c) The almost exclusive foci of trait improvement were herbicide tolerance^a and insect (Bt) resistance^b.

The above observations are very significant because they call attention to and explain to a large extent the opposition and unease which genetically modified crops have elicited from significant sectors of society as well as highlight the challenges and opportunities for us in the Philippines and the rest of the developing world as far as exploiting the benefits of modern biotechnology for food and agriculture.

An essential feature of modern agricultural biotechnology is its increasing proprietary nature. Unlike the agricultural sciences in the past which have come out of publicly supported laboratories, the new biotechnologies are locked into patents, and other private intellectual property rights.

In order to recover their massive investments, the private companies must create value added for which there is effective demand - i.e., from farmers, consumers, food manufacturers and traders, etc. who are willing and have the capacity to pay. Thus it should not come as a surprise that their initial targets are commodities grown by commercial producers in developed countries.

Likewise, their objects of innovations are those characters of high value to commercial growers. Among the possible target traits, crop protection against weeds and insect pests were obvious priorities in as much as commercial growers expend lots of money on herbicides and insecticides to control these pests. Moreover, these Western farmers are fully aware of the health hazard they expose themselves to and the pollution they cause their own environments with excessive use of pesticides.

Were the initial priorities high levels of essential vitamins and minerals in food crops, public perception would have been different although for people in Europe and USA who have adequate nutrition these may still not be attractive enough. Better if the breeding objectives were low cholesterol, low sodium, high antioxidant, and "lite" farm produce.

These statistics in the initial commercialization of genetically modified crops demonstrate clearly the bias in the application to developed country needs. With food surpluses and consumers with more than sufficient purchasing power to acquire adequate and balanced diets, the developed countries can very well do without agricultural biotechnology. It is really the developing countries who need biotechnology for agriculture. Should the anti-biotechnology lobbies in the West succeed in discouraging public and private investments in agricultural biotechnology, the poor developing countries will be the biggest losers.

It is therefore in the interests of the developing country themselves that the frontiers of agricultural biotechnology science be pushed to the limits through continuing investments by the private and public sectors globally. Additionally, it is in our national interest to develop capacity for biotechnology research ourselves to address those food, agricultural and environmental problems and opportunities which are uniquely ours.

IV. MANAGING RISKS ASSOCIATED WITH GENETICALLY MODIFIED CROPS

Modern biotechnology could be a powerful tool for improving productivity and sustainability of agriculture in developing countries. However, as with all other innovations and changes involving complex systems, there will always be trade-offs; there will always be unintended unwanted consequences that accompany the gains. It is a matter of weighing the risks against the benefits, of avoiding or mitigating the unwanted consequences and intelligently deciding which aspects of change to accept and which to reject.

It is useful at this point to recognize that the objections to the use of transgenic crops can be differentiated into two - those risks inherent to the technology and those that transcend it (Leisinger, 1999).

The risks inherent to genetically modified organisms include the danger of unintentionally introducing allergens and other anti-nutrition factors in our foods; the possibility of the new introduced genes escaping to other organisms by outcrossing thus creating superweeds, and in the case of insect-killing genes, the possibility of adversely affecting beneficial non-target arthropods. Moreover, antibiotic resistance has been used as a marker for selecting genetically modified plants. There is fear that the genes for antibiotic resistance might be transferred to bacteria that cause disease in man.

As far as the food risks are concerned, in the developed countries where legislation and regulatory institutions are in place, there are elaborate steps or protocols to precisely avoid or mitigate those dangers. There are standard tests for known specific allergens and anti-nutrition factors. At the molecular level, there are now DNA sequence tests which identify gene combinations which have the potential to generate allergenic substances.

On the matter of environmental risks, the possibility of introduced genes "escaping" to the wild through outcrossing between the genetically manipulated transgenic plants with wild relatives, can not be ruled out. Obviously if there are no known interfertile relatives as in the case of corn in most parts of the world, the risk is miniscule. Moreover, it depends on what genes may be "escaping" into the wild. A weedy rice plant which by chance acquired the novel beta carotene gene from daffodil (a GM rice plant developed in Switzerland) is clearly no threat to anybody including the insects who feed on them.

And even when such outcrossings do occur, the chances that these rare hybrid plants will survive and flourish over their competitors in the wild are extremely low not unless the gene confers a selection advantage for hybrid plants possessing the new gene. However, experience to date indicate that varieties bred and selected by man for specific purposes are less weedy and generally lose their ability to compete in the wild.

The so-called superweeds that may come out of outcrossing herbicide-resistant transgenic plants with weed relatives will be superweeds only in cultivated fields as long as the specific herbicide is used. In the wild where no herbicides are sprayed, there is no reason such rare hybrid plants should outcompete other plants which do not possess the herbicide-resistance gene. In any case, there is a ready agronomic expedient: switch to other modes of weed control such as cultivation and use of other herbicides.

The risk of genetically modified insect-inhibiting plants affecting non-target organisms is no worse than the current practice of broad-spectrum insecticides decimating both harmful and beneficial insects. In fact on the contrary, the transgenic plants like the Bt crops tend to be more specific and discriminating.

With regard to the concern about the use of antibiotic resistance genes, the U.K. Royal Society noted that the widespread use of antibiotics as feed additives for animals, and as over-the-counter and prescribed medicines for humans carry a greater risk of creating antibiotic resistant bacteria than transfer of marker genes from genetically modified plants (UK Royal Society, 1999a). Indeed, a large number of bacteria present in the gut already carry resistance to several antibiotics, including kanamycin and ampicillin. Nevertheless, the U.K. British Royal Society considers the presence of antibiotic resistance marker genes in genetically modified crops unacceptable and encourages the development and use of alternative marker systems.

However, what is more urgent is the real possibility that insects may quickly build up resistance to the new genes rendering the utility of the improved varieties very short-lived. It is clearly in the interest of the plant breeders and the private seed companies which developed the new varieties to manage the deployment of their genetically modified resistant varieties in such a way that insect-resistance build-up is discouraged by, for example, creation of insect refuges amidst fields sown to Bt crops.

These remarks were not meant to dismiss the concerns for food safety and biosafety inherent with biotech-derived foods and organisms. It is the obligation of the technology innovators, the producers and of government to assure the public of the safety of the novel food and drugs they offer as well as their benign effect on the environment. However, hazard identification and risk assessment ought to be scientifically based and on a case-by-case basis i.e., regulating the end product rather than the process (Juma and Gupta, 1999). Risk assessment should consider the characteristics of the organism being assessed, intended use of the organism and features of the recipient environment.

It is very important that we set in place the appropriate legislation and regulatory mechanisms to govern biotechnology not only as a matter of good science and sound governance but also to effectively respond to the genuine concerns for food safety and environmental safety of the general public.

On the other hand, technology-transcending risks as opposed to technology-inherent risks, emanate from the political and social context in which a technology is used (Leisinger, 1999). Included under this category are differential access to the new technology leading to a further widening of the economic gap between developed countries (technology users) versus the developing countries (non-users); further disparity in income between rich versus poor farmers within the same communities, and the further loss of biodiversity should the new transgenic varieties become too successful displacing other varieties.

However, in the case of technology-transcending risks relating to access, the solution is not to ban the use of the new technology by everybody, but by developing technologies tailor-made for the needs of the poor and by instituting measures so that the poor producers will likewise have ready, affordable access to the new technology.

As Leisinger (1999) contends, technology-transcending risks mostly materialize because a gap opens between human scientific technical ability and human willingness to shoulder moral and political responsibility.

This differentiation between technology-inherent risks and technology-transcending risks is very germane to our situation in the Philippines because we have to aggressively address both concerns if we were to succeed in exploiting the potential of modern biotechnology to advance our national purposes now, and not much later.

V. LABELLING OF GM FOODS AND INTELLECTUAL PROPERTY RIGHTS

There are two other very important concerns related to the adoption of genetically modified crops – segregation and labelling of GM crops and GM-derived foods and protection of intellectual property rights.

A debate is raging on in developed countries on the need to legally require the labelling of GM crops and foods derived from GM crops. The prevailing

position in the United States is that if the GM crop or GM-derived food is substantially similar to the conventional product, there is no need for labelling. However, in Europe there is a powerful lobby to require labelling of all GM crops so that consumers can exercise the right of choice. The UK Royal Society (1999b) strongly supports the labelling of foods containing GM material but hedges its support by qualifying "... where the new food stuff is substantially changed (according to specific criteria) from that of its conventional counterpart".

Segregation of GM products and labelling will incur additional costs which ultimately will be passed on to the consumer. There is no point of legally requiring segregation and labelling when there are no demonstrated or anticipated risks. However if by labelling, the producers and the food processors expect to receive a premium for their products, they may do so voluntarily. The consumers can exercise their choice of paying a little more in exchange for the guarantee of the product being GM-free.

We import each year hundreds of thousands of metric tons of corn and soybean from the United States. Since easily half of these commodities grown in the US are from GM crops we can assume that we, as well as the American public and other importers, have been consuming GM-derived corn and soybean products for the last five years. So far there has not been a single report of food allergy and poisoning from GM corn and soybean.

However this may not be necessarily true for other GM crops that may follow.

In any case there is no rush for the Philippines to legislate the segregation and labelling of GM crops. If there is a real risk from GM corn and soybean, the US regulatory agencies and the consumer watchdog organizations will be the first to blow the whistle on the US GM corn and soybean crops.

However we should strengthen our capability to monitor, assess and regulate these new foods alongside the conventional ones. Should we in the future develop our own transgenics for our own unique crops like the coconut, we have to rely on our own capacity to test them. We can not expect help from the developed countries who produce soybean oil and rapeseed oil with which our coconut oil competes in the world market.

Much of the new agricultural biotechnology have been generated by the private sector. During 1997-1999, the transactions of the major bioscience companies in the seeds industry are reported to have reached about \$18 billion (M. Kern in Persley, 1999). Thus the new knowledge and genetic materials are for the most part protected by intellectual property rights.

Since copying and infringement of patent rights can be easy with biological materials which can self-reproduce, the private sector is naturally reluctant to transfer their knowledge where there is no protection of intellectual property rights.

All countries who have joined the World Trade Organization (WTO) are bound to implement the provisions of the Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS), which lay down the minimum lev-

els of protection and ensures that enforcement procedures are available under national law.

Thus to facilitate transfer and dissemination of proprietary agricultural biotechnology and to promote technological innovation, the Philippines must comply with the minimum requirements under the TRIPS soon.

We must bring our business people, scientists and lawyers together to craft legislation which will satisfy the minimum requirements under the TRIPS while securing the freedom to operate of our national researchers and looking after the interests of our agribusiness sector and the small farmer sector.

However the new legal, regulatory and business arrangements could be very complex and very difficult for our national scientists to manage. We need to train our scientists and research administrators on how to assess, secure ownership and market intellectual property rights and how to enter into all kinds of licensing and material transfer agreements.

VI. CONCLUSION

The need for sustainably productive agriculture looms larger and larger in the horizon as we begin the 21st century. During the next 20 years, the population of the Philippines is projected to increase from 77 million to 108 million. We will need 40% more rice by the year 2025 but we shall have less arable land and less water to produce it.

Modern biotechnology has great potential to contribute to agricultural productivity and sustainability. The biological processes which underpin the growth and development of crops, fish, forest trees, livestock and microorganisms can be manipulated through their genomes. With the new science of molecular biology, it is now possible to identify specific genes; understand their functions in the whole organism; clone, move and transfer the genes across natural species barriers, and make the genes express their products in specific tissues at specific growth stages in the recipient organisms. This new tool allows man to perform a lot of manipulations of the biological factors of production which were impossible before. In conjunction with other conventional tools of science, many essential operations can be performed with more precision, quicker and eventually cheaper.

A major application of modern biotechnology is the development and use of genetically modified or transgenic crops. Crops may be genetically modified to raise yield ceilings, improve resistance to pests and diseases, develop tolerance to drought, excessive temperatures, soil acidity and salinity and other abiotic stresses and improve the nutritional, processing and keeping quality of produce.

The applications of modern biotechnology in health, industry and on the environment are widely accepted. However there are objections and unease in their uses in food and agriculture, particularly in the use of genetically modified crops.

As with all other innovations and changes involving complex systems, there will always be trade-offs, there will always be unwanted consequences that come

with the gains. It is a matter of weighing the risks against the benefits, of avoiding or mitigating the unwanted consequences and intelligently deciding which aspects of change to accept and which to reject.

There are risks associated with biotechnology - risks inherent to the technology and those that transcend it.

The risks inherent to biotechnology in particular to genetically modified crops include the danger of unintentionally introducing allergens and other anti-nutrition factors in our foods, introducing and/or creating novel genes which can in turn create and let loose in the environment unwanted and harmful organisms. Technology-transcendent risks as opposed to technology-inherent risks emanate from the political and social context in which a technology is used. Differential access to biotechnology may engender serious economic gaps between users and non-users and further loss of diversity.

A clear distinction between these two sets of risks is important as they call for different responses.

Technology inherent risks are susceptible to scientific analyses and technological corrections. Protocols for assessing food safety and biosafety are in place for many organisms or products. If they are not yet available, further research can be conducted. There is no substitute to strengthening our national capacity to manage this type of risks.

What is important is that hazard identification and risk assessment are scientifically based and made on a case-to-case basis, regulating the end product rather than the process. Risk assessment should consider the characteristics of the organisms being assessed, intended use of the organism, and features of the recipient environment.

Technology-transcendent risks on the other hand have their roots in social, economic and political inequalities or differences. Their solutions must for the most part be sought from the same realms of human activity e.g. agrarian reform, access to rural credit, more effective extension and rural institutions, better rural infrastructure and access to markets, and more agriculture-friendly policies.

The transcendent risk of unequal access to biotechnology is a very real dilemma to developing countries like the Philippines. Much of the new biotechnology are proprietary and are not exactly relevant to the needs of the poor in developing countries.

We must do two things: We must strengthen our national capacity to conduct agricultural biotechnology research and development. We must also put in place the proper intellectual property rights environment to encourage the private sector to invest on the problems of Philippine agriculture as well as the appropriate incentives so the new technology will get into the hands of our poor farmers who need them most.

VII. REFERENCES:

- Hossain, M. and M.C.A. Sombilla. 1999. World grains market: Implications for a food security strategy. In *Food Security in the Philippines*. Cabanilla L. and M. Paunlagui, eds. pp. 21-48.
- James, C. 1999. Global review of commercialized transgenic crops. ISAAA Brief No. 12.
- Juma, C. and A. Gupta. 1999. Safe use of biotechnology. In *Biotechnology for Developing Country Agriculture: Problems and Opportunities*. IFPRI.
- Leisinger, K.M. 1999. Ethical challenges of agricultural biotechnology for developing countries. In *Agricultural Biotechnology and the Poor: Proceedings of an International Conference*, 21-22 October 1999, Washington DC 20433 CGIAR. G. Persley and M. Lantin, eds. pp. 173-180.
- Persley, G.J. 1999. Agricultural biotechnology and the poor: Promethean science. In *Agricultural Biotechnology and the Poor: Proceedings of an International Conference*, 21-22 October 1999, Washington DC 20433, CGIAR. G.J. Persley and M.M. Lantin, eds. pp. 3-21.
- Persley, G. J. and J.J. Doyle. 1999. Overview. In *Biotechnology for Developing Country Agriculture: Problems and Opportunities*. IFPRI.
- Serageldin, I. 1999. The challenge of poverty in the 21st century: The role of science. In *Agricultural Biotechnology and the Poor: Proceedings of an International Conference*, 21-22 October 1999, Washington DC 20433, G. Persley and M. Lantin, eds. CGIAR, pp. 25-31.
- UK Royal Society. 1999a. Genetically modified plants for food use. Statement of the U.K. Royal Society. p. 11.
- UK Royal Society. 1999b. GMOs and the environment. Statement of the U.K. Royal Society. p.5.
- Vogt, D.U. and M. Parish. 1999. Food biotechnology in the United States: Science, Regulation, and Issues. CRS Report to Congress.

HENSTOCK INTEGRATION IN A HILBERTIAN COUNTABLY NORMED SPACE WITH NUCLEARITY

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ABSTRACT

Henstock integration of real-valued functions has been extended to functions with values in normed spaces. Cao, who considered Banach-valued functions, showed that Henstock's lemma, which plays an important role in the real-valued case, does not always hold in infinite dimensional Banach spaces. Nakanishi showed that Henstock's lemma holds in a ranked space called Hilbertian CN-space with nuclearity. In this paper, we revisit this space, define r -differentiability of a function with values in an r -separated ranked space, and give results concerning the primitives of Henstock integrable functions with values in this space. Further, we shall give a descriptive definition of the Henstock integral defined by Nakanishi.

Key words: Henstock integral, ranked CN-space, Hilbertian, nuclearity, r -separated, r -differentiable, HL-integral, Strong Lusin

INTRODUCTION

Nakanishi (1994) used a special ranked space called Hilbertian CN-space with nuclearity and defined the Henstock integral of a function with values in the said space. She showed that Henstock's lemma, which does not always hold in infinite dimensional spaces, holds in such a space. In this paper, we give another look at ranked spaces as well as concepts associated with them, and recall some of the important results obtained by Nakanishi. Some of these results will be used to prove our present results concerning the primitives of Henstock integrable functions. As a main result of this study, we characterize the Henstock integral defined by Nakanishi in terms of the Strong Lusin condition of a function.

Preliminaries

Definition 2.1 Let X be a nonempty set such that, for each $x \in X$, there exists a nonempty class $V(x)$ consisting of subsets of X , called *preneighborhoods* of x such that $x \in U(x)$ whenever $U(x) \in V(x)$. Put $V = \bigcup_{x \in X} V(x)$. Suppose further that for each $n \in \mathbb{N}$ ($\mathbb{N} = \{0, 1, \dots\}$), there is assigned a nonempty class $V_n \subset V$ satisfying the following: For every $U(x) \in V(x)$ and for every n , there exists a $V_m(x) \in V_m$ for some $m > n$ such that $V_m(x) \subset U(x)$. Then the space X endowed with the classes $V(x)$ and V_n for each $x \in X$ and for each $n \in \mathbb{N}$ is called a *ranked space*. It is sometimes denoted by the ordered-triple (X, V, V_n) . Further, if $U(x)$ is a preneighborhood of x and $U(x) \in V_n$, then we say that it is of *rank* n . In this case, x is the *center* of $U(x)$.

Example 2.2 Let $X = [a, b]$. For each $x \in X$, let $V(x)$ be the (usual) neighborhood system of x and for each $n \in \mathbb{N}$, let $V_n = \{(x - 1/2^{n+1}, x + 1/2^{n+1}) \cap X : x \in [a, b]\}$. If V is the union of all $V(x)$, then (X, V, V_n) is a ranked space.

Definition 2.3 A sequence of preneighborhoods $\{U_i, \{x_i, n(i)\}\}$, i.e., a sequence of preneighborhoods U_i of x_i with ranks $n(i)$, is called a *fundamental sequence* (f.s. for brevity) if it satisfies the following conditions:

- (C1) The sequence of preneighborhoods is decreasing, i.e., $U_0 \supset U_1 \supset \dots$;
- (C2) $n(0) < n(1) < \dots < n(k) < n(k+1) < \dots$; and
- (C3) for every $n \in \mathbb{N}$, there exists a k such that $k \geq n$, $x_k = x_{k-1}$ and $n(k) < n(k+1)$.

Definition 2.4 A ranked space (X, V, V_n) is said to be *r-separated* if it satisfies the ff. condition: For every $x, y \in X$, $x \neq y$, and for every f.s. $\{U_i(x)\}$ of center x and f.s. $\{V_i(y)\}$ of center y , there exists a k such that $U_k(x) \cap V_k(y) = \emptyset$.

Definition 2.5 Let X be a vector space with a countable sequence of compatible norms $\{p_n\}$. Then X is called a *countably normed space*. It is sometimes denoted by $(X, \{p_n\})$. Further, in this space, we have the ff:

- (a) A sequence $\{x_j\}$ in X is a *convergent sequence* if there is a vector $x \in X$ such that $p_n(x_j - x) \rightarrow 0$ as $j \rightarrow \infty$ for every norm p_n .
- (b) A sequence $\{x_j\}$ in X is a *Cauchy sequence* in X if it is a Cauchy sequence for every norm p_n .
- (c) X is *complete* if every Cauchy sequence in X converges.

Theorem 2.6 [Nakanishi] Let X be a CN-space with a sequence $\{p_n\}$ of increasing norms, i.e., $p_0(x) \leq p_1(x) \leq \dots$ for every $x \in X$. Then $(X, V(x), V_n)$ is a ranked space, where

$$\begin{aligned} V(x) &= \{x + S_n : n \in \mathbb{N}\} \quad (x \in X), \\ V_n &= \{x + S_n : x \in X\} \quad (n \in \mathbb{N}), \text{ and} \\ S_n &= \{y \in X : p_n(y) < 1/2^n\} \quad (n \in \mathbb{N}). \end{aligned}$$

Definition 2.7 Let X be a CN-space with a sequence $\{p_n\}$ of increasing sequence of norms, i.e., $p_0(x) \leq p_1(x) \leq \dots$ for every $x \in X$. We call the ranked space $(X, V(x), V_n)$ described in Theorem 2.6 as a *ranked countably normed space* or simply *ranked CN-space*.

Lemma 2.8 Every ranked CN-space $(X, \{p_n\})$ is r -separated.

Definition 2.9 Let X be an r -separated ranked space, and $f: [a, b] \rightarrow X$ a function. We say that the *r -limit* of $f(t)$ is L , as t tends to t^* , if for every f.s. $\{U_j(t^*)\}$ of center t^* in $[a, b]$, there is a f.s. $\{V_j(L)\}$ of center L in X such that $f(\bigcup_j U_j(t^*)) \subset \bigcup_j V_j(L)$. In this case we write,

$$\begin{aligned} r - \lim_{t \rightarrow t^*} (f(t)) &= L. \end{aligned}$$

Definition 2.10 Let X be an r -separated ranked space, and $F: [a, b] \rightarrow X$. F is r -differentiable at the point $t^* \in [a, b]$ if

$$r - \lim_{t \rightarrow t^*} \frac{F(t) - F(t^*)}{t - t^*} = F'_r(t^*) \text{ exists}$$

F is *r -differentiable* on $[a, b]$ if it is r -differentiable at every point in $[a, b]$.

Definition 2.11 Let $(X, \{p_n\})$ be a complete CN-space such that $\{p_n\}$ is an increasing sequence of compatible norms. If each norm p_n is induced by an inner product $(\cdot, \cdot)_n$ on X , then we call $(X, \{p_n\})$ a *Hilbertian CN-space*.

Let $(X, \{p_n\})$ be a Hilbertian CN-space. Note that the term "Hilbertian" comes from the fact that if X_n is the completion of X with respect to p_n , then X_n is a Hilbert space. Further, from Gelfand & Shilov's Generalized Functions, if $\{p_n\}$ is an increasing sequence of compatible norms, then the sequence $\{X_n\}$ can be considered to have the relationship $X_0 \supset X_1 \supset \dots \supset X$. Hence, for $m < n$, the mapping $\varphi_{m,n}: X \rightarrow X$ defined by $\varphi_{m,n}(x_{(n)}) = x_{(m)}$, where $x_{(n)}$ and $x_{(m)}$ denote the same element x of X considered as element of X_n and X_m respectively, is a continuous linear operator from the everywhere dense subset X of X_n onto the everywhere dense subset X of X_m . Thus, by the Hahn Banach Theorem, $\varphi_{m,n}$ can be extended to a continuous linear operator $T_{m,n}$ from X_n onto a dense subset of X_m .

Definition 2.12 Let $(X, \{p_n\})$ be a Hilbertian CN-space with increasing sequence $\{p_n\}$ of compatible norms. We say that $(X, \{p_n\})$ has the *nuclearity* property if for each m , there exists an $n > m$ satisfying the following property:

$$(N) \quad T_{m,n}(x) = \sum_{k=1}^{\infty} \lambda_{m,n,k} (x, e_{n,k})_n e_{m,k} \text{ for every } x \in X, \text{ where } (\cdot)_n \text{ is the inner}$$

product on X_n that induces p_n , $\{e_{n,k}\}$ and $\{e_{m,k}\}$ are orthonormal systems of vectors in the spaces X_n and X_m , respectively, each $\lambda_{m,n,k} > 0$, and the series $\sum_{k=1}^{\infty} \lambda_{m,n,k}$ converges.

Definition 2.13 Let $(X, \{p_n\})$ be a complete ranked CN-space. An X -valued function f defined on $[a, b]$ is said to be *Henstock integrable* to a vector $z \in X$ on $[a, b]$ if for every $n \in \mathbb{N}$ there exists $\delta_n(\xi) > 0$ on $[a, b]$ such that for any δ -fine division $D = \{([u, v]; \xi)\}$ of $[a, b]$, we have

$$p_n((D) \sum f(\xi)(v - u) - z) < 1/2^n.$$

Definition 2.14 Let (X, p) be a normed space. An X -valued function f defined on $[a, b]$ is said to be strongly *Henstock integrable* on $[a, b]$ if there exists an additive function $F: [a, b] \rightarrow X$ satisfying the following property: For every $\varepsilon > 0$ there exists $\delta(\xi) > 0$ on $[a, b]$ such that for any δ -fine division $D = \{([u, v]; \xi)\}$, we have

$$(D) \sum p(f(\xi)(v - u) - F(v) + F(u)) < \varepsilon.$$

The above integral is known as the *HL-integral* and the function F , where we assume that $F(a) = 0$, is called the *HL-primitive* of f . The following theorem is due to Cao.

Theorem 2.15 [Cao] Let (X, p) be a Banach space. If $f: [a, b] \rightarrow X$ is strongly Henstock integrable with HL-primitive F on $[a, b]$, then F is differentiable almost everywhere on $[a, b]$ and $F'(t) = f(t)$ a.e. on $[a, b]$.

Definition 2.16 A function $F: [a, b] \rightarrow X$ is said to satisfy the *Strong Lusin* condition if for every subset E of $[a, b]$ of measure zero and for every $\varepsilon > 0$, there exists a $\delta(\xi) > 0$ such that for any δ -fine division $D = \{([u, v]; \xi)\}$ of $[a, b]$ with $\xi \in E$, we have

$$(D) \sum p(F(v) - F(u)) < \varepsilon.$$

Theorem 2.17 [Canoy] If $F: [a, b] \rightarrow X$ is a primitive of a strongly Henstock integrable function f , then F satisfies the Strong Lusin condition and $F'(x) = f(x)$ a.e. on $[a, b]$.

Theorem 2.18 [Canoy] A function $f: [a,b] \rightarrow X$ is strongly Henstock integrable on $[a,b]$ if and only if there is an SL-function $F: [a,b] \rightarrow X$ such that $F'(x) = f(x)$ a.e. on $[a,b]$.

3. RESULTS

The first three results are proved by the author in his earlier paper.

Lemma 3.1 Let $(X, \{p_n\})$ be a ranked CN-space. If an X -valued function F defined on $[a,b]$ is differentiable at a point $t^* \in [a,b]$ with respect to p_n , then it is differentiable there with respect to p_m for all $m < n$. Moreover, $F'_m(t^*) = F'_n(t^*)$ ($m < n$), where $F'_m(t^*)$ denotes the derivative of F at t^* with respect to p_m .

A direct consequence of the above lemma is the following

Corollary 3.2 Let $(X, \{p_n\})$ be a ranked CN-space. If an X -valued function F defined on $[a,b]$ is differentiable at a point $t^* \in [a,b]$ for every p_n , then $F'_n(t^*) = F'_m(t^*)$ for all m and n .

Theorem 3.3 Let $(X, \{p_n\})$ be a ranked CN-space. An X -valued function F defined on $[a,b]$ is r -differentiable at $t^* \in [a,b]$ if and only if it is differentiable at t^* for every p_n . Moreover, $F'_n(t^*) = F'(t^*)$ for every n , where $F'_n(t^*)$ denotes the derivative of F at t^* with respect to p_n .

The following theorem is due to Nakanishi.

Theorem 3.4 (Henstock's Lemma) Let $(X, \{p_n\})$ be a Hilbertian CN-space with nuclearity, and $f: [a,b] \rightarrow X$ a Henstock integrable function on $[a,b]$ with primitive $F(t) = \int_a^t f(s)ds$. Then, for every n , there exists a positive function $\delta_n(\xi)$ on $[a,b]$ such that for any δ_n -fine division $D = \{([u,v], \xi)\}$ of $[a,b]$, we have

$$(D) \sum p_n(f(\xi)(v-u) - F(v) + F(u)) < 1/2^n.$$

Corollary 3.5 Let $(X, \{p_n\})$ be a Hilbertian CN-space with nuclearity, and $f: [a,b] \rightarrow X$ a Henstock integrable function on $[a,b]$ with primitive $F(t) = \int_a^t f(s)ds$. Then for every n , the following holds: Given $\varepsilon > 0$, there exists a positive function $\delta(\xi)$ on $[a,b]$ such that for any δ -fine division $D = \{([u,v], \xi)\}$ of $[a,b]$, we have

$$(D) \sum p_n(f(\xi)(v-u) - F(v) + F(u)) < \varepsilon.$$

Lemma 3.6 Let $(X, \{p_n\})$ be CN-space with $p_1(x) < p_2(x) < \dots$ for all x in X , and $f: [a,b] \rightarrow X$. If f is strongly Henstock integrable on $[a,b]$ with HL-primitive F_n with respect to p_n , then it is strongly Henstock integrable on $[a,b]$ with respect to p_m for every $m < n$. Moreover, $F_m = F_n$ on $[a,b]$ for all $m < n$.

Theorem 3.7 Let $(X, \{p_n\})$ be a Hilbertian CN-space with nuclearity, and $f: [a,b] \rightarrow X$. Then f is Henstock integrable on $[a,b]$ with primitive F if and only if f is strongly Henstock integrable on $[a,b]$ with HL-primitive F for every p_n .

Proof: (\Rightarrow) Suppose f is Henstock integrable on $[a,b]$ with primitive

$$F(t) = \int_a^t f(s)ds. \text{ Then, by Corollary 3.5, } f \text{ is strongly Henstock integrable}$$

on $[a,b]$ with HL-primitive F for every norm p_n .

(\Leftarrow) Suppose that f is strongly Henstock integrable on $[a,b]$ for every p_n . By Lemma 3.6, we may take a common HL-primitive $F = F_1$. Then for each n , there exists a $\delta_n(x) > 0$ such that for any δ_n -fine division $D = \{(u,v]; \xi\}$ of $[a,b]$, we have

$$(D)\sum p_n((f(\xi)(v-u) - F(v) + F(u)) < 1/2^n.$$

Thus,

$$\begin{aligned} p_n((D)\sum f(\xi)(v-u) - F(b) + F(a)) &= p_n((D)\sum (f(\xi)(v-u) - F(v) + F(u))) \\ &\leq (D)\sum (p_n(f(\xi)(v-u) - F(v) + F(u))) \\ &< 1/2^n \end{aligned}$$

for all n . Therefore, f is Henstock integrable to $F(b) - F(a) = F(b)$ on $[a,b]$. \square

Theorem 3.8 Let $(X, \{p_n\})$ be a Hilbertian ranked CN-space with nuclearity, and $f: [a,b] \rightarrow X$ a Henstock integrable function on $[a,b]$ with primitive $F(t) = \int_a^t f(s)ds$. Then F is r -differentiable almost everywhere on $[a,b]$.

Proof: By Theorem 3.7, f is strongly Henstock integrable on $[a,b]$ with HL-primitive F for every p_n . Thus, by Theorem 2.15, F is differentiable almost everywhere on $[a,b]$ for every p_n . By Theorem 3.3, F is r -differentiable almost everywhere on $[a,b]$. \square

Theorem 3.9 Let $(X, \{p_n\})$ be a Hilbertian CN-space with nuclearity and $f: [a,b] \rightarrow X$ a function. Then f is Henstock integrable on $[a,b]$ if and only if there exists an SL-function $F: [a,b] \rightarrow X$ such that $F'_r(x) = f(x)$ a.e. on $[a,b]$.

Proof: (\Rightarrow) Suppose f is Henstock integrable with primitive F . By Theorem 3.7, f is strongly Henstock integrable with primitive F for every norm p_n . Now, by Theorem 2.17, F is an SL-function and $F'(t) = f(t)$ a.e. on $[a,b]$ for every norm p_n . By Theorem 3.3, $F'_r(t) = f(t)$ a.e. on $[a,b]$.

(\Leftarrow) Suppose there exists an SL function F such that $F'_r(t) = f(t)$ a.e. on $[a,b]$. By Theorem 3.3, $F'(t) = f(t)$ a.e. on $[a,b]$ for every norm p_n . It follows from Theorem 2.18 that f is strongly Henstock integrable on $[a,b]$ with HL-primitive F for each norm p_n . Thus, by Theorem 3.7, f is Henstock integrable on $[a,b]$. \square

ACKNOWLEDGMENTS

The author would like to extend his heartfelt gratitude to the officers, members and staff of the National Academy of Science and Technology, Philippines for the opportunity they gave him to be one of the speakers during the 22nd Annual Scientific Meeting of NAST last July 5-6, 2000. Special thanks must go to the MSU-IIT and the Commission on Higher Education for the moral and financial support they had extended.

REFERENCES

- Canoy, S. R. The SL-Condition und the Strong Henstock Integral. 1996. *Matimyas Matematika*, 19(2) : 1-5.
- Canoy, S. R. On the ϵ -differentiability of the Primitives of Henstock Integrable Functions with values in a Hilbertian CN-space with Nuclearity. 2000. *Matimyas Matematika*, 23(2): 1-8.
- Can, S. S. The Henstock Integral for Banach-Valued Functions. 1992. *SEA Bull. Math.* 16: 35-40.
- Gelfand, I.M., and Shilov, G.E. Generalized Functions. 1968. Vol. 2. Academic Press, New York and London, pp. 1-17.
- Kunugi, K. Sur la Methode des Espaces Ranges. 1966. *Proceedings of Japan Acad.* 42: 318-322.
- Nakanishi, S. The Henstock Integral for Functions With Values in Nuclear Spaces. 1994. *Math Japonica* 39(2): 309-335.

NEW MEASUREMENT OF COSMIC RAY FLUX IN ILIGAN CITY

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ABSTRACT

Sea level cosmic ray flux is measured anew in Iligan City using two plastic scintillators of area 100x10 cm² which are stacked vertically by a spacing of 150 cm. Each scintillator is separately coupled to a Hamamatsu photomultiplier tube which when high voltage is applied, constitutes a detection-amplification system. This assembly of detectors is then connected to Nuclear Instrumentation Modules to discriminate and count coincident signals over a fixed interval of time through the months of August -- September 1999 at the MSU-IIT High Energy Physics (HEP) Laboratory. Data show that there is no significant difference between the measured average nighttime and daytime cosmic ray fluxes and that the average hourly and daily fluxes is a constant throughout the measurement period. All these results are in agreement with the first measurements of cosmic ray flux done at the same laboratory in 1999 using an entirely different detector-electronics assembly. The measured values are also shown to be consistent with Monte Carlo simulations and with the predicted, internationally accepted values

Key words: Cosmic ray, plastic scintillator detectors, NIM modules

INTRODUCTION

Studies on cosmic rays are generally conducted at different locations, depending on the objectives of the study. High Altitude measurements offer the advantage of obtaining direct measurements of the chemical composition and energy spectra of highly energetic primary cosmic rays and allow observation of higher occurrence rate of cosmic ray events, such as atmospheric muon production processes (Maeda, 1973). Moreover measurement of horizontally incident cosmic ray muons at higher altitudes is accurate enough for studies on very high-energy muons. However, such measurement, especially if aimed at higher energies, requires huge detectors and sufficiently long exposure time (Hong, 1995). It is also susceptible to uncontrolled deviations in thermodynamic parameters of the atmosphere, such as atmospheric temperature, pressure, and particularly, vertical temperature gradient (Maeda, 1973). To compensate this limitation, sea level and underground studies are carried by measuring the extensive air showers at the surface as well as the penetrating high-energy muons to obtain indirect information on the chemical composition of the primary cosmic rays (Maeda, 1973).

Cosmic rays are high-energy subatomic particles that originate from outer space. They initiate ionization of the upper atmosphere of the earth thereby producing extensive air showers of secondary particles that, in turn, decay in flight to form the atmospheric muons. These cosmic ray fragments traverse the atmosphere in all directions and interact with surface materials. This fact made it possible for such interactions to be detected and measured using proper absorbing materials, apparatus, and method of observation.

Studies of terrestrial cosmic ray flux is a very important aspect of radiation monitoring. For instance, it has been estimated that the exposure of aircrew to cosmic radiation is 10x at plane altitudes that at sea level and health issues have been raised (New Zealand NRL, 1995). It has also been documented that cosmic rays cause soft fails in electronic logic or memory and the knowledge of local cosmic ray flux is essential in predicting electronic fail rates (Zeigler, 1996).

METHODS OF OBSERVATION

A. Set-up

The experimental set-up shown in Fig. 1 is composed of two plastic scintillators of area $100 \times 10 \text{ cm}^2$. Each scintillator is coupled to a photomultiplier tube (PMT) to form a detection-amplification system. The output of the assembly of detectors is then channeled to the Nuclear Instrument Modules (NIM) as input for signal processing and counting of coincident signals. The two scintillation counters, together with the nuclear instrumentation modules, form the pulse-type system of counting used in the experiment.

The two scintillation counters are mounted horizontally on a supporting wooden stand, as shown in Fig. 1, so that one runs parallel to the other at a



Figure 1. The Experimental Set-up

vertical distance of 150 cm. The counters are then connected to a high voltage power supply using two high voltage cables. The output terminals of the scintillation counter assembly are then connected to the *NIM* modules via small co-axial cables of the same length. The cables must be of the same length to avoid discrepancy in the delaying of signals from the counters to the coincidence circuit.

B. The Detector

The two detectors used in this experiment are each composed of plastic scintillators, of dimensions $100 \times 10 \text{ cm}^2$, 0.50 cm thick and coupled optically to identical *PMTs* of type Hamamatsu H1161-RB9038. The scintillators are made of scintillating materials that produce sparks or scintillations of light when an ionizing radiation passes through them (Tsoulfanidis, 1983). Light from the scintillator is very weak and this necessitates a photomultiplier tube for amplification of the order of 10^6 . The *PMT* then converts the amplified light into a strong electric pulse at its output.

C. Nuclear Instrumentation Module (NIM)

The *NIM* modules that were used in this study shown in Fig. 2 consist of a delay module, an 8-channel discriminator module, a 4-channel coincidence module, and the dual scaler or counter with an LCD display.

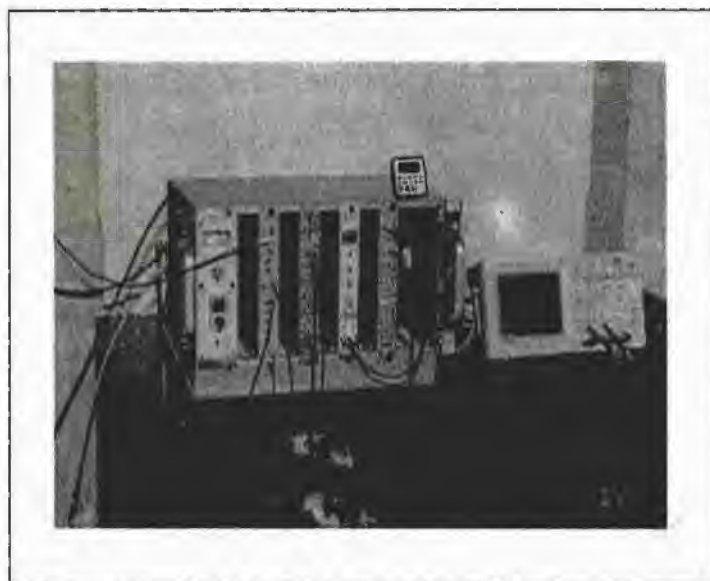


Figure 2. The Nuclear Instrumentation Module

With high voltage on, observable electric pulse from the *PMT* anodes actually represent the amplified photoemissions coming from cosmic ray particles hitting the plastic scintillators. Low-level pulses collectively called electronic noise, however, usually accompany these signals and tend to distort them. Hence, a standard logic circuit unit called the *NIM* discriminator module is used to select the desired input pulses and eliminate electronic noise by properly setting the threshold voltage level of the module. Input pulses whose corresponding energies are lower than the indicated threshold are rejected while pulses whose corresponding energies exceeded or at least equaled the threshold are allowed for subsequent counting.

Measuring cosmic ray flux using a single detector renders the experiment ineffective since signals from the detector represent a mixture of particles hitting the detector as mentioned. Therefore at least two detectors should be used. The particle of interest must hit both detectors coincidentally, with the signal coming from the second serving as a confirmation of its arrival at the first detector (Niño-franco, 1999). In order to ensure that signals are emanating from both detectors another standard circuit is used which is the *NIM* coincidence module. This unit is responsible for accepting two or more input pulses and conducts signals at its output only if the input pulses arrived at a specified time interval known as the resolving time. In order to register the desired coincidence, detector signals are first allowed to pass through delay lines prior to discrimination process and coin-

cidence counting. Once proper delay is implemented (using coaxial cables and a *NIM* delay module), the signals are then guided to the discriminator, and then to the coincidence unit. Finally, pulses that are observed to be coincident are gated to the output for subsequent recording by the scaler.

Preparatory Measurement

The set-up shown in Fig. 1 cannot be used directly to have the cosmic ray flux measurement. Preparatory measurements should be done first in order that set-up is the appropriate assembly for the said measurement.

A. The PMT Operating Voltage

Photomultipliers can be operated over a wide range of working voltages extending over 1000 volts or more before reaching the maximum rating. The maximum high voltage ratings of the two *PMTs* used is -25000 volts. However, the actual operating voltage used in this experiment is set to -1800 volts. Figs. 3 and 4 show the actual operating voltage as determined by the plateau measurement process.

B. The Coincidence Method

To obtain a reasonable discriminator threshold voltage, both detectors A and B are operated one at a time and the schematic diagram shown in Fig. 5 is used. Figs. 6 and 7 show the resulting curve for threshold voltage determination. In addition, the detector (A/B) is paired to a reference detector C whose discriminator was set to minimum and the discriminator of the detector (A/B) is then varied from its minimum to maximum in steps of -100 mV. The ratio of the counts from the two scalers (N_1/N_2) is plotted against the threshold voltage of the discriminators and coincidence unit width was set to 50 ns. The very low ratio of N_1/N_2 corresponds to the noise, and the very high ratio of N_1/N_2 already neglects more significant coincidence. Based on Fig. 6 and 7, the discriminator threshold voltage is chosen at -500 mV. At this level, most of the noise are eliminated while all or most of the desired cosmic ray signals are kept.

The relative delay to be imposed on the branch of the set-up that delivers the delayed or advanced signals to the coincidence unit is measured using the schematic diagram shown in Fig. 8. This time both detectors A and B are now operated at the same time while no more reference is made to detector C. Delays on A and B are adjusted and the appropriate threshold voltage voltages were set. The ratio of the counts on the scalers (N_1/N_2) was then recorded and plotted against the relative difference of Delay A and Delay B as $T_a - T_b$. The correct setting for the delay must then be the value of $T_a - T_b$ corresponding to the middle of the plateau shown in Fig. 9. For this experiment, detector A is delayed by $T_a = 5$ nanoseconds and detector b delayed by $T_b = 10$ nanoseconds, thus making the relative delay, $T_a - T_b = -5$ nanosecond.

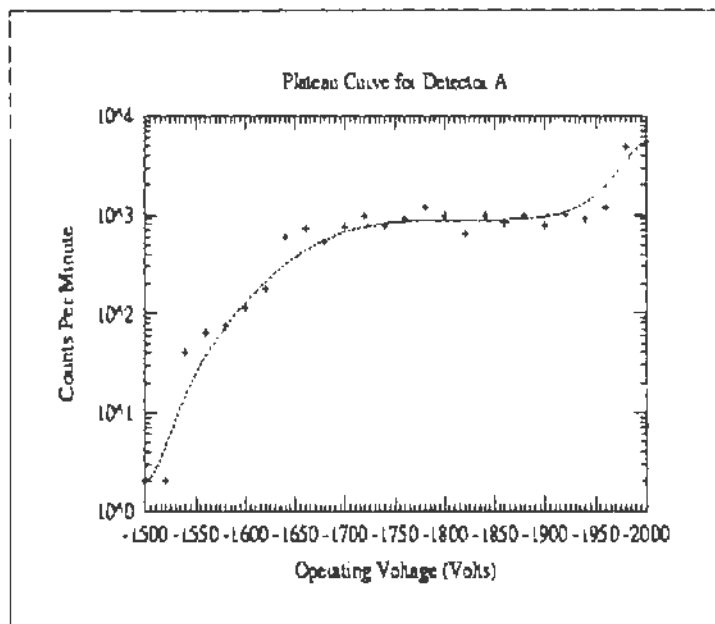


Figure 3. Plateau Curve for Detector A

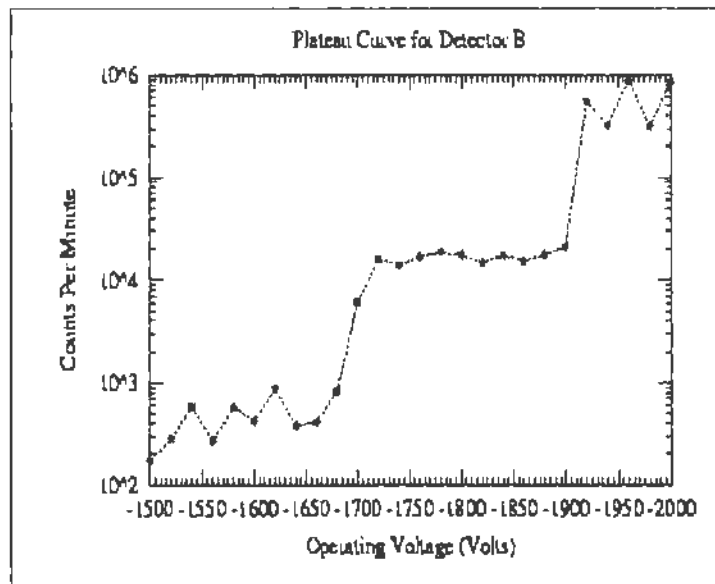


Figure 4. Plateau Curve for Detector B

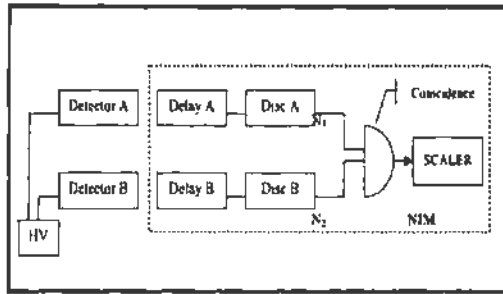


Figure 5. Schematic diagram of the connection for the determination of the threshold voltage

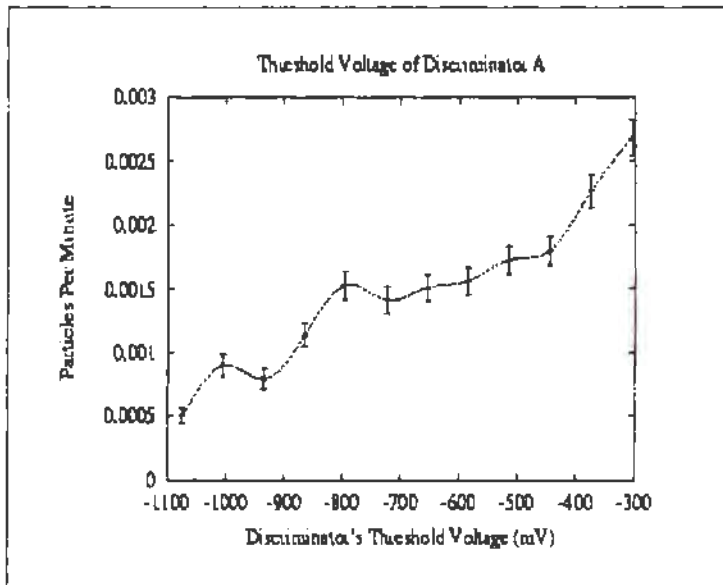


Figure 6. Threshold Curve for Discriminator A

RESULTS

Once the proper settings for the discriminator and delay are done we can then proceed to the measurement of cosmic ray flux. The set-up is then made to operate from 8:00 AM to 4:00 PM during daylight and from 8:00 PM to 4:00 AM during the night. The counts taken directly from the coincidence circuit are recorded on an hourly basis, which means that for daytime and for the nighttime we obtain 8 samples each.

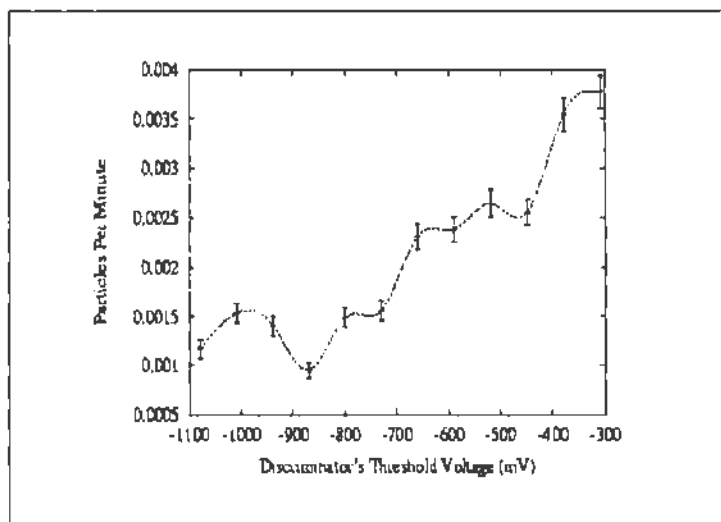


Figure 7. Threshold Curve for Discriminator 9

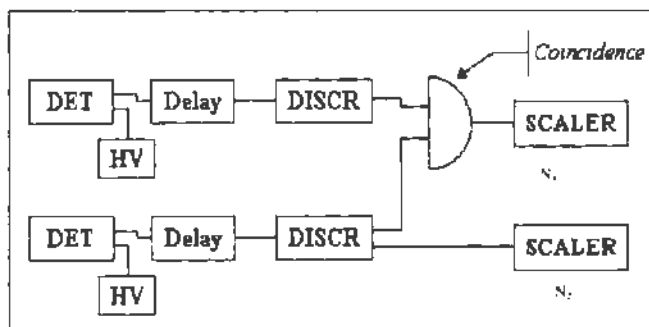


Figure 8. Schematic diagram of the connection used in the determination of the appropriate delay

A. Measured Flux

The flux measurement on a per hour basis are converted to a per minute basis of measurement. They are then grouped by eight, which corresponds to the number of per hour measurement from 8:00 AM to 4:00 PM during daytime and from 8:00 PM to 4:00 AM during nighttime. For each group of eight we get averages and corresponding errors. The average \bar{n} of each group of eight were computed using equation.

$$\bar{n} = \left(\frac{1}{N}\right) \sum_{i=1}^N n_i \quad (1)$$

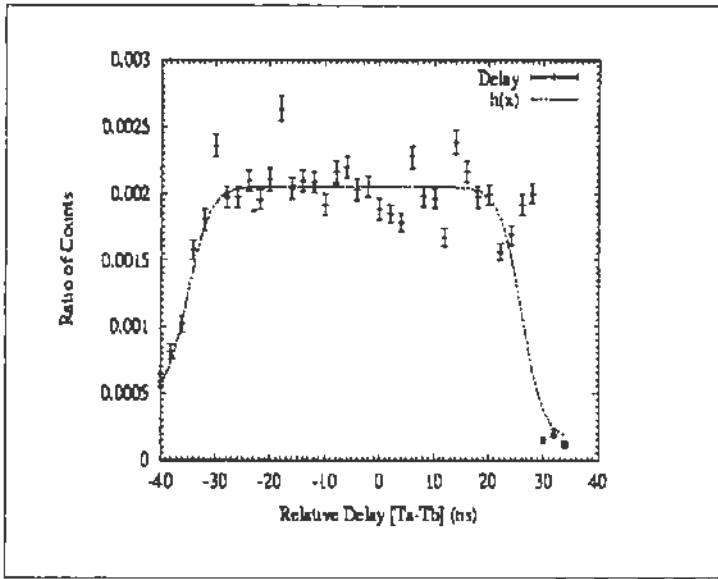


Figure 9. Relative Delay Curve

and the corresponding error σ computed according to,

$$\sigma^2 = \left(\frac{1}{N-1}\right) \sum_{i=1}^N (n_i - \bar{n})^2 \quad (2)$$

where $N=8$ for both equations. Equation 2 is used in the computation for error since $N=8$ is relatively a small number of sample per day or night. The average and error computations are done separately for the data during daytime and nighttime.

Comparison of the 34 averages and corresponding errors from the graph revealed that for daytime measurements no significant day-to-day variations of flux are observed. The same observation holds for nighttime measurement of fluxes. Figs. 10 and 11 typify this insignificance of daytime and nighttime variations of cosmic ray flux.

Fig. 12 shows a flat or constant value for the measured average flux which means that there is no significant variation between the daily averages of the cosmic flux for the entire measurement period. The horizontal dashed line drawn across the plot in Fig. 12 represents the overall average computed using Eq. 1 with $N = 592$, the total number of per hour measurements done during the data gathering period. The overall average flux n_m , is calculated to be 26.86 particles/min with an error of ± 0.69 particles/min, or

$$Flux_{measured}, \bar{n}_m = 26.86 \pm 0.69 \text{ particles/min} \quad (3)$$

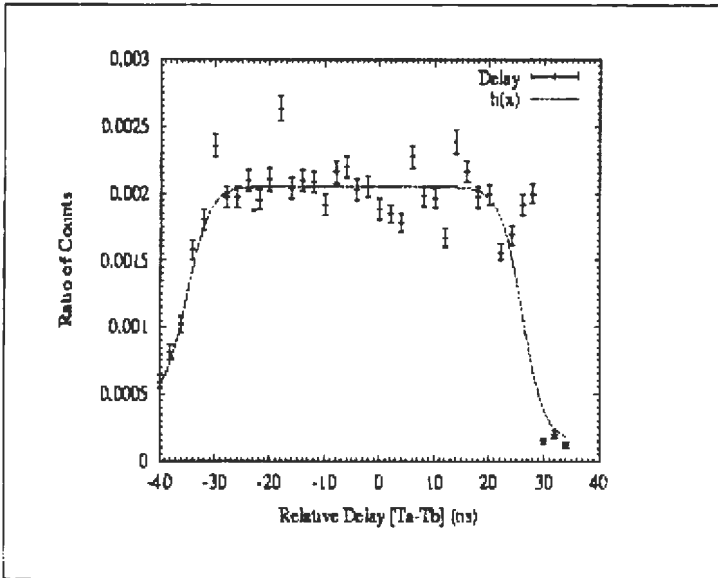


Figure 9. Relative Delay Curve

and the corresponding error σ computed according to,

$$\sigma^2 = \left(\frac{1}{N-1} \right) \sum_{i=1}^N (n_i - \bar{n})^2 \quad (2)$$

where $N=8$ for both equations. Equation 2 is used in the computation for error since $N=8$ is relatively a small number of sample per day or night. The average and error computations are done separately for the data during daytime and nighttime.

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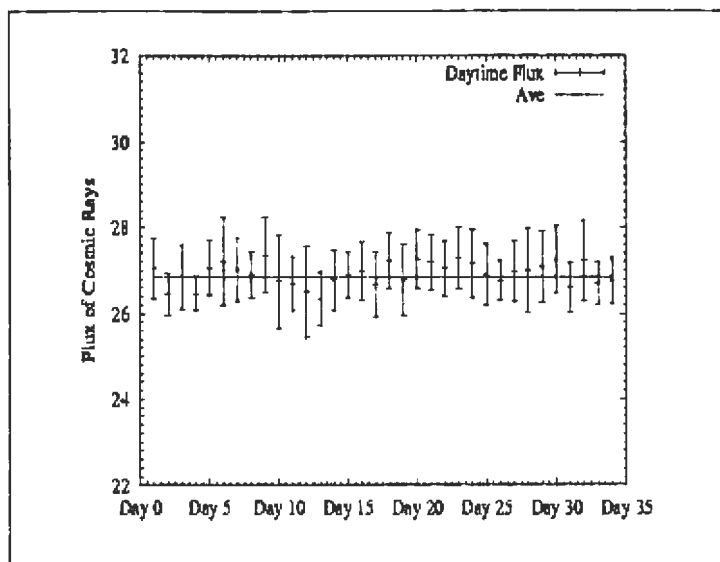


Figure 10. Day Time Flux

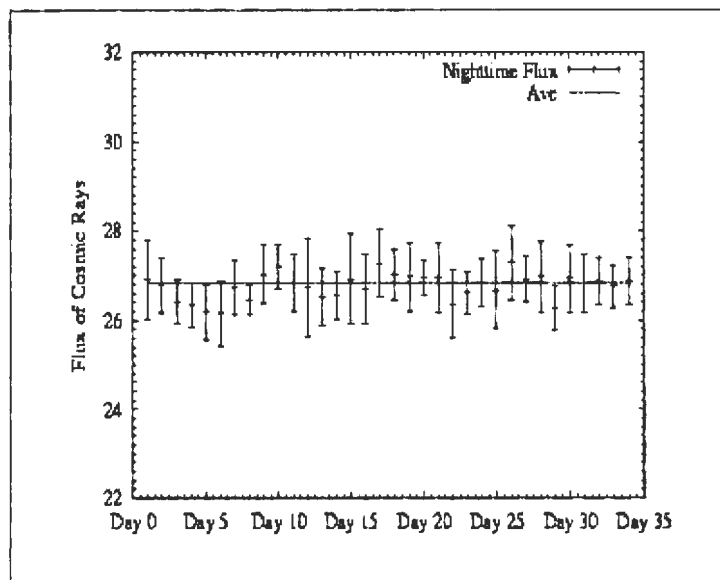


Figure 11. Night Time Flux

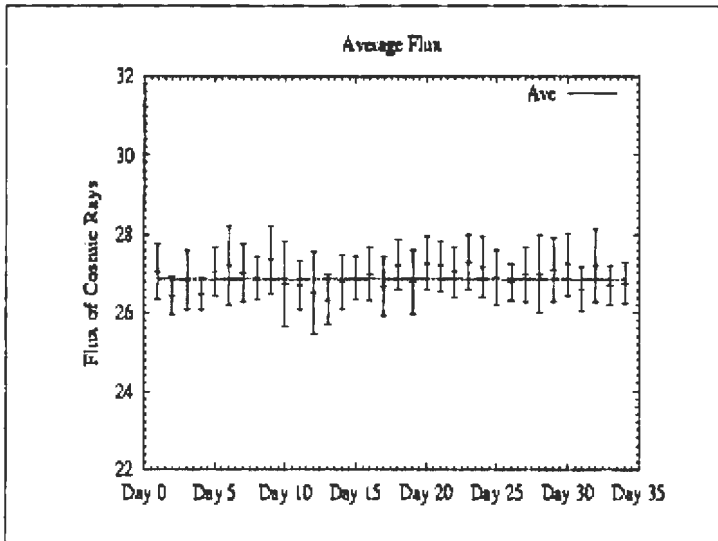


Figure 12. Average Flux

B. Comparison with Measured Values

Previous measurement of cosmic ray flux done in the same location gave an average flux equal to 10.52 particles/min with an error ± 0.43 particles/min, or $\text{Flux}_{(\text{measured})} = \bar{\mu}_m = 10.52 \pm 0.43$ particles/min using plastic scintillators of size $20 \times 20 \text{ cm}^2$ and different sets of *NIM* electronic modules.

To obtain an exact comparison between the 1999 and present measurements, the fluxes are converted into the units of number of particles per square meter per minute, by dividing the raw data with the scintillator areas. This gives a flux of 0.0269 ± 0.0007 particles/ m^2/min for the $100 \times 10 \text{ cm}^2$ scintillator while it is 0.0263 ± 0.0004 particles/ m^2/min for the $20 \times 20 \text{ cm}^2$ scintillator. These results are again shown in Table 1 to emphasize that the two independent measurements are entirely consistent with each other.

Table 1. Comparison of Fluxes for different dimensions of scintillators.

Scintillator used	Flux	
	# of particles min-1	# of particles cm-2 min-1
100 x 10 x 0.5 cm3	26.86 \pm 0.69	0.0269 \pm 0.0007
20 x 20 x 1.0 cm3	10.52 \pm 0.43	0.0263 \pm 0.0004

C. Estimated Flux

A straightforward way to check the measured flux is by using a rough measurement of solid angle. This estimate is done by considering the set-up to be enclosed by a sphere of radius equal to the distance from the center of the top counter to one corner of the bottom counter. It can only subtend a fraction of the 4π -geometry called $\Delta\Omega$, the fractional solid angle and is equal to the ratio of the area of the detector opening to the area enclosing the space where the events to be detected takes place multiplied by 4π :

$$\Delta\Omega = \frac{100 \times 10}{4\pi R^2} 4\pi = 0.039 \text{ sterad} \quad (4)$$

where we have measured $R = 158.20$ cm in our set-up. The standard reference put up by the Particle Data Group (PDG) lists typical values of sea level cosmic ray flux in terms of the flux I_γ per unit solid angle per unit horizontal area per unit time about the vertical direction and this is equal to 110 particles/ $\text{cm}^2/\text{sec}/\text{sterad}$ (PDG, 1988, 1994). This is equivalent to 0.66 particles/ $\text{cm}^2/\text{min}/\text{sterad}$. Hence our measured values agrees with

$$Flux_{\text{(estimate)}} = \Delta\Omega \times I_\gamma \times (100 \times 10 \text{ cm}^2) = 26.37 \text{ particles/min.}$$

Thus this crude estimate agrees with our measured average value as given in Eq. 3.

D. Simulated Flux

A more accurate way, and the standard practice, is to test our measurements by doing a Monte Carlo simulation of cosmic radiation hitting the detector assembly. A computer program in high level language is written that uses a random number generator to simulate cosmic rays hitting the specified detector geometry. Again using the reference values given by PDG for the solid angle flux, I_γ , the computer simulation program outputs a result which gives the average flux to be 26.183 ± 0.262 particles/min which is again consistent with our measured values.

CONCLUSIONS

From the results obtained in the experiment, daytime and nighttime variation of flux is negligible. Fluxes measured in the same location though of different scintillator dimensions gave consistent measurements of the flux in terms of number of particles per square cm per min. For the 20×20 cm^2 scintillator detector assembly, the average measured flux is 0.0263 ± 0.0004 particles/ cm^2/min and for the 100×10 cm^2 scintillator detector assembly the value is 0.0269 ± 0.0007 particles/ cm^2/min . Both the estimated and the Monte Carlo generated flux are consistent with these actual measurements.

ACKNOWLEDGMENTS

It is with deep gratitude that we acknowledge the contribution of Prof. Kazuo Abe, of High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. Prof. Hitoshi Miyata of Niigata University, Dr. Susumu Igarashi of the Accelerator Division of KEK, without whose help this research would not have been possible. Two of us (R.M.T. & E.F.N.) acknowledge the graduate scholarship grants given to us by the Commission on Higher Education's Mindanao Advanced Education Project.

LITERATURE CITED

- Bevington, P. R., Robinson, D. Keith. *Data Reduction and Error Analysis for the Physical Sciences*. McGraw-Hill, Inc., 1992.
- Das, A., Ferbel, T. *Introduction to Nuclear and Particle Physics*. John Wiley and Sons, Inc., 1994.
- Gaisser, T. K., Stanev, T. Cosmic Rays. *Review of Particle Physics*. 1996. pp. 122-126.
- Hong, J. T., The MACRO Collaboration. "Multiple Muon Measurements with MACRO", hep-ex/9410001 (1995).
- Leo, W. *Techniques for Nuclear and Particle Physics Experiments*. Springer-Verlag, New York, 1987.
- Maeda, Kaichi., "Energy and Zenith Angle Dependence of Atmospheric Muons" *Fortschritte der Physik* 21, 113-154 (1973).
- Melissinos, A. C. *Experiment in Modern Physics*. Academic Press Inc., New York, 1966. pp. 151 - 225.
- Neddermeyer, S. H. and Anderson, C. D. Nature of Cosmic-Ray Particles. *Review of Modern Physics*. 11, 1939.
- New Zealand National Radiation Laboratory. 1995. *The Exposure of New Zealand Aircrew to Cosmic Radiation*. Information Sheet 19. <http://www.nrl.govt.nz/>.
- Niño Franco, E., *Measurement of Cosmic Ray Flux*. Masteral Thesis, Unpublished. Graduate School, Mindanao State University - Iligan Institute of Technology, Iligan City, 1999.
- Particle Data Group, Particle Physics Booklet. 1994. Lawrence Berkeley Laboratory., Berkeley, CA 94270, USA, July 1994.
- Particle Data Group. 1988. *Review of Particle Properties*. Phys. Lett. B. 204:1-486.
- Tsoufanidis, N. *Measurement and Detection of Radiation*. McGraw-Hill, Inc., 1983. pp. 197 - 215.
- Zeigler, J.F., Curtis, H. W., Muhlfield, F.P., Montrose, C.J., Chin, B., Nicewicz, M., Russel, C. A., Wang, W. Y., Freeman, L. B., Houser, P., LaFave, L. E., Walsh, J. L., Otto, J. M., Unger, G. J., Ross, J. M., O'Gorman, T. J., Messina, B., Sullivan, T. D., Sykes, A. J., Yourke, H., Enger, T. A., Tolat, V., Scott, T. S., Taber, A. H., Sussman, R. J., Klein, W. A. and Wahaas, C. W. 1996. *IBM experiments in soft fails in computer electronics (1978-1994)*. IBM Journal of Research and Development. 40:3-18.

INFLUENCE OF ELEVATED CO₂ AND PHOTON FLUX DENSITY ON GROWTH, CARBOHYDRATE CONTENT AND SURVIVAL OF PINUS RADIATA SHOOT CULTURES SUPPLIED WITH VARYING SUCROSE LEVELS

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ABSTRACT

The observation that sucrose supply increased growth, despite the presence of CO₂ in the headspace during the light period and provision of higher photon flux density (PPFD), indicated that carbohydrates were limiting at 350 $\mu\text{L CO}_2 \text{ L}^{-1}$. Hence, in the present study, CO₂ in the headspace was enriched to 2,000 $\mu\text{L L}^{-1}$ to investigate whether sucrose could be eliminated from the media and fully autotrophic *Pinus radiata* plants produced *in vitro*. In the first experiment, sucrose was supplied at 0, 3 and 6% at a PPFD of 150 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. Dry matter production and shoot height were greatly enhanced by CO₂ enrichment at all sucrose levels indicating that CO₂ enhances autotrophy. In addition, vitrified shoots were never observed at 2,000 $\mu\text{L CO}_2 \text{ L}^{-1}$ and the number of senescent shoots were reduced. Increasing the sucrose supply from 0 to 3% stimulated growth even at elevated CO₂. On the assumption that more photosynthetic reducing power may have been required to fully develop autotrophy, the PPFD was raised to 280 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ in the second experiment and sucrose was supplied as in the first experiment. Substantial improvement of growth was achieved with a combination of high PPFD and elevated CO₂ showing that these factors could partially substitute for an external sucrose supply. Maximum growth was achieved at 6% sucrose, 280 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and 2,000 $\mu\text{L CO}_2 \text{ L}^{-1}$. The requirement for extra sucrose was observed despite a large accumulation of starch at high CO₂. Although the reason for this starch accumulation is unknown it may prove beneficial at planting out.

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Key words: *Pinus radiata*, vitrification, photon flux density, CO₂, autotrophy, *in vitro*, sucrose, senescent, shoot culture

INTRODUCTION

P. radiata shoots grown *in vitro* require CO₂ availability during the light period to acquire photosynthetic competence (Antone-Chan, 1993). Achievement of photosynthetic competence enhanced growth and, perhaps most importantly, eliminated the problem of "wet" shoots (Antone-Chan, 1993). However, the addition of sucrose to the media further stimulated growth. Higher PFD was also shown to enhance photosynthetic competence possibly by producing more carbohydrates. These results suggest that carbohydrate supply may still limit growth *in vitro* even when photosynthesis is fully functional at 350 $\mu\text{L L}^{-1}$.

A large number of studies with C₃ plants grown in controlled conditions have shown that increasing the CO₂ above ambient increases the rate of photosynthesis, carbohydrate production and growth (Conroy, 1992). It is therefore likely that increasing the CO₂ concentration in the vial may enhance carbohydrate production and consequently remove the requirement for carbohydrate supply in the media. Removal of soluble sugars from the media would partly solve the problem of contamination, enhance autotrophy in the plantlets (Kozai, 1991) and may enhance survival at planting out. Given that vitrification and contamination are currently the problems which limit the financial success of micropropagation, solving these two problems would be a major achievement with economic consequences. Earlier studies showed CO₂ enrichment increased growth of plants *in vitro* (Courmar et al., 1991; Figueira et al., 1991; Infante et al., 1989; Kozai, 1991 and references therein and Mousseau, 1986); however no studies on *P. radiata* have been reported.

In an earlier study on *P. radiata*, it was demonstrated that increasing PFD could not completely substitute for no sucrose in the medium (Antone-Chan, 1993). It is not known whether increasing the CO₂ levels can eliminate the need for an external carbohydrate supply or whether higher PFD is needed to support the higher rates of photosynthesis at elevated CO₂. An advantage of CO₂ enrichment for glasshouse-grown plants aside from increasing growth is that accumulation of carbohydrates facilitates osmotic adjustment during drought (Conroy et al., 1988). If this occurred in *in vitro*-grown plants, death at the planting out stage should be reduced. This would further reduce the cost of micropropagated plants.

This study investigates the influence of CO₂ enrichment at different PFD and sucrose supplies on the growth and survival of *P. radiata* shoots *in vitro*. The soluble and structural carbohydrate and starch concentrations are also measured to determine whether extra carbohydrate is accumulated as a result of the treatments.

Materials and Methods

Plant Material

Shoot tip explants (about 10 mm long) were obtained from the stock plantlets of *P. radiata* D. Don family 20010 which had been maintained and routinely subcultured. Stock plantlets were produced by remultiplication of the adventitious shoots which had been initiated from excised cotyledons. The initiation of *P. radiata* shoots from excised cotyledons was adapted from Aitken-Christie and Thorpe (1984). The stock plantlets were then maintained and routinely subcultured every four weeks on a Modified Le Poivre nutrient medium (Aitken-Christie and Thorpe, 1984) containing 0.6% (w/v) Bacto Davis agar and 3% (w/v) sucrose. There was 30 ml of nutrient-agar medium in each 200 ml sealed jar which was maintained at sterilized culture conditions by autoclaving at 115 psi for 15 to 20 minutes.

The cultures were grown under Philips white, TL 65/80W/33RS fluorescent lights with a photon flux density of 80 to 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (measured at the level of the lids of culture jars using an Li-188 quantum sensor, LiCor, Inc., USA) over a 14-h photoperiod. The growth room temperature was maintained at $23 \pm 2^\circ\text{C}$.

Experimental Design and Culture Conditions

Experiment 1

Shoot explants were randomly chosen from the stock plantlets and transferred to LP/agar media. The media had either 0, 3 or 6% (w/v) sucrose contained in 200 ml unsealed jars. Unsealed jars are culture jars with two holes drilled unto the screwtop lids. One hole was fitted with a rubber seal and the other hole was fitted with a perspex tube 3 cm long and 1.5 cm in diameter. The perspex tube which allowed the atmosphere in the jars to equilibrate with ambient air was loosely covered with a cotton wool, thus preventing any bacterial contamination. The rubber seal facilitated the measurement of gases within the jars.

Each jar contained four explants. There were eight jars (replicates) for each sucrose concentration. Half of the jars containing each sucrose concentration were transferred to a controlled-environment cabinet at the ambient CO_2 concentration of 340 $\mu\text{L L}^{-1}$. The other half were transferred to a matched cabinet in which the air was enriched with CO_2 to 2,000 $\mu\text{L L}^{-1}$. The PFD was 150 $\text{mmol m}^{-2} \text{s}^{-1}$ for the 14-h photoperiod. In an earlier study (Antone-Chan, 1993), analysis of the gases in the headspace of the unsealed culture jars have shown that its CO_2 concentration increased parallel to that of the chamber atmosphere.

Experiment 2

Shoot explants chosen from the stock plantlets were also transferred to LP/agar media which contained either 0, 3 or 6% (w/v) sucrose as described above. There were also eight jars (replicates) for each sucrose concentration and each jar contained four explants. The CO₂ concentration in the growth cabinet was maintained at 2,000 $\mu\text{L L}^{-1}$ for all jars. However, half the jars at each sucrose concentration were exposed to a PFD of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and the other half at 280 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 14-h photoperiod. Explants were cultured for 50 days.

Measurements

All measurements were made after 50 days in culture. The foliar condition and the number of the plants which survived were assessed. If the number of dead or senescing primary needles per plantlet was less than 25% of the total, the plantlet was counted as surviving. Healthy plants were those which remained green and turgid, with new fascicles and leaf buds formed. The dying or dead plants include those which had stunted growth and had more than 25% yellow or etiolated leaves and dead aerial parts.

The fresh weights and heights of the shoots were measured before these were oven-dried (at 70°C) for 48 hours for dry weight measurements. The length of the three longest fully expanded primary needles of each plant was also measured (in Experiment 1 only).

Approximately 170 mg FW of fully expanded primary needles (pooled samples) from the remaining plants from each jar was used for carbohydrate determination. Tissue extraction and determination of total soluble sugars and starch were done. Total soluble sugars was determined by the anthrone hexose assay, where 0.7 ml sample (tissue extract) was mixed with 1.4 ml of anthrone reagent (2 mg anthrone/ml concentrated sulphuric acid). The mixture was then heated for 8 minutes in a boiling water bath and the concentration of glucose was measured spectrometrically at 620 nm.

Starch was determined by dispersing pellet in 0.2 N KOH at 100°C for 30 minutes. After adjusting the pH to 5.5 by the dropwise addition of 1M acetic acid, glucoamylase reagent was added and then incubated at 30°C overnight. Starch analysis was done on the supernatant using the anthrone hexose assay after boiling the suspension for one minute.

Statistical Analysis

The difference between the treatments and the significance of their interactions were assessed by analysis of variance (Student-Newman-Keuls test). The Fisher's exact test for independence in a 2x2 table was used to assess the data on survival. Standard errors were calculated to show the variation about the mean. The analyses were done using the software program CoStat (CoHort Software, Berkeley, CA, 1990).

Results

Experiment 1

Increasing the CO_2 concentration outside the unsealed jars with shoot cultures generally increased dry and fresh weights ($P < 0.01$) (Figure 1). Increasing the sucrose supply also on average increased the dry and fresh weights ($P < 0.01$). However, there was an interaction between the treatments ($P < 0.001$) because of the following: at $350 \mu\text{L L}^{-1} \text{CO}_2$, growth continued to increase with each sucrose addition. In contrast, at elevated CO_2 , dry weight was maximum at 3% and declined at 6% sucrose. Consequently, at 6% sucrose the ambient-grown plants were heavier than those grown at high CO_2 .

Shoot height was mainly affected by sucrose content in the medium ($P < 0.01$), however there was a significant interaction between CO_2 enrichment and increasing sucrose supply ($P < 0.0001$) (Figure 2) in the same manner as the dry weight. Thus, at 0 and 3% sucrose high CO_2 increased height, but at 6% sucrose it was decreased by CO_2 enrichment.

Needles were generally longer with CO_2 enrichment and increasing sucrose supply ($P < 0.05$) (Figure 3).

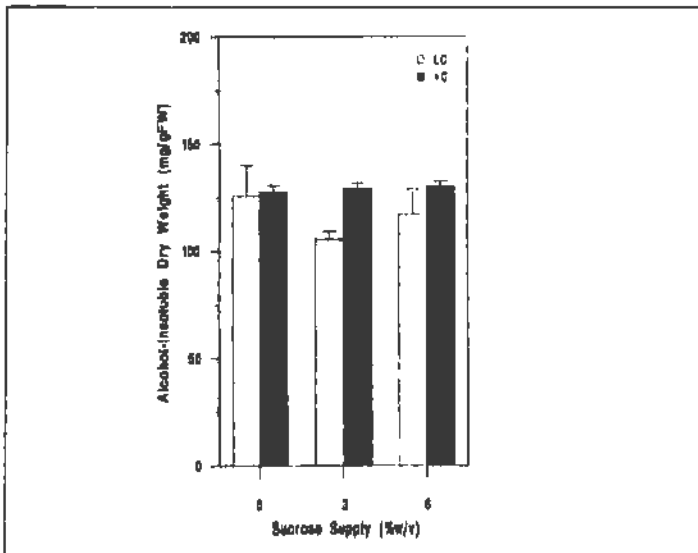


Figure 1. Influence of elevated CO_2 on the fresh weight (A) and dry weight (B) of *P. radiata* shoots after 50 days in culture. The plants were grown at $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ with varying levels of sucrose in the medium and they were continually exposed to either $350 \mu\text{L L}^{-1}$ of CO_2 (LC) or $2,000 \mu\text{L L}^{-1}$ of CO_2 (+C). Each bar represents the mean of four replicates. One standard error is indicated at the top of each bar.

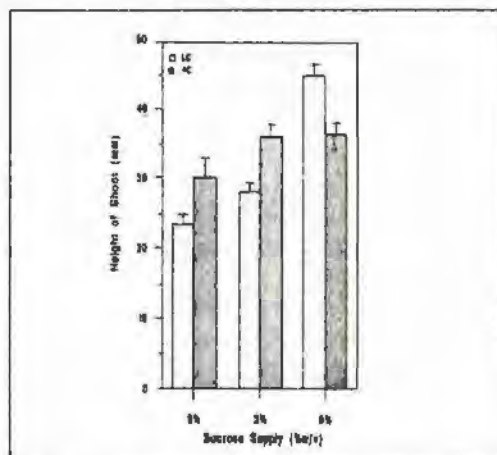


Figure 2. Height of *P. radiata* shoots after 50 days in culture at $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ with varying levels of sucrose in the medium. The plants were continually exposed to either $350 \mu\text{L L}^{-1}$ of CO_2 (LC) or $2,000 \mu\text{L L}^{-1}$ of CO_2 (+C). Each bar represents the mean of four replicates. One standard error is indicated at the top of each bar.

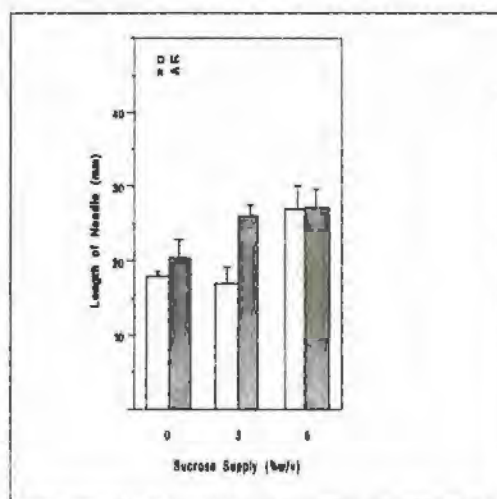


Figure 3. Length of longest fully expanded primary needle of *P. radiata* after 50 days in culture at $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ with varying levels of sucrose in the medium. The plants were continually exposed to either $350 \mu\text{L L}^{-1}$ of CO_2 (LC) or $2,000 \mu\text{L L}^{-1}$ of CO_2 (+C). Each bar represents the mean of four replicates. One standard error is indicated at the top of each bar.

Enriching the air with CO₂ also markedly improved survival of plants at each sucrose concentration ($P < 0.01$) (Table 1). Higher sucrose concentrations also enhanced survival and thus a combination of high CO₂ and 6% sucrose completely eliminated shoot death.

Elevated CO₂ and higher sucrose availability generally increased total soluble sugar concentration ($P < 0.001$) (Figure 4). The significant interaction between the treatments resulted from the large accumulation of sucrose due to CO₂ enrichment at 0% sucrose. The increase was negligible at the 3 and 6% sucrose.

The CO₂ treatments had a dramatic effect on starch accumulation ($P < 0.001$) and there was on average a four-fold increase in starch due to CO₂ enrichment (Table 2). Increasing sucrose availability also tended to cause an increase in starch content but not to the same extent as CO₂ enrichment. Consequently, the starch to soluble sugar ratio was greatly enhanced at the higher CO₂ level ($P < 0.001$) (Table 2). The effects due to sucrose supply on starch production were also significant ($P < 0.05$), showing that a strong interaction existed between sucrose supply and CO₂ enrichment.

The alcohol-insoluble dry weight represents the structural carbohydrate component of the plant, the major proportion of which is the cell wall. Neither sucrose supply nor CO₂ enrichment had a significant effect on this carbohydrate fraction (Figure 5).

Table 1. Influence of CO₂ enrichment at a PFD of 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on the survival of *P. radiata* shoots after 50 days in culture with varying levels of sucrose in the nutrient medium. The values (%) refer to the number of plants which either survived (healthy) or were dying/dead in relation to the total number of explants used.

Sucrose supply (%w/v)	CO ₂ $\mu\text{l L}^{-1}$	Condition of the plant ^a	
		Healthy	Dying/dead
0		350	44 56
	2,000	94	6
3	350	75	25
	2,000	94	6
6	350	100	0
	2,000	100	0

^aThe main effects due to CO₂ enrichment and sucrose supply were significant ($P < 0.05$).

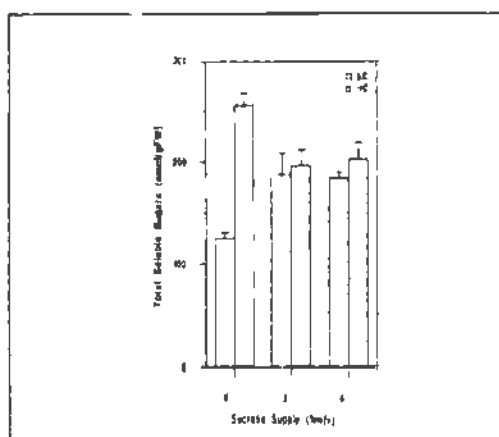


Figure 4. Influenced of elevated CO_2 on total soluble sugars (glucose equivalents) of *P. radiata* after 50 days in culture at $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ with varying levels of sucrose in the medium. The plants were continually exposed to either $350 \mu\text{L L}^{-1}$ of CO_2 (LC) or $2,000 \mu\text{L L}^{-1}$ of CO_2 (+C). Each bar represents the mean of four replicates. One standard error is indicated at the top of each bar.

Table 2. Influence of CO_2 enrichment at a PFD of $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ on starch production of *P. radiata* shoots after 50 days in culture with varying levels of sucrose in the nutrient medium. Each value represents the mean of four replicates + standard error.

Sucrose supply (%w/v)	CO_2 $\mu\text{L L}^{-1}$	Starch production	
		Starch ($\mu\text{mol/gFW}$)	Starch soluble sugars ratio _b
0	350	41.7 ± 6.7	0.34 ± 0.07
	2,000	146 ± 13	0.57 ± 0.056
3	340	27.1 ± 1.9	0.15 ± 0.01
	2,000	188 ± 9	0.88 ± 0.15
6	340	72.5 ± 8.4	0.37 ± 0.04
	2,000	195 ± 13	0.96 ± 0.05

^aThe main effects due to CO_2 enrichment and sucrose supply were significant ($P < 0.001$ and $P < 0.05$, respectively) and there was also a significant interaction between these factors ($P < 0.05$).

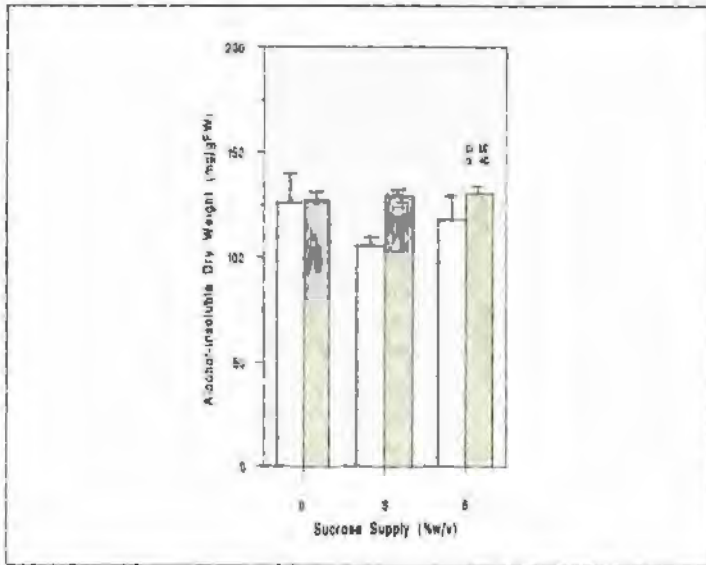


Figure 5. Influenced of elevated CO_2 on alcohol-insoluble dry weight of *P. radiata* shoots after 50 days in culture at $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ with varying levels of sucrose in the medium. The plants were continually exposed to either $350 \mu\text{L L}^{-1}$ of CO_2 (LC) or $2,000 \mu\text{L L}^{-1}$ of CO_2 (-C). Each bar represents the mean of four replicates. One standard error is indicated at the top of each bar.

Experiment 2

Increasing the PFD at a CO_2 concentration of $2,000 \mu\text{L L}^{-1}$ increased both the dry and fresh weights at all sucrose concentrations ($P < 0.01$) (Figure 6). The largest relative increase was at 6% sucrose giving a significant interaction between PFD and sucrose supply. Consequently, weights continued to increase with higher sugar availability at the higher PFD. In contrast, there was a decline in plant weight with increasing sucrose concentration at the lower PFD (Figure 6). This confirms the results of experiment 1 (Figure 1).

Increasing PFD increased shoot height at each sucrose level ($P < 0.01$) (Figure 6A). There was an interaction between sucrose supply and PFD because higher sucrose availability only increased height when the PFD was $280 \mu\text{mol m}^{-2} \text{s}^{-1}$. The same general trend was observed in experiment 1, however there was a small increase in height when the sucrose supply was increased from 0 to 3% (Figure 2).

Because of the elevated CO_2 used in this experiment there were very few unhealthy shoots even at 0% sucrose (Table 3).

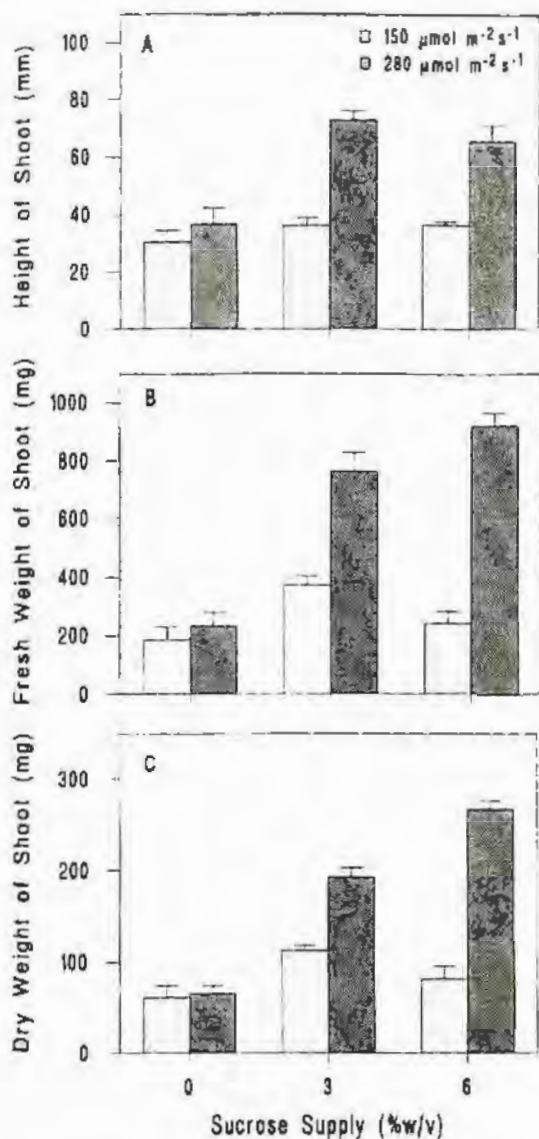


Figure 6. Influenced of elevated CO₂ on alcohol-insoluble dry weight of *P. radiata* shoots after 50 days in culture at 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with varying levels of sucrose in the medium. The plants were continually exposed to either 350 $\mu\text{L L}^{-1}$ of CO₂ (LC) or 2,000 $\mu\text{L L}^{-1}$ of CO₂ (+C). Each bar represents the mean of four replicates. One standard error is indicated at the top of each bar.

Table 3. Influence of photon flux density (PFD) on the survival of *P. radiata* shoots after 50 days in culture at 2,000 $\mu\text{L L}^{-1}$ of CO_2 . The plants were grown in an LP agar medium with varying levels of sucrose. The values (%) refer to the number of plants which either survived (healthy) or were dying/dead in relation to the total number of explants used.

Sucrose supply (%w/v)	PFD $\mu\text{mol m}^{-2} \text{s}^{-1}$	Condition of the plant ^a	
		Healthy	Dying/dead
0	150	94	6
	280	83	17
3	150	94	6
	280	100	0
6	150	100	0
	280	100	0

^aThe differences due to PFD were not significant.

Discussion

The highest shoot productivity of *P. radiata* over 50 days in culture was achieved by providing a PFD of 280 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at a CO_2 concentration of 2,000 $\mu\text{L L}^{-1}$ and a 6% sucrose concentration in the medium (Figure 6). Failure to provide sufficiently high PFD resulted in a depression of growth by high CO_2 at 6% sucrose (Figure 1). Shoot height was affected in a similar way (Figures 2 and 6A). These findings indicate that elevated CO_2 , PFD and sucrose supply all played a role in shoot growth. The effect of these vial factors were probably mediated through effects on photosynthesis and water relations (discussed in Chapter 4 and 5 in Antone-Chan, 1993).

CO_2 enrichment has the potential to increase photosynthesis *in vitro* especially in the presence of high light levels (Desjardins et al., 1988; Fujiwara et al., 1988). The enhancing effects of elevated CO_2 on the rate of CO_2 assimilation has long been known in most C_3 species (Kimball, 1983). The reason is that the first enzyme in the C_3 photosynthetic pathway, ribulose-1,5-bisphosphate carboxylase (Rubisco) has an affinity for oxygen as well as CO_2 . Dissipation of energy through photorespiration reduces the rate of photosynthesis by 40-50% (Sharkey, 1985). Raising the CO_2 concentration increases the ratio of CO_2 to O_2 at the site of fixation in the chloroplast thereby, increasing photosynthetic rates. Thus, the response of photosynthesis to increasing CO_2 levels from 0 to approximately 1,000 $\mu\text{L L}^{-1}$ is linear at saturating PFD (Farquhar and Von Caemmerer (1982).

At CO_2 concentrations above this, RuBP regeneration becomes limiting because insufficient reducing power is produced by the light reactions of photosynthesis. Hence, a family of CO_2 response curves, plateauing at increasing CO_2 levels can be constructed by measuring photosynthesis at increasing PFD. Consequently, in my experiments, at CO_2 concentrations of $2,000 \mu\text{L L}^{-1}$, which would have almost completely suppressed photorespiration, higher PFD was required to promote maximum growth (Figure 6). It is therefore, essential to increase the PFD if gains in growth are to be made by increasing the concentration of CO_2 .

A third limitation to photosynthesis has been described by Sharkey (1985), i.e., triose phosphate utilization limitation. When there are insufficient sinks for the carbohydrates produced by photosynthesis, feedback inhibition of photosynthesis occurs possibly via altering Rubisco activity. In many species, feedback inhibition can be delayed by diverting triose phosphate from sucrose to starch production in the chloroplast. Starch accumulation was observed in the pine plantlets grown at $2,000 \mu\text{L CO}_2 \text{ L}^{-1}$. The starch to soluble carbohydrate ratio was on average of 0.8 at elevated CO_2 compared with 0.3 at ambient CO_2 (Table 2). Hence, the increase in shoot dry weight at elevated CO_2 was partly attributable to the accumulation of starch. Although increasing the PFD would have also increased photosynthetic rates (as demonstrated in previous experiments (Chapter 5 in Antone-Chan, 1993)), the starch to soluble carbohydrate ratio was not substantially increased. Morin et al. (1992) have suggested that the increase in starch in glasshouse-grown clover at elevated CO_2 was caused by altering the ratio between photorespiration and photosynthesis. Changes in Pi flux into the chloroplast and/or nitrogen flux through the photorespiration cycle may be responsible for changing the starch to soluble sugar ratio at elevated CO_2 (Conroy, 1992).

The finding that starch accumulation partly accounted for the increase in dry weight is significant for plant survival on planting out because starch reserves could be degraded to soluble sugars and serve as substrate for growth or for osmotic adjustment. An interesting observation was that structural carbohydrate (estimated as alcohol-insoluble dry weight) was unaffected by either CO_2 enrichment or higher sucrose supply. It is a common observation that leaves of high CO_2 -grown plants appear "stiffer". It is therefore likely that changes in cell wall properties on water relations are responsible. This is supported by the findings in an earlier experiment (Antone-Chan, 1993) that the relationship between relative water content, osmotic potential and water potential was altered when CO_2 was available in the culture vessels.

Higher CO_2 availability eliminated senescent shoots. Earlier (Antone-Chan, 1993), it was demonstrated that vitrified (or "wet") shoots could be eliminated by facilitating gas exchange between the air and the jars. It was concluded that the presence of CO_2 as substrate for photosynthesis during the light period may have contributed. Increasing the CO_2 even higher, may have further enhanced longevity by providing carbohydrate reserves in the form of starch. The finding that higher sucrose supply also eliminated dead shoots supports this idea (Table 1).

Increasing CO_2 from 350 to 2,000 $\mu\text{L L}^{-1}$ resulted in increased needle length as well as starch accumulation, irrespective of sucrose level at a PFD of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$. This increase in expansion may have been due to greater substrate availability, improved water relations or changes in the elastic properties of the cell walls. It was demonstrated that P was maintained better when CO_2 concentration was increased and sucrose was provided (Chapter 4 in Antone-Chan, 1993). In the present study, the response of elongation at high PFD and CO_2 irrespective of sucrose may indicate an effect of CO_2 on water relations and/or direct effect on elongation.

Conclusion

Enrichment of the atmosphere of the unsealed jars with CO_2 to a concentration of 2,000 $\mu\text{L L}^{-1}$ enhanced the growth of *P. radiata* shoots in vitro provided that sufficient reducing power could be generated. Consequently, the growth increases were only achieved at a PFD of 280 $\mu\text{mol m}^{-2} \text{s}^{-1}$. CO_2 enrichment also reduced the number of senescent plants.

Increasing the CO_2 concentration also greatly enhanced starch accumulation. It was, therefore, surprising that despite the accumulation of carbohydrate, increasing the concentration of sucrose in the media to 6% further increased growth. At the lower PFD this was not observed.

The results indicate that maximum growth is achieved at high CO_2 concentrations and PFD and with 6% sucrose. In practical terms it would be possible to remove the sucrose from the media because the decrease in growth may be more than offset by the reduced likelihood of contamination. High CO_2 is also advantageous because it leads to accumulation of starch which on planting out would be a valuable carbohydrate reserve.

The results are also interesting in terms of basic plant physiology. Many questions have been raised about the influence of external sucrose supply, CO_2 enrichment and PFD on accumulation of carbohydrates and their influence on shoot elongation. Whether high CO_2 has an impact on growth other than via its effect on photosynthesis remains to be investigated.

ACKNOWLEDGEMENTS

Assoc. Prof. E.W.R. Barlow

Dr. Malcolm L. Reed

Patrick O'Connell (CSIRO Division of Food Research)

Jacob Mileczanowski

Dr. Benjamin O. Chan

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REFERENCES

- Auken-Christie, J. and Thorpe, T.A. 1984. Clonal propagation: Gymnosperms. In: Vasil, I.K. (ed.), Cell Structure and Somatic Cell Genetics of Plants, Vol. 1. Academic Press, Florida, pp. 82-95.
- Antone-Chan, M. 1993. Effects of Vial Environment on the Growth and Development of *Pinus radiata* Shoot Cultures. Ph.D. Thesis. School of Biological Sciences, Macquarie University, NSW, Australia.
- Conroy, J. 1992. Influence of elevated atmospheric CO₂ concentrations on plant nutrition. Australian J. Botany 40: 445-456.
- Conroy, J., Koppers, M., Koppers, B., Virginia, J. and Barlow, F.W.R. 1988. The influence of CO₂ enrichment, phosphorus deficiency and water stress on the growth, conductance and water use of *Pinus radiata* D. Don. Plant Cell and Environment 11:91-98.
- Coumar, L., Dimon, B., Carrier, P., Lohou, A. and Chagvardieff, P. 1991. Growth and photosynthetic characteristics of *Solanum tuberosum* plantlets cultivated *in vitro* in different conditions of aeration, sucrose supply and CO₂ enrichment. Plant Physiology 97: 112-117.
- Desjardins, Y., Laforge, F., Lussier, C. and Gosselin, A. 1988. Effect of CO₂ enrichment and high photosynthetic photon flux on the development of autotrophy and growth of tissue cultured strawberry, raspberry and asparagus plants. Acta Horticulturae 230: 45-53.
- Farquhar, G.D. and von Caemmerer, S. 1982. Modeling of photosynthetic response to environmental conditions. In: Lange, O.L., Nobel, P.S., Osmond, C.B. and Ziegler, H. (eds.), Physiological Plant Ecology II. Water Relations and Carbon Assimilation. Encyclopedia of Plant Physiology, New Series Vol. 12B, Springer-Verlag, Berlin, pp. 549-585.
- Figueira, A., Whipkey, A. and Janick, J. 1991. Increased CO₂ and light promote *in vitro* shoot growth and development of *Theobroma cacao*. J. American Soc. Hort. Sci. 116(3):585-589.
- Fujiwara, K., Kozai, T. and Watanabe, I. 1988. development of a photoautotrophic tissue culture system for shoots and/or plantlets at rooting and acclimatization stages. Acta Horticulturae 230:151-158.
- Infante, R., Magnanini, E. and Righetti, B. 1989. The role of light and CO₂ in optimising the conditions for shoot proliferation of *Actinidia deliciosa* *in vitro*. Physiologia Plantarum 77: 191-195.
- Kimball, B.A. 1983. Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. Agron J. 75: 774-788.
- Kozai, T. 1991. Micropropagation under photoautotrophic conditions. In: Debergh, P.C. and Zimmerman, R.H. (eds), Micropropagation: Technology and Application, Kluwer Academic Publishers, Dordrecht, Boston, London, pp. 447-469.
- Moin, F., Marcel, A. and Betsche, T. 1992. Growth kinetics, carbohydrate and leaf phosphate content of clover (*Trifolium subterraneum* L.) after transfer to a high CO₂ atmosphere or to high light and ambient air. Plant Physiology 99:89-95.
- Mousseau, M. 1986. CO₂ enrichment *in vitro*. Effect on autotrophic and heterotrophic cultures of *Nicotiana glauca* (var. Samsun). Photosynthesis Research 8: 187-191.
- Sharkey, T.D. 1985. Photosynthesis in intact leaves of C₃ plants: Physics, physiology and rate limitations. The Botanical Review 51: 54-105.

CYTOGENETIC EFFECTS OF MEDICINAL PLANTS FOR DIABETES (AQUEOUS PLANT EXTRACT OR TABLET) ON HUMAN LEUKOCYTES TREATED IN VITRO

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ABSTRACT

The cytogenetic effects of aqueous extracts from bark of "duhat" (*Syzygium cumini* L.), leaves of "damong manis" (*Artemesia vulgaris* L.) and periwinkle (*Catharanthus roseus* L.) and tablets of "ampalaya" (*Momordica charantia* L.) were determined using human leukocytes cultured in vitro. Three to four drops of blood from five to seven volunteers aged 20-21 were cultured in chromosome medium with 0, 3% and 5% sterile extracts. Mitotic index was obtained by counting 500 cells per treatment while chromosomal aberrations were observed in at least 30 cells. Mean mitotic indices using the three medicinal plant extracts were reduced significantly as the concentrations of treatments were increased. For Artemesia, the control showed a mean mitotic index of 16.86% and 22.95 and 25.96% for periwinkle leaves and "duhat" bark, respectively. "Ampalaya" tablets did not significantly affect the mitotic index. Loose sister chromatids, gaps and breaks were the chromosomal abnormalities observed. The frequency of cells with no chromosomal aberration was reduced from 66.67 to 36.00% for Artemesia leaves, 97.00 to 79.00% for periwinkle and 94.00 to 81.00% for "duhat" bark. Artemesia, periwinkle and "ampalaya" significantly induced chromosome condensation. Mean frequency reached 45.00%. Results indicated that all four medicinal plants are possible mutagens. "Duhat" bark is a mitotic inhibitor and as such can be tapped as possible tumor growth suppressor.

Key words: leukocytes, mitotic index, cytogenetic effects, mitotic inhibitor, periwinkle, mutagenic, *Syzygium cumini* L., *Catharanthus roseus* L., *Momordica charantia* L., *artemesia vulgaris* L.

INTRODUCTION

About 35,000 of third world plants are medicinal. Here in the Philippines, medicinal plants have been part of our cultural heritage (de Padua, 1990). Considering the vast number of species with medicinal potentials, in 1969, the National Institute of Science and Technology now DOST (Department of Science and Technology) initiated the program called "Drugs from Plants." It aims to develop safe, effective and low cost drugs from local sources. Manuals and pamphlets were published. seminar workshops have been conducted and herbal gardens were set up all over the country.

Diabetes mellitus is known to affect approximately 120 million people worldwide and in the Philippines 3.1M. It is a metabolic disorder manifested by loss of weight despite an overly good appetite. Cells are depleted of fats and proteins to fulfill the cellular energy requirement not supplied through glucose metabolism. The cells of the pancreas of diabetics are unable to produce enough insulin, which allows glucose derived from food to enter the cells to be broken down as a source of energy. Blood sugar rises and glucose appears in the urine, carrying water with it. These results in increased urine output and thirst. Fat metabolism may be enhanced leading to the accumulation of ketoacid, which if unchecked can result to coma or death.

Medicinal Plants for Diabetes

There are plants, which possess medicinal values and are known to be included in folklore list of insulin substitutes. Morales (1996) conducted a survey of medicinal plants in Olongapo City and eight (8) towns of Pangasinan used to treat *Diabetes mellitus*. A total of 79 plant species were identified and number one in the list is the bark of "duhat" (*Syzygium cumini* L.). Also included are "damong maria" (*Artemisia vulgaris* L.), periwinkle (*Catharanthus roseus* L.) and "ampalaya" (*Momordica charantia* L.). "Duhat" bark as a decoction in water is taken three times a day. The medicinal value of "duhat" bark for diabetes was first studied by Garcia (1987). A light brownish white, amorphous, odorless and slightly bitter powder was isolated from "duhat". One gram of powder has 440 units of insulin. *Artemisia* is used as expectorant, antiseptic, can treat dysmenorrhea, stomach pain, dysentery, pulmonary tuberculosis and many others (Co, 1989) Periwinkle or locally known as chichirica can cure stomachache, toothache, indigestion, and dysentery. It contains vincristine and vialbine used in treating Hodgkin's lymphoma (Prati, 1994). "Ampalaya", a vegetable famous for its bitter taste, is used in China as anti-tumor, anti-infective agent in the US, against HIV infection, stomachache, and constipation (Paño, 1999). Worldwide it is accepted as a strong anti-diabetic because it contains polypeptide p, a plant insulin with less side effects compared to the insulin obtained from the pancreas of animals. In the Philippines, the potential of "ampalaya" as antidiabetic drug was noted by Zamora

(1992). The Department of Health's Institute for traditional Medicine and the U.P. College of Pharmacy are continuously doing research on the antidiabetic potential of "ampalaya". Decoction from "ampalaya", eggplant (*Solanum melongena* L.) and rose apple or "makopa" has been discovered to remedy diabetes and is now owned by a US Company (Bengwayan, 1999). The initial sale in the first year is expected to reach 500 Million dollars. Patent for "ampalaya" has been applied and it is now privately owned by US National Institute of Health, US Army and New York University.

The Philippine government with its increasing population must provide the health needs of the Filipinos. In the event that the of cost synthetic medicine is continuously increasing, the popularity of herbal medicine to cure different diseases rises tremendously. Any new medicine must be subjected to proper screening. Cytogenetic analysis of somatic cells is one of the major methods of testing. The most common source of cells for clinical cytogenetic study is the peripheral blood lymphocytes (Life Technologies, 1998). Routine cytogenetic studies performed on peripheral blood lymphocytes include detection of chromosomal aberrations associated with numerical and structural changes. Knowing that Filipinos are using aqueous medicinal plants and tablets for diabetes, it is but necessary to assess how safe it is to use these medicinal plants.

This study was conducted to determine the cytogenetic effects of aqueous medicinal plant extracts and tablets for diabetes on human leukocytes treated in vitro. Medicinal plants include *Artemesia* (Fig. 1a) and periwinkle (Fig. 1b) leaves, "duhat" bark and "ampalaya" tablet. Specific objectives are to determine the effects of the medicinal plant extracts on mitotic index and evaluate if they can induce chromosomal aberrations.

The study was conducted at the Genetics and Molecular Biology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños College, Laguna.

MATERIALS AND METHODS

Three to four drops of blood from 5-7 male donors (20-21 years old) were cultured on chromosome medium. The blood was treated separately with different concentrations (0, 1%, 3%, 5%) of sterile aqueous medicinal plant extracts and tablet on the 48th hour of culture. Colcemid was added to the culture (on the 71st hour) to arrest the chromosomes at the metaphase plane. After one hour and fifteen minutes of colcemid treatment, cells were fixed and harvested. Smears were prepared for each treatment. Five hundred cells were observed to score for mitotic index (number of dividing cells/total number of cells observed). Fifty cells per treatment were scored for chromosomal aberrations. Analysis of variance was done and means were compared using LSD.



a



b

Figure 1. Pictures of (a) periwinkle (*Catharanthus roseus* L.) and (b) *Artemisia vulgaris* L.

RESULTS AND DISCUSSION

Effects on Cell Division

Photomicrographs of dividing leukocytes arrested at metaphase (from the control group) are shown in Fig. 2. Chromosomes are clearly visible. The centromere location of each chromosome is distinct.

Analysis of variance on mean mitotic index revealed that the treatments are significantly different. The number of dividing cells decreased as the concentrations of treatments increased. For *Artemesia* (Table I), comparison of means revealed that the mean mitotic index of control and 1% (16.86 and 16.80, respectively) is significantly different from 5% (14.53). Using periwinkle leaves, mean mitotic indices are significantly different except for 3 and 5%. Using "duhat" bark, the mitotic index was significantly reduced compared with the control (25.91). A very low proportion of dividing cells [5% (14.53%)] was noted. "Duhat" bark is a mitotic inhibitor and as such can be tapped as a possible tumor growth suppressor. The story of the discovery of the antitumor value of periwinkle extract is worth repeating. Periwinkle, being cited in folk medicine as an aid in treating diabetes was included in the survey to identify oral insulin substitute by Noble and Beer at the University of Western Ontario and Svoboda at Eli Lilly in Indianapolis. Studies showed that crude extract of periwinkle drastically reduced white blood cell counts especially granulocytes. It tremendously decreased bone marrow activity in rats. The effects of the active leukopenic extract against leukemia cells were further tested *in vitro*. This led to the isolation of vincaleukoblastine (Lewis and Elvir-Lewis, 1977).

Effects of Medicinal Plants on Chromosomal Morphology

Changes in chromosome structure offer readily scored morphological evidence of damage to the genetic material (Evans, 1984). These include gaps, breakages,

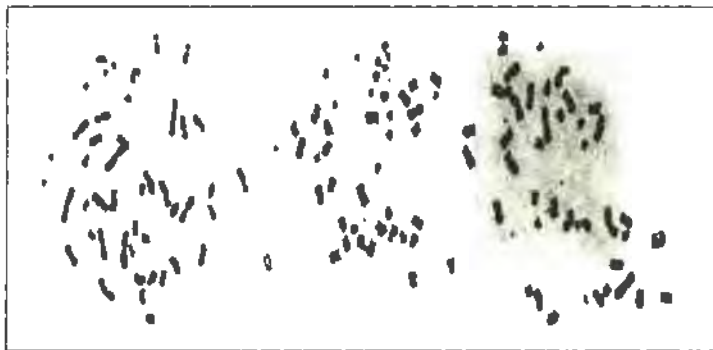


Figure 2. Chromosomes of untreated (control) human leukocytes arrested at metaphase plane. Magnification (4,000X).

Table 1. Mean mitotic index of human leukocytes treated in vitro with different concentrations of aqueous medicinal plant extract for diabetes.

Treatment Concentrations	Mean Mitotic Index (%)		
	Medicinal Plants		
	<i>Artemesia leaves</i>	<i>Periwinkle leaves</i>	"Duhai" bark
Control			
	16.86a	22.95a	25.96a
1%	16.80a	19.28b	9.96b
3%	15.66ab	16.36bc	8.60b
5%	14.53b	14.32c	0.04c

loose sister chromatids and condensed chromosome sets. These chromosomal aberrations are gross structural changes, which can be detected without detailed karyotype analysis (Engle *et al.*, 1986).

The mean frequency of chromosomal aberrations noted is presented in Table 2. Using *Artemesia* leaves, the number of normal cells decreased as treatment concentration increased. The number of cells with loose sister chromatid (characterized by early sister chromatid separation) increases from 23.33 in control to 32.00% in 5%. Figure 3 shows loose sister chromatid observed using 1 to 3% *Artemesia* and 5% periwinkle. The frequency of gap (defined as the separation in the chromatid arm without loss of alignment or dislocation of the distal fragment) also increased with increasing concentrations of treatment. A similar observation was made with the frequency of break, a discontinuity of the chromatid arm with either loss of alignment or dislocation of the distal fragment (Ford, 1973). Figure 4 shows a break observed using 3% *Artemesia*. Compared to *Artemesia*, the number of normal cells is higher when periwinkle leaves are used (97.00-79.00%). The frequency of other chromosomal aberrations was noted to be low as well. A low frequency of cells with chromosomal aberrations was observed using "duhai" bark. This would explain why higher percentage of cells with normal chromosomes was noted (94.00-81.00%).

Effects on Chromosome Condensation

Artemesia and periwinkle leaves induced chromosome condensation (Fig. 5). This is when chromosomes contracted to at least half the normal size as those found in the control. The chromosomes could no longer be classified according to groups. The frequency of cells that showed condensed chromosomes as treatment increases is highly significant. Mean frequencies when compared are significantly different (Table 3). Both for *Artemesia* and periwinkle, the frequency of cells with condensed chromosomes increased from 5 to 44.00% and 9 to 45.00%, respectively. Shortening of the chromosomes could possibly be an indication of chromatin

Table 2. Mean frequency of chromosomal aberrations observed on human leukocytes treated *in vitro* with different concentrations of aqueous medicinal plant extract for diabetes.

Medicinal Plant Used	Chromosomal Aberrations Observed	Mean Frequency (%)			
		Treatment Concentration			
		Control	1%	3%	5%
<i>Artemisia</i> leaves	Normal	66.67	48.34	39.99	36.00
	Loose sister chromatid	23.33	26.67	33.33	32.00
	Gap	10.00	23.33	21.68	23.00
	Break	0.0	1.66	5.00	9.00
Periwinkle leaves	Normal	97.00	92.00	86.60	79.00
	Loose sister chromatid	20.00	6.60	10.60	19.5
	Gap	1.0	0.66	-	-
	Break	-	0.66	2.66	1.5
"Duhai" bark	Normal	94.00	92.00	91.00	-
	Loose sister chromatid	1.33	2.66	3.00	-
	Gap	4.66	4.66	8.00	-
	Break	-	0.66	8.00	-

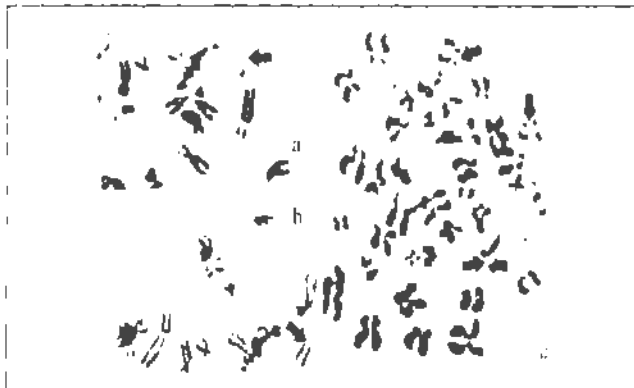


Figure 3. Loose sister chromatids observed when human leukocytes were treated *in vitro* with 1 and 3% *Artemisia* and 5% periwinkle aqueous leaf extract. Magnification (4,000X). (a-b) treated with 1 and 3% *Artemisia*; (c) treated with 5% periwinkle.

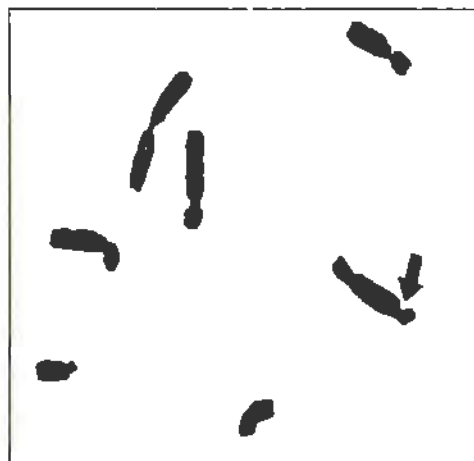


Figure 4. Break observed when human leukocytes were treated *in vitro* with 3% *Artemesia* aqueous leaf extract. Magnification (4,000X).

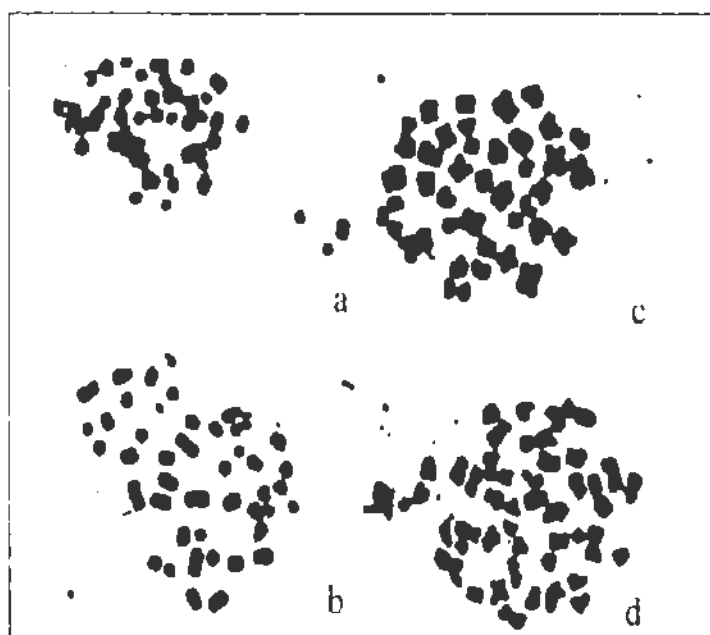


Figure 5. Chromosome condensation observed when human leukocytes were treated *in vitro* with *Artemesia* (a, b) and periwinkle (c,d) leaf extracts. Magnification (4,000X).

Table 3. Mean frequency of chromosome condensations observed on human leukocytes treated *in vitro* with different concentrations of aqueous medicinal plant extracts for diabetes.

Treatment Concentration (%)	Mean Frequency (%) of Chromosome Condensation	
	<i>Artemisia leaves</i>	<i>Periwinkle leaves</i>
Control	5.00a	9.00a
1	16.00b	21.00b
3	32.00c	33.00c
5	44.00c	45.00d

condensation (Engle *et al.* 1986). Polyamines could be present in the extract, which can induce chromosome condensation because of their ability to form stable complexes with DNA proteins (Davidson and Anderson, 1960; Du Praw, 1970).

Effects of "Ampalaya" Tablets on Cell Division

The different concentrations of "ampalaya" tablet did not significantly affect mean mitotic index (Table 4). The control has 13.00% while 3 and 5% have 11.30 and 10.90%, respectively. "Ampalaya" tablets do not show toxicity to the cells at the concentration tested, thus allowing for normal cell division to occur.

Effects of "Ampalaya" Tablets on Chromosome Morphology and Condensation

As shown in Table 5, "ampalaya" tablets are not potent chromosome breaking agents. The frequency of cells with normal chromosomes was high (88.70-95.10%) at 0% to 5%. The frequency of gaps, breaks and loose sister chromatids was low at all concentrations of ampalaya.

However, "Ampalaya" tablets significantly affected chromosome condensation (Table 6). At 3 and 5%, chromosome condensation was 2.3 % and 1.7%, respectively. There was no difference between control and 1% treatment.

SUMMARY AND CONCLUSION

The three medicinal plant extracts affected cell division of human leukocytes cultured *in vitro*. "Duhai" bark is a probable inhibitor of cell division and as such can be tapped as a tumor growth suppressor. The depressive effects of the different extracts on mitotic index may also indicate the ability of the extracts to hamper the production of new cells in the body such as blood cells and sex cells. The chromosomal aberrations observed like gaps, breaks and loose sister chromatids

Table 4. Mean mitotic index of human leukocytes treated *in vitro* with different concentrations of "ampalaya" (*Momordica charantia* L.) tablet.

Treatment Concentration (%)	Mean Mitotic Index (%)
Control	13.00
1	12.30
3	11.30
5	10.90

Table 5. Mean frequencies (%) of chromosomal aberrations observed on human leukocytes treated *in vitro* with different concentrations of "ampalaya" (*Momordica charantia* L.) tablet.

Chromosomal aberrations	Treatment Concentration			
	Control	1%	2%	5%
Normal	94.30	95.10	88.70	92.50
Loose sister chromatid	2.60	2.90	5.60	3.10
Gap	2.00	1.10	3.10	3.00
Break	1.10	0.90	2.60	1.40

Table 6. Mean frequencies (%) of chromosome condensation observed on human leukocytes treated *in vitro* with different aqueous concentrations of "ampalaya" (*Momordica charantia* L.) tablet.

Treatment Concentration (%)	Chromosome Condensation (%)
Control	8.6a
1%	8.6a
3%	20.6b
5%	15.1bc

according to Evans (1984) are morphological evidences of damage to the genetic material. All four treatments significantly induced chromosome condensation. Highly condensed chromosomes are not available for transcription. Thus, synthesis of important proteins or enzymes necessary for the cells' primary processes may be inhibited and this condition is detrimental to a dividing cell.

Considering that the medicinal plants tested produced visible cytogenetic effects, caution must be exercised against the excessive and frequent oral intake to minimize the possibility of detrimental effects.

ACKNOWLEDGMENT

The project was funded by the Institute of Biological Sciences, College of arts and Sciences, UPLB and the Commission on Higher Education (CHED).

LITERATURE CITED

- Bengwayan, M. 1999. Lowly "talang", "ampalaya", makops" now under patent, US Biopirates. Strikes Again. Today. Sept. 7. P.4
- Co, L.L. 1989. Common Medicinal Plants of the Cordillera Region. Busmanian Press. Quezon City Phil. 487 pp
- Davidson, D. and N.G. Anderson. 1960. Chromosome coiling induced by polyanines. Exp. Cell. Res. 20: 610-613.
- De Padua, L.S. 1990. Philippine Medicinal Plants. Island Pub. House. Manila. P.5.
- Du Praw, E.J. 1970. DNA and Chromosomes. Holt, Rinehart and Winston. Inc. New York. 283-301.
- Engle, L.M., C.B. Dela Villa, and S.T. Bacud. 1986. Cytogenetics Effects of Different Chemical Agents on Human Leucocytes *In Vitro*. UPLB Basic Research Terminal Report. 29 p.
- Evans, H.J. 1984. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen test. In Handbook of Mutagenicity Procedures. 2nd ed. Kilbey, B.S., M. Legator, W. Nichols and C. Ramel. Elsevier Science Pub. B.V. Amsterdam. N.Y. Oxford. 405-427.
- Ford, E.H.R. 1973. Human Chromosomes. Acad. Press Inc. New York. USA. 361 p.
- Garcia, F. 1987. Distribution of insulin-like principle in different plants and its therapeutic application to a few cases of *Diabetes mellitus*. Abstract of Science. Papers. 3876 (3) 3-20
- Kelly, T.E. 1986. Clinical Genetics and Genetic Counselling. 2nd ed. P.23.
- Lewis, W.H. and M.P.F. Elvir-Lewis. 1997. Medical Botany. Plants Affecting Man's Health. A Wiley-Interscience Pub. John Wiley and Sons. New York. 127-134.
- Life Technologies. 1998. GIBCO-BRL. Form. 3812.
- Morales, M.S. 1996. A Survey of Herbal Plants used in the Treatment of *Diabetes mellitus* in Olongapo City and the Ilokano and Pangasinan-speaking Areas of Pangasinan Province. B.S. Thesis UPLB. 45 pp.
- Pano, A.R. 1999. "Ampalaya". Bitter is better. Phil. Star. July 29:25.
- Pratt, W.B. 1994. The Anticancer Drugs. Oxford Press Inc. New York. 2nd ed. (89-19)
- Therman, E. 1980. Human Chromosomes. Holt, Rinehart Pub. 21-32.
- Zamora, F. 1992. "Ampalaya" as an antidiabetic. Greenfields. 222:39-40

ANTAGONISTIC PLANTS FOR THE MANAGEMENT OF THE ROOT-KNOT NEMATODE, *Meloidogyne graminicola* IN RICE-ONION SYSTEM

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ABSTRACT

A microplant experiment with the following treatments: *Tagetes* sp., two species of *Crotalaria* (*C. incana* and *C. macronata*), rice and fallow, was conducted to determine the effects of *Tagetes* and *Crotalaria* plants on the population and development of the rice root-knot nematode, *Meloidogyne graminicola*. In the two trials conducted, it was observed that no galls were formed on the roots of *Tagetes* and *Crotalaria* plants 60 and 90 days after soil infestation. The rice plants however, had a mean root gall of 305.70 at harvest (90) days. Onion grown on the plots planted previously with *Tagetes* and *Crotalaria* did not show root galls, however, galls were observed in onion planted in microplots previously planted with rice. Fresh weight of onion were higher in *Tagetes* and *Crotalaria*-treated plots compared to the plots planted to rice and the clean fallow treatments, however differences were insignificant. This could be due to the nematode control and to the added fertility when the biomass of these plants was incorporated in the soil before onion was planted. Rice planted in these treatments showed significant reduction in the number of galls and nematode density in the soil after 60 days. These results showed that planting *Tagetes* sp. or *Crotalaria* spp. in nematode-infested soil is effective and also feasible in managing the rice-knot nematode.

Key words: Antagonistic plants, Root-knot nematode, *Meloidogyne graminicola*, *Tagetes*, *Crotalaria incana*, *C. macronata*, fallow, rice, onion

INTRODUCTION

Many genera of plant parasitic nematodes are associated with rice but not all are proven of potential economic importance. Among the nematodes associated with rice, the root-knot nematode, *Meloidogyne graminicola* has been considered one of the most destructive. Infected rice root tips become swollen and hooked. Above ground symptoms include tip drying, leaf bronzing, chlorosis, growth reduction, unfilled spikelets, reduced tillering and poor yield (Babatola, 1984). Yield reduction of upland rice as reported is about 3-32% (Barsalote and Gapasin, 1995). In the Philippines, the nematode is widely distributed occurring in about 40% of the 18 provinces surveyed (Soriano and Prot, 1992). In 1994, *M. graminicola* was found in rice growing areas in Central Luzon (Unpublished report).

Rice is normally grown twice a year where there is continuous irrigation. In Central Luzon, specifically in Nueva Ecija, farmers practice the rice-vegetable cropping pattern. Problems of pests in rice being carried over in vegetables can not be avoided. *M. graminicola*, which has become a pest of rice also infest several vegetable crops including weeds (Gapasin et al., 1996; Zamora and Gapasin, 1996). Most farmers in San Jose and Bongabon, Nueva Ecija grow onion after rice and *M. graminicola* has been reported a potential pest in onion (Gapasin et al., 1996). Therefore management of the nematode is of prime importance.

Several management options are available against the root-knot nematode like chemical, cultural, physical, mechanical, use of resistant varieties and biological control. Antagonistic plants have been used effectively in the management of some species of nematode in the soil. Caswell et al. (1991) reported a decline of *Rotylenchulus reniformis* population after *Crotalaria juncea* and *Tagetes patula* were grown. The mode of action of alpha-tertibienyl and related compounds may explain the suppressive effects of *Tagetes* species. Root lesion nematode (*Pratylenchus penetrans*) were nearly eliminated from the plots with marigold (*Tagetes patula* L. cv Sparky).

This study was conducted to determine the effects of *Tagetes* sp. and *Crotalaria* sp. on the population and development of the rice root-knot nematode, *Meloidogyne graminicola*.

MATERIALS AND METHODS

Microplots with an area of 2.4 x 0.75 meters were prepared and inoculated with 1,000 eggs of *M. graminicola* and later sown with 30 seeds of rice (var. UPLR15). The rice plants were allowed to grow in the microplots for 60 days. Rice plants were harvested and their galled roots together with the egg masses were evenly buried in the plots. Constant watering was done to enhance egg hatching to build-up the nematode population in the microplots. After one week

seeds of *Crotalaria incana*, *C. mucronata*, *Tagetes* sp. and rice (UPLRi5) were sown in the microplots. A clean fallow treatment was included and the treatments were replicated three times. The plants were allowed to grow for 90 days. After 60 days, 10 plants from each of the microplots were harvested and the roots were washed carefully in running water and later cut into small pieces. From each root system, 5 grams were taken and the number of galls were counted and assayed for nematode larvae using the modified Baermann funnel method. Likewise, ten-200 g soil was taken from each plot and the nematode larvae were extracted using also the modified Baermann funnel method. The larvae extracted from the roots and soil were counted under the stereomicroscope with the aid of a hand tally counter. The same procedures were followed when plants were harvested at 90 days. Two trials were conducted.

One month seedlings of bulb onion (var. Yellow Granex) were planted in the microplots after harvesting the plants in the different treatments. The onion plants were allowed to grow for 90 days. The fresh weight, number of galls and nematode population in the soil were collected and statistically analyzed. Rice seeds were then sown in the microplots for bioassay. The rice plants were allowed to grow for 60 days and number of galls and nematode population in the soil were collected and statistically analyzed.

RESULTS AND DISCUSSION

Crotalaria spp. and *Tagetes* sp. effectively reduced nematode densities in soil and affected development of the root-knot nematode in the microplot experiment (Tables 1 and 2). No galls were formed in the roots of these plants suggesting that they are antagonistic to the rice root-knot nematode while the roots of rice after 60 days were heavily galled with mean galls of 49.30 and 81.96 in the first and second trials, respectively. The number of galls in the rice plants at 90 days increased and had mean galls of 305.70 and 267.90 in the first and second trials, respectively. The number of galls in the rice plants at 90 days increased and had mean galls of 305.70 and 267.90 in the first and second trials, respectively. The increasing trend of nematode density was also observed in the roots. Nematode density in the soil was low in *Crotalaria* spp and *Tagetes* sp. treatments while those grown to rice increased tremendously in both trials. Chitwood (1993) reported that naturally occurring phytochemicals with biological activity against plant parasitic nematodes include polythienyls, alkaloids, phenolics, polyacetylenes, fatty acids, terpenoids and others. Several workers have shown the efficacy of *Tagetes* in suppressing nematodes in soil. Cultivation of marigolds (*Tagetes* spp.) significantly suppressed the population and build-up of noxious nematodes (Oostenbrink, 1960; Alam et al., 1977). Likewise, McSorley and Frederick (1994) reported little or no galling or egg production from any *Meloidogyne* isolate on *Lageratum houstonianum* cv. Blue Mink, *Lobularia maritima* cv. Rosie O'Day, or *Tagetes patula* cv. Dwarf Primrose. Two compounds [α -terthienyl and 5-(3-buten-1-ynyl)-

Table 1. Effect of *Tagetes*, *Crotalaria* and clean fallow treatments on the number of galls and *M. graminicola* population in roots and soil 60 and 90 days after soil infestation. (1st Trial.)

Treatments	Numbe of Galls	Nematode Density in Roots	Nematode Density in Soil
60 days			
<i>Tagetes</i> sp.	0.00a	0.00a	5.23a
<i>Crotalaria mucronata</i>	0.00a	0.00a	7.46a
<i>Crotalaria incana</i>	0.00a	0.00a	6.23a
Clean fallow	-	-	3.20a
Rice (UPLRi5)	49.30b	199.10b	496.30b
90 days			
<i>Tagetes</i> sp.	0.00a	0.00a	2.56a
<i>Crotalaria mucronata</i>	0.00a	0.00a	2.56a
<i>Crotalaria incana</i>	0.00a	0.00a	2.96a
Clean fallow	-	-	2.53a
Rice (UPLRi5)	305.70 b	354.70b	368.60b

5 g per root system and 200 g soil per plant; 10 sample plants per plot

Table 2. Effect of *Tagetes*, *Crotalaria* and clean fallow treatments on the number of galls and *M. graminicola* population in roots and soil 60 and 90 days after soil infestation. (2nd Trial.)

Treatments	Numbe of Galls	Nematode Density in Roots	Nematode Density in Soil
60 days			
<i>Tagetes</i> sp.	0.00a	0.00a	63.11a
<i>Crotalaria mucronata</i>	0.00a	0.00a	43.66a
<i>Crotalaria incana</i>	0.00a	0.00a	55.22a
Clean fallow	-	-	23.88a
Rice (UPLRi5)	81.96b	253.66b	283.66b
90 days			
<i>Tagetes</i> sp.	0.00a	0.00a	28.88a
<i>Crotalaria mucronata</i>	0.00a	0.00a	33.44a
<i>Crotalaria incana</i>	0.00a	0.00a	26.66a
Clean fallow	-	-	20.66a
Rice (UPLRi5)	305.70 b	354.70b	316.44b

5 g per root system and 200 g soil per plant; 10 sample plants per plot

2,2'-bithienyl] from *Tagetes* were found effective against several nematodes including root-knot species. The alkaloid monocrotaline in *Crotalaria* may be inhibitory to nematodes (Chitwood, 1993). Nematode density in the soil were tremendously reduced by the treatments. The clean fallow treatment had the lowest nematode density in the soil after 60 and 90 days. This was expected since the plots were weeded and turning the soil may have profound effect on the nematode larvae due to the heat of the sun. The result of the microplot experiment confirms the efficacy of these antagonistic plants in reducing root galls thus affecting the nematode development and reducing significantly the nematode densities in the soil. *Tagetes* sp. and *Crotalaria* spp. can be effective in suppressing the rice root-knot nematode population in the soil. Planting these antagonistic crops during fallow period in nematode infested soil is feasible.

Onion grown in the *Crotalaria* spp. and *Tagetes* sp. treatments had higher fresh weight compared to the clean fallow and rice treatments, however, differences were insignificant (Table 3). Likewise, no galls were observed in onion roots grown under the different treatments except those grown in the rice treatment. The nematode density in these treatments was also low compared to the rice treatment, however, differences were insignificant. This increase could be due to the added fertility afforded by the decomposed plants since their biomass was incorporated in the soil after harvest and may also be due to nematode control. Mulches of decaying organic matter can improve the growth of plants and in some cases actually reduced the damage caused by the nematode due to some metabolic by-products in the decomposition of organic matter (Watson, 1944 and Smith and Batista, 1942). Rice grown after onion in *Tagetes*, *Crotalaria* and fallow plots has significantly lower numbers of galls and nematode density in the soil compared to the plots planted to rice (Table 4). This result clearly shows the antagonistic effects of *Tagetes* and *Crotalaria*. The carry over effects of these antagonistic plants are long lasting and therefore feasible in managing the rice root-knot nematode.

Table 3. Effect of *Tagetes*, *Crotalaria* and clean fallow treatments on fresh weight of bulb onion, number of galls and *M. graminicola* population in soil after 90 days.

Treatments	Fresh weight (g)	Number of Galls	Nematode Density in Soil
<i>Tagetes</i> sp.	10.11	0.00	17.33
<i>Crotalaria mucronata</i>	10.80	0.00	20.99
<i>Crotalaria incana</i>	10.45	0.00	25.67
Clean fallow	6.92	0.00	16.00
Rice (UPLR15)	6.49	12.47	32.44

5 g per root system and 200 g soil per plant; 10 sample plants per plot

Table 4. Effect of *Tagetes*, *Crotalaria* and clean fallow treatments on number of galls in rice and *M. graminicola* population in soil after 60 days.

Treatments	Number of Galls	Nematode Density in Soil
<i>Tagetes</i> sp	6.70	9.60
<i>Crotalaria mucronata</i>	13.87	13.07
<i>Crotalaria incana</i>	6.40	9.43
Clean fallow	6.63	8.40
Rice (UPLR15)	86.20	54.03
LSD (1.0t)	12.18	14.84

5 g per root system and 200 g soil per plant, 10 sample plants per plot

ACKNOWLEDGEMENT

We are grateful for the funding provided by the United States Agency for International Development (USAID) through PhilRice with grant No. LAG-4196-G-00-3053-00 to the Integrated Pest Management- Collaborative Research Support Program (IPM-CRSP) Project.

LITERATURE CITED

- Alant, M.M., S.K. Saxena and A.M. Khan. 1977. Influence of interculture of marigold and mungbean with some vegetable crops on plant growth and nematode population. *Acta Bot. Indica* 5:33-39.
- Babatola, J.O. 1984. Rice nematode problems in Nigeria, their occurrence, distribution and pathogenesis. *Tropical Pest Management* 30:256-265.
- Barsalote, L.B. and R.M. Gapasin. 1995. Pathogenicity of the rice root-knot nematode, *Meloidogyne graminicola* in upland rice. *Philipp. Phytopath.* 31:95-102.
- Chitwood, D.J. 1993. Naturally occurring nematocides. *A.C.S. Symp. Ser. Am. Chem. Soc.* p. 300-315.
- Gapasin, R.M., F.B. Gergon and J.C. Prot. 1996. *Meloidogyne graminicola*: a potential problem in rice-onion systems. A paper presented during the Symposium on IPM in Rice-Vegetable Systems. Status, Constraints and Prospects held at PCARRD, Los Baños, Laguna on October 2-4, 1996.
- Meserley, R. and J.J. Frederick. 1994. Response of some common annual bedding plants to three species of *Meloidogyne*. *J. Nematol.* 26:773-777.
- Osteubrunk, M. 1960. Population dynamics in relation to cropping, manuring and soil disinfection. In: *Nematology* (Sasser, J.N. and Jenkins, W.R. (Eds.), Univ. North Carolina Press, Chapel Hill. 439-442.
- Smith, F.B. and J.W. Bausta. 1942. The nematode problems from the soil microbiological stand point. *Proc. Soil Sci. Fla.* 4:13-144-147.
- Soriano, L.R.S. and J.C. Prot. 1992. Plant parasitic nematodes associated with irrigated rice in the Philippines. *Phil. J. Crop Sci.* 17:8-28.
- Watson, J.R. 1944. Mulches to control root-knot. *Proc. Fla. Acad. Sci.* 7:151-153.
- Zamorá, O.B. and R.M. Gapasin. 1996. Reaction of different corn, legume and root crop varieties to the rice root-knot nematode, *Meloidogyne graminicola* Golden and Birchfield. *Phil. Phytopath.* 33:37-44.

A MODEL FOR SCHOOL-BASED CONTROL OF COMMON INTESTINAL HELMINTHS USING MASS TREATMENT: PARASITOLOGIC ASSESSMENT

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ABSTRACT

A model for school-based control of common intestinal helminths was developed and tested in San Vicente Elementary School in Biñan, Laguna, Philippines integrating health care with education programmes, monitoring parasitologic parameters, and comparing the efficacy of once versus twice yearly mass treatment. Mebendazole (Antiox) 500 mg choco-flavored tablets were administered to schoolchildren with parental consent following a treatment schedule determined by randomization. Parasitologic assessment and monitoring were conducted among third grade pupils considered the indicator group. Stool specimens were collected and processed using Kato Katz method which allowed qualitative and quantitative diagnosis in terms of parasite species and egg counts, respectively. Parasitologic parameters were monitored at Days 0, 7 to 14, 180, 187 to 194, 360 and 367 to 374. There were significant reductions of infection rates and intensities of infection post-treatment. Twice yearly deworming was shown to be superior to once yearly treatment. Continuation of periodic mass treatment with mebendazole initially twice yearly with monitoring of parasitologic and nutritional progress as well as school performance parameters are strongly recommended for the school. Larger scale application of the results of this and other similar studies are recommended.

Key Words: mass treatment, common intestinal helminths, Aseans, Trichuris, school-based control, deworming, parasitologic assessment, monitoring

INTRODUCTION

There is overwhelming evidence that roundworms, whipworms and hookworms are leading causes of morbidity in pre-school and school age children especially in developing countries like the Philippines. Intestinal parasitism causes adverse effects on health, growth and school performance further causing underdevelopment. It is believed that the proportion of the world's population infected with these worms have remained virtually unchanged over the past 50 years.

Among a few anthelmintics, mebendazole (Antiox) has been shown to be effective in the treatment of common intestinal helminthiasis and is included in the World Health Organization (WHO) Model List of Essential Drugs. Since safe, effective, and easy to administer drugs are available, a strategy of periodic mass treatment through schools could target school age children, as advocated by the Health Promoting Schools initiative of WHO. This same strategy emphasizes the role of schoolteachers as potential partners in making possible a healthy school populace.

Mass treatment has been recommended in areas or target groups with prevalence of more than 50% according to the WHO Panel of Experts. They have suggested that monitoring may be done utilizing third grade students who may provide a good cross-sectional picture of the overall extent of the worm problem in the school. Chemotherapy targeted at population groups like schoolchildren will have benefits to other non-treated populations by clearing the source of infection, thereby reducing fecal contamination of the environment and eventually diminishing transmission. There may be a need therefore to develop and test an affordable school-based model for control of common intestinal helminths using mass treatment.

The main objectives of this study were:

- 1) To develop a model for school-based control of common intestinal helminths which integrates health care with education programmes;
- 2) To test the model for school-based worm control using the following parameters:
 - a) cure rates
 - b) egg reduction rates
- 3) To compare the efficacy of once yearly versus twice yearly mass treatment.

MATERIALS AND METHODS

Study Design Overview

This was a comparative study in children belonging to an indicator group, the third grade pupils. All pupils meeting the study entry criteria received a single

oral dose of mebendazole 500 mg choco-flavored tablet once-yearly or twice-yearly after randomization. Infection rates, mean arithmetic and geometric egg counts, cure and egg reduction rates were derived. All the other pupils from the other grades were treated at the beginning of the project period and a year later. The study was conducted according to Good Clinical Practice Guidelines and the Declaration of Helsinki.

Study Site and Population

San Vicente Elementary School (SVES) is located in Barangay San Vicente, Biñan, Laguna, which is 33 kilometers south of Manila. As part of the Biñan School District, the school has provided public elementary education since it opened in 1958. For academic year 1999-2000, the school had an enrollment of 2,904 with 1,552 boys and 1,352 girls, with ages ranging from 5 to 15 years.

All pupils with parental consent from the third grade comprised the indicator group (Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level, WHO, 1998) in which parasitologic parameters were measured, while all pupils in all grade levels were targeted to be included in the mass treatment strategy.

Inclusion and Exclusion Criteria

Inclusion criteria for mass treatment were the following: 1) male or female, of elementary school age; and 2) written informed consent of the parent or guardian. Exclusion criteria were: 1) demonstration of previous hypersensitivity reaction to a benzimidazole or any related compound; 2) intake of any anthelmintic in the 2 weeks prior to enrolment into the study; and 3) concomitant infection or any other underlying disease which would compromise the evaluation of the response to the study medication.

Mass Treatment

A training of teachers was undertaken in September 1999 by way of a seminar-workshop that aimed to achieve the following:

- 1) To describe the problem of common intestinal helminthiasis;
- 2) To discuss mass treatment as a control strategy for common worms;
- 3) To discuss the role of the school, its students and teachers in worm control;
- 4) To describe possible problems which may be encountered in the implementation of school-based mass treatment for control of common intestinal helminthiasis;
- 5) To propose solutions to these possible problems; and
- 6) To finalize an action plan for implementation of school-based mass treatment for control of common intestinal helminthiasis.

Strategies used to achieve the above mentioned goals included video showing, short lecture-discussions, and a workshop to discuss possible problems and solutions as well as to draw up an action plan for implementation. Based on outputs of the seminar-workshop, the Guidelines for Mass Treatment for SVES Teachers were formulated.

Health education was also conducted among schoolchildren using posters and wall news-type material, known as the *Kontra Bulate Bulletin*. Two meetings with the Parent-Teacher Association (PTA) were conducted aiming to raise awareness of parents regarding common worms and the need for mass treatment. A billboard on the worm control project and the importance of SVES in worm control efforts in the future was also erected in a strategic place in the school grounds. A poster-making contest among schoolchildren was also conducted along with the teachers' bulletin board and jingle presentation contest per grade level with the winners announced in the Treatment Day program.

Mass Treatment Day was held on 5 October 1999 participated in not only by the school community and the project team but also by local officials, the parish priest, the PTA officers, and a representative from Janssen Pharmaceutica. Mebendazole (Antiox) 500 mg chocolate flavored tablets were distributed to classroom teachers by the project team, and teachers administered treatment to pupils with parents' consent overseen by the project team. Teachers recorded and reported who among their pupils were treated, and summary sheets indicating coverage were submitted. Treatment coverage rates were determined, while drug use and inventories were taken note of.

A second Mass Treatment Day was held in February 2000, six months after the first, but only for children who were randomly selected to receive two doses of mebendazole in a year in the indicator grade. Children who were to receive only one dose of the drug were given placebo tablet, a type of candy that simulated a tablet in appearance. A third Mass Treatment Day was held in August 2000 for all consenting pupils in all grade levels.

Adverse experiences were monitored during each Treatment Day and recorded across the name of each pupil who experienced such on the teacher's class list. Teachers referred each pupil experiencing an adverse event to the medical team/project staff for further assessment and appropriate management. An Adverse Experiences Recording Sheet was accomplished per pupil referred. A guide was devised to help the project staff in assessing the severity and causality of the adverse experience.

Parasitologic Assessment

Stool specimens were collected from all consenting third grade pupils at Day 0 (baseline or pre-treatment 1), Day 7 to 14 (post-treatment 1), Day 180 (+/- 14 days, pre-treatment 2), Day 187 to 194 (post-treatment 2), Day 360 (+/- 14 days, pre-treatment 3), Day 367 to 374 (post-treatment 3). Specimens were processed using Kato Katz method that allowed qualitative assessment of infection

according to species that allowed derivation of infection rates and quantitative assessment that allowed estimation of burden of infection through the number of eggs per gram (epg) of feces. Intensity of helminth infection per pupil was assessed as light, moderate or heavy, according to the Classes of Intensity proposed for use by the WHO Expert Committee in 1987 (Prevention and Control of Intestinal Parasitic Infections: Report of a WHO Expert Committee, WHO, 1987).

Post-treatment stool examinations allowed assessment of response at the end of therapy as one of the following:

- 1) Clinical cure defined as the absence of helminth eggs in stool examinations at Day 7 to 14;
- 2) Clinical improvement defined as a reduction, compared to baseline, in stool egg counts in patients not cured in stool examinations on Day 7 to 14;
- 3) Clinical failure defined as no change compared with baseline in the number of helminth eggs in stool examinations on Day 7 to 14; and
- 4) Clinical outcome indeterminate when a valid assessment of clinical efficacy cannot be made due to poor patient cooperation or extenuating circumstances.

Efficacy measures consisted of cure rates and egg reduction rates (arithmetic and geometric). Reinfection data were also collected from pupils who were infected at baseline and were cured at Day 7 to 14 by doing parasitologic assessments at Days 180 and 360 (\pm 14 days).

Data Handling and Analysis

Pre-treatment infection rates (Days 0 and 180) were compared using McNemar Chi-square. The trend of infection rates (having at least one parasite), *Ascaris* and *Trichuris* infection rates of pupils in the two treatment schedules were compared using the Generalized Estimating Equations. The two treatment schedules being compared were once per year (mebendazole at Day 0, placebo at Day 180 and mebendazole at Day 360) and twice per year (mebendazole given at Days 0, 180 and 360).

Efficacy analysis included only those who satisfied the following criteria in all the follow-up periods: 1) pupils with parental consent; 2) pupils who were treated; and 3) pupils who were able to submit stool specimens for examination.

Cure rates at Day 7 to 14 and reinfection rates at Day 180 were described using proportions. At Day 187 to 194, cure rates were compared for pupils given once versus twice per year using chi-square test. The cure rates at Day 7 to 14 among the different intensity of infections were compared using Chi-square test of homogeneity. Reinfection rates at Day 360 were compared similarly. Fishers exact test was used when necessary.

Egg counts were described using arithmetic and geometric means. The egg counts were increased by 1 and transformed using log transformation. Mean log transformed egg counts of the once versus twice per year treatment were compared

using the repeated measures analysis of variance. The effect of time and its interaction with treatment regimen on egg counts were also assessed. If the time and treatment interaction was significant, tests of contrasts were performed to determine the follow-up periods by which a significant difference between the two treatment schedules was observed.

RESULTS

Mass Treatment - Consent Rates and Treatment Coverage

Consent rates (for mass treatment) were from 79.1% to 92.4% with an overall consent rate of 84.5%. Among 2454 pupils with consent, 2279 or 92.9% were treated. Treatment rates per grade level ranged from 89.9% to 96.5% among those with consent. The total number of consenting pupils who were treated represented 78.5% of the school population.

For the second round of mass treatment, only Grade III pupils were targeted for treatment and were randomly selected to receive once or twice yearly mebendazole. A total of 460 Grade III pupils still had consent from parents or guardians for inclusion in the second round of mass treatment. This represented 92.2% of the total Grade III population. This was not markedly different from the 92.4% consent rate achieved for the first round of mass treatment. In both instances, consent rates may be considered excellent. For the third round of mass treatment, overall consent rate was 90.9% which was an improvement of the 84.5% consent rate achieved almost a year earlier. Overall treatment coverage was 88.5%, which was likewise an improvement of the 79.1% treatment coverage achieved almost a year earlier.

Baseline Parasitologic Assessment (Pre-treatment 1)

Of the 499 pupils in the indicator group, 418 pupils or 83.8% submitted stool specimens for baseline parasitologic assessment. Of these, 329 pupils or 78.7% were positive for at least one intestinal helminth infection. Positivity rate per section ranged from 57.9% to 90.4%, with increasing positivity rate the lower the section. Of the 418 pupils who were examined, *Trichuris* was the most common intestinal parasite seen with 292 Grade III pupils infected (69.8%). The next most common intestinal parasite was *Ascaris* with 181 pupils or 43.3% in the same grade infected. Only 3 pupils or 0.7% were found to have hookworm, while only 4 pupils or 1.0% were found to have *Enterobius*.

Overall, majority of pupils infected with *Ascaris* (51.4%) were classified as having light intensity of infection with most pupils (84.0%) having light to moderate intensity. In the same manner, majority of pupils infected with *Trichuris* (51.7%) were classified as having light intensity of infection with most pupils (92.1%) having light to moderate intensity. Only 16.0% and 7.9% of pupils examined had heavy intensity *Ascaris* and *Trichuris* infections, respectively (Tables 1 and 2).

Table 1 Distribution of infected Grade III pupils with ascariasis according to intensity of infection* at baseline (Day 0) SVES, Biñan, Laguna, July 1999

Section	No. of pupils examined	No. of pupils infected (%)	Light		Moderate		Heavy	
			No.	%	No.	%	No.	%
1	57	19 (33.3)	18	94.7	1	5.3	0	0.0
2	62	20 (32.3)	10	50.0	7	35.0	3	15.0
3	65	25 (38.5)	12	48.0	11	44.0	2	8.0
4	63	35 (55.6)	17	48.6	10	28.6	8	22.9
5	68	32 (47.1)	14	43.7	11	34.4	7	21.9
6	51	22 (43.1)	13	59.1	6	27.3	3	13.6
7	52	28 (53.8)	9	32.1	13	46.4	6	21.4
Total	418	181 (43.3)	93	51.4	59	32.6	29	16.0

*Intensity of *Ascaris* infection:

Light: 1 - 4,999 epg

Moderate: 5,000 - 49,999 epg

Heavy: > 50,000 epg

Source: WHO Guidelines for the evaluation of soil transmitted helminthiasis and schistosomiasis at the community level, 1998

Table 2 - Distribution of infected Grade III pupils with trichuriasis according to intensity of infection* at baseline (Day 0) SVES, Biñan, Laguna, July 1999

Section	No. of pupils examined	No. of pupils infected (%)	Light		Moderate		Heavy	
			No.	%	No.	%	No.	%
1	57	20 (35.1)	17	85.0	3	15.0	0	0.0
2	62	43 (69.4)	26	60.5	16	37.2	1	2.3
3	65	47 (72.3)	27	57.4	18	38.3	2	4.3
4	63	43 (68.3)	20	46.5	19	44.2	4	9.3
5	68	51 (75.0)	23	45.1	23	45.1	5	9.8
6	51	42 (82.4)	23	54.8	14	33.3	5	11.9
7	52	46 (88.5)	15	32.6	25	54.3	7	14.8
Total	418	292 (69.9)	151	51.7	118	40.4	23	7.9

*Intensity of *Trichuris* infection:

Light: 1 - 999 epg

Moderate: 1,000 - 9,999 epg

Heavy: > 10,000 epg

Source: WHO Guidelines for the evaluation of soil transmitted helminthiasis and schistosomiasis at the community level, 1998

Arithmetic means of *Ascaris* and *Trichuris* eggs fell within the category of moderate intensity of infection. There was a tendency for arithmetic mean egg counts to be higher as the section became lower for both ascariasis and trichuriasis. Geometric mean egg counts for trichuriasis also had the tendency to be higher as the section became lower.

Follow-up Parasitologic Assessments

Day 7 to 14 (Post-treatment 1)

A total of 426 third grade pupils submitted stool specimens on the first follow-up (post-treatment 1, Day 7-14). This represented 85.4% of all Grade III pupils. Clinical cure was noted in 58.2% of those pupils treated with ascariasis, while clinical improvement was noted in 31.5%. Clinical failure was evident in 3.0% of pupils with ascariasis, while 7.3% of pupils did not follow up. Clinical cure was noted in only 37.2% of pupils with trichuriasis, while clinical improvement was noted in 45.2%. Clinical failure was seen in 7.7% of pupils with trichuriasis, while 9.9% of pupils did not follow up.

For pupils with ascariasis, overall cure rate was 62.7%. Cure rate was highest at 79.8% among those with light intensity of infection on baseline, while it was lowest at 21.7% among those with heavy intensity of infection on baseline. As intensity of infection at baseline increased, cure rate decreased. There was a significant difference in the cure rates for ascariasis with different levels of intensity of infection ($p=0.000$). For pupils with trichuriasis, overall cure rate was 41.3%. Cure rate was highest at 55.9% among those with light intensity of infection on baseline, while it was lowest at 6.3% among those with heavy intensity of infection on baseline. Similar to the findings in ascariasis, intensity of infection at baseline increased, cure rate decreased. There was a significant difference in the cure rates for trichuriasis with different levels of intensity of infection ($p=0.001$).

Overall, egg reduction rate (ERR) in pupils with ascariasis at baseline using arithmetic mean egg counts was 85.6%, while it was 70.7% among those with trichuriasis at baseline using arithmetic mean egg counts. With geometric mean egg counts, ERR was 90.8% for those with ascariasis and 90.1% for those with trichuriasis.

Day 180 (Pre-treatment 2) and Day 187 to 194 (Post-treatment 2)

The stool submission rate increased from 90.7% (pre-treatment 1) to 93.9% (pre-treatment 2). The infection rates for all sections in pre-treatment 2 were lower than the levels in pre-treatment 1. Positivity rates ranged from 57.9% to 90.4% (overall infection rate=78.7%) at pre-treatment 1, while at pre-treatment 2, it ranged from 16.1% to 77.0% (overall infection rate=55.8%). Overall infection rate 180 days after treatment was 29.1% lower than the baseline overall infection rate. Of 432 pupils who underwent parasitologic assessment prior to the next round of mass treatment (pre-treatment 2), 241 or 55.8% were found to be positive for at

least one infection. Of these, 226 (52.3%) had *Trichuris* and 63 (14.6%) had *Ascaris*. The proportion of *Ascaris* infections was 66.3% lower than that observed at baseline or pre-treatment 1, while the proportion of *Trichuris* infections was 25.1% lower than in pre-treatment 1. Three pupils (only 0.7%) had *Enterobius*, and there was no hookworm infection reported.

Majority of Grade III pupils with *Ascaris* infection per section, except section 3, had light intensity infections. Overall, 93.7% of these children were classified as having light to moderate intensity of *Ascaris* infection. Heavy intensity roundworm infection was seen in 6.3% of infected pupils. This was 60.6% lower than the proportion of heavy intensity infections at baseline. No pupil in section 1 was reported to harbor *Ascaris* infection. *Trichuris* infection was seen in 52.3% of those examined. A majority of infected pupils (66.0%) were classified as having light intensity infections with only 1.3% classified as heavy intensity infection. This represented an 83.5% reduction of the proportion of heavy intensity *Trichuris* infections at baseline.

Arithmetic and geometric mean *Ascaris* egg counts 180 days after treatment were 94.6% and 92.7% lower, respectively, compared to baseline levels. Arithmetic and geometric mean *Trichuris* egg counts 180 days after treatment were 24.3% and 79.0% lower, respectively, compared to baseline levels. Arithmetic mean egg counts for both helminth infections were classified as light intensity infections. Arithmetic mean egg count of *Ascaris* per section showed no distinct trend but the geometric mean egg count showed a tendency to be higher as the section became lower as seen also at baseline. For *Trichuris*, this tendency was observed both for the arithmetic and geometric mean egg counts but more clearly in the former. Reinfection rates at pre-treatment 2 for *Ascaris* per section were from 0.0% to 36.4% while for *Trichuris*, it ranged from 26.7% to 53.8%. There was no distinct trend in terms of reinfection rate and section. The overall reinfection rates 180 days after treatment were 14.4% and 38.1% for *Ascaris* and *Trichuris*, respectively.

Out of the 63 pupils infected with *Ascaris* at pre-treatment 2, 30 (47.6%) received a second round of mebendazole, 23 (36.5%) received placebo and the remaining 10 (15.9%) did not receive either because they were not present during the treatment day. On the other hand, of the 226 pupils with *Trichuris*, 105 (46.5%) received mebendazole, 88 (38.9%) received placebo and 33 (14.6%) did not receive either because they were not present during the treatment day. Among those who were given mebendazole, clinical cure was observed in 22 or 73.3% of those with ascariasis while 6 (20.0%) were found to show clinical improvement. One pupil was noted to have clinical failure and another one classified as clinical outcome indeterminate. Clinical cure was found in 45.7% of those pupils with *Trichuris* at pre-treatment 2 who received a second round of mebendazole. Forty-two pupils or 40.0% were observed to have had clinical improvement while 11.4% and 2.9% of pupils were classified as having clinical failure and clinical outcome indeterminate, respectively.

The ERRs for those pupils with ascariasis at pre-treatment 2 who received a second round of mebendazole were 78.8% using arithmetic mean egg counts and 99.9% using geometric mean egg counts. For those with trichuriasis, ERR was 83.7% using arithmetic mean egg counts and 96.9% using geometric mean egg counts.

Overall, the infection rates (positive for at least one parasite) in both treatment groups were much lower in post-treatment 1, pre-treatment 2 and post-treatment 2 as compared to their original levels in pre-treatment 1. It can also be noted that levels of infection in post-treatment 1 and pre-treatment 2 for both treatment groups were not much different. Levels of infection at post-treatment 2 showed that there was a big drop in the infection rates for those who received twice yearly treatment and practically not much change for those who received once-yearly treatment, as expected ($p=0.000$, for both ascariasis and trichuriasis).

Day 360 (Pre-treatment 3) and Day 367 to 374 (Post-treatment 3)

Overall, stool submission rate was 97.9%. Positivity rates ranged from 0.0% to 100.0%. The overall infection rate was 56.7%. Of the 423 pupils who were found to be infected at pre-treatment 3 follow-up, 210 (49.6%) were positive for *Trichuris* and 113 (26.7%) had *Ascaris*. These rates were 28.9% and 38.3% lower than those observed at pre-treatment 1. The infection rate for *Ascaris* at pre-treatment 3 was 82.3% lower than those observed at pre-treatment 2. On the other hand, the infection rate for *Trichuris* at pre-treatment 3 was 5.2% lower compared to pre-treatment 2. Two pupils (0.5%) had *Enterobius*. The other two pupils (0.5%) had hookworm. One (0.2%) was seen to have heterophyid egg.

Majority of pupils with *Ascaris* infection per section had light intensity infections. Overall, 47.8% of these children were classified as having moderate to heavy intensity *Ascaris* infection and 74.8% of pupils with *Trichuris* infection were classified as light intensity with only 0.9% classified as heavy intensity. Only sections 3 and 6 had pupils with heavy intensity infections at pre-treatment 2.

Arithmetic mean egg counts were classified as moderate intensity of infection. The arithmetic and geometric mean egg counts of *Ascaris* and *Trichuris* among pupils who were assigned in the once-a-year treatment group were classified as moderate and light intensity infections, respectively. On the other hand, the arithmetic and geometric mean egg counts of *Ascaris* and *Trichuris* among pupils who were assigned in the twice-a-year treatment group were classified as light intensity infections. Arithmetic and geometric mean egg counts of *Ascaris* per section showed no distinct trend. For *Trichuris* however, geometric mean egg count showed a tendency to be higher as the section became lower.

Pupils who belonged to the once-a-year group had higher infection rate having 58.4% of the pupils infected compared to those pupils who belonged to the twice-a-year group having only 51.2% of the pupils infected. Pupils who had a twice-a-year treatment schedule had 21.4% and 43.8% of the pupils infected with *Ascaris* and *Trichuris*, respectively. These were relatively lower than those pupils

who belonged to the once-a-year group that had 30.7% and 50.6% of the pupils infected with *Ascaris* and *Trichuris*, respectively.

Reinfection rates were not found to be significantly different between pupils belonging to once versus twice yearly treatment schedules for both ascariasis ($p=1.000$) and trichuriasis ($p=0.687$). Reinfection rates for *Ascaris* among pupils who were assigned in once-a-year and twice-a-year treatment schedules were 33.3% and 45.0%, respectively. Reinfection rates for *Trichuris* were seen among 55.6% of the pupils assigned in the once-a-year treatment group and 63.4% of the pupils assigned in a twice-a-year treatment group. At pre-treatment, among pupils with a once-a-year treatment schedule, 1 out of 3 pupils were reinfected with *Ascaris*, while 5 out of 9 pupils were reinfected with *Trichuris*. Among pupils in the twice-a-year treatment schedule, 9 out of 20 pupils (45.0%) were reinfected with, while 26 out of 47 pupils (63.4%) were reinfected with *Trichuris*.

Overall, among those who received treatment in August 2000, clinical cure was observed in 73.9% of pupils with *Ascaris* while 18.9% showed clinical improvement. Two pupils with clinical failure and 6 others with clinical outcome indeterminate were noted. For those pupils with *Trichuris*, clinical cure was observed in 57.1% of pupils while 29.8% showed clinical improvement. Fourteen pupils with clinical failure and 13 others with clinical outcome indeterminate were noted.

Among those who received once-a-year treatment, clinical cure was observed in 70.6% of those with ascariasis, while 19.6% showed clinical improvement. One pupil with clinical failure and 4 others with clinical outcome indeterminate were noted. For those pupils who received twice-a-year treatment, clinical cure was observed in 76.8% of those pupils having the same infection, while 16.3% showed clinical improvement. One pupil with clinical failure and 2 others with clinical outcome indeterminate were noted. Cure rates between the pupils in the two treatment schedules were not significantly different from each other ($p=0.178$). Among those who received once-a-year treatment, clinical cure was observed in 58.3% of those with trichuriasis, while 28.6% showed clinical improvement. Five pupils manifested with clinical failure, while 6 others with clinical outcome indeterminate were noted. For those pupils who received twice-a-year treatment, clinical cure was observed in 55.2% of those pupils having the same infection while 29.9% had improved conditions. Eight pupils with clinical failure and 5 others with clinical outcome indeterminate were noted. Cure rates between the two treatment schedules were not significantly different from each other ($p=0.667$).

The ERR for those pupils with ascariasis at pre-treatment 3 were 93.1% using arithmetic mean egg counts and 97.6% using geometric mean egg counts. For those with trichuriasis, ERR was 88.1% using arithmetic mean egg counts and 98.5% using geometric mean egg counts.

The ERR for those pupils with ascariasis who received once-a-year treatment were 84.3% and 99.9% using arithmetic and geometric egg counts, respectively. For those pupils who received twice-a-year treatment and had the same infection,

who belonged to the once-a-year group that had 30.7% and 50.6% of the pupils infected with *Ascaris* and *Trichuris*, respectively.

Reinfection rates were not found to be significantly different between pupils belonging to once versus twice yearly treatment schedules for both ascariasis ($p=1.000$) and trichuriasis ($p=0.687$). Reinfection rates for *Ascaris* among pupils who were assigned in once-a-year and twice-a-year treatment schedules were 33.3% and 45.0%, respectively. Reinfection rates for *Trichuris* were seen among 55.6% of the pupils assigned in the once-a-year treatment group and 63.4% of the pupils assigned in a twice-a-year treatment group. At pre-treatment, among pupils with a once-a-year treatment schedule, 1 out of 3 pupils were reinfected with *Ascaris*, while 5 out of 9 pupils were reinfected with *Trichuris*. Among pupils in the twice-a-year treatment schedule, 9 out of 20 pupils (45.0%) were reinfected with, while 26 out of 47 pupils (63.4%) were reinfected with *Trichuris*.

Overall, among those who received treatment in August 2000, clinical cure was observed in 73.9% of pupils with *Ascaris* while 18.9% showed clinical improvement. Two pupils with clinical failure and 6 others with clinical outcome indeterminate were noted. For those pupils with *Trichuris*, clinical cure was observed in 57.1% of pupils while 29.8% showed clinical improvement. Fourteen pupils with clinical failure and 13 others with clinical outcome indeterminate were noted.

Among those who received once-a-year treatment, clinical cure was observed in 70.6% of those with ascariasis, while 19.6% showed clinical improvement. One pupil with clinical failure and 4 others with clinical outcome indeterminate were noted. For those pupils who received twice-a-year treatment, clinical cure was observed in 76.8% of those pupils having the same infection, while 16.3% showed clinical improvement. One pupil with clinical failure and 2 others with clinical outcome indeterminate were noted. Cure rates between the pupils in the two treatment schedules were not significantly different from each other ($p=0.178$). Among those who received once-a-year treatment, clinical cure was observed in 58.3% of those with trichuriasis, while 28.6% showed clinical improvement. Five pupils manifested with clinical failure, while 6 others with clinical outcome indeterminate were noted. For those pupils who received twice-a-year treatment, clinical cure was observed in 55.2% of those pupils having the same infection while 29.9% had improved conditions. Eight pupils with clinical failure and 5 others with clinical outcome indeterminate were noted. Cure rates between the two treatment schedules were not significantly different from each other ($p=0.667$).

The ERR for those pupils with ascariasis at pre-treatment 3 were 93.1% using arithmetic mean egg counts and 97.6% using geometric mean egg counts. For those with trichuriasis, ERR was 88.1% using arithmetic mean egg counts and 98.5% using geometric mean egg counts.

The ERR for those pupils with ascariasis who received once-a-year treatment were 84.3% and 99.9% using arithmetic and geometric egg counts, respectively. For those pupils who received twice-a-year treatment and had the same infection,

ERR were noted to be 97.2% and 99.9% with arithmetic and geometric egg counts, respectively. For those pupils with trichuriasis, ERR among pupils who received once-a-year treatment were 89.8% and 98.7% using arithmetic and geometric mean egg counts, respectively. For those pupils who received twice-a-year treatment and had the same infection, ERR were noted to be 80.2% and 98.0% with arithmetic and geometric egg counts, respectively.

Efficacy Analysis

The number of pupils satisfying the criteria for efficacy analysis was 244 with 123 pupils randomized to the once-a-year treatment schedule and 121 pupils to the twice-a-year treatment schedule. With comparison of pre-treatment infection rates (Days 0 and 180), of 244 pupils considered, 194 (79.5%) were positive for at least one infection at Day 0. At Day 180, the positivity rate was 52.9% (129/244) which was significantly lower than the baseline positivity. (McNemar Chi-square = 50.9, $p < 0.01$). The overall trend in terms of infection rates in the two treatment schedules was found to have slight significant difference ($p = 0.055$), with a marked difference observed at Day 187-194 (Table 3).

The overall trend in terms of *Ascaris* infection in the two treatment schedules was found to have significant difference ($p = 0.048$) where infection rates in pupils in the twice yearly treatment schedule was significantly lower than those in the once yearly treatment schedule beginning at Day 194 onwards. The overall trend in terms of *Trichuris* infection in the two treatment schedules was not significantly different ($p = 0.097$) (Table 4).

As for the overall trend of *Ascaris* egg counts, repeated measures analysis of variance of transformed data showed that there was significant interaction between time effect and treatment schedule effect on log egg counts ($p = 0.04$). Test of contrasts showed the time periods when a significant difference between the two treatment schedules was seen. The test of contrasts showed that the change in the mean log egg counts from Day 0 to Day 194 in the twice yearly group is significantly higher compared to that of the once yearly group. There was an increase in mean log egg counts from Day 180 to Day 194 in the once yearly group while a decrease was observed in the twice yearly group. In both treatment schedules, there was an observed increase in the mean log egg count from Day 194 to Day 360, but the increase was not statistically significant. The mean log egg counts at Day 360 were lower than at baseline in both treatment schedules, but the decrease in the twice yearly group was significantly greater compared to the once yearly group (Tables 5 and 6).

As for the overall trend of *Trichuris* egg counts, repeated measures analysis of variance of transformed data showed that there was significant interaction between time effect and treatment schedule effect on log egg counts ($p = 0.01$). Test of contrasts showed the time periods when a significant difference in the two treatment schedules. The test of contrasts showed that the difference in the mean log egg counts at Day 194 and mean log egg counts in other follow-up periods in

Table 3. Overall trend of infection rates SVES, Biñan, Laguna
Days 0 to 374

Treatment group	Day 0	Day 7	Day 180	Day 194	Day 360	Day 374
Once-a-year (n=123)	95 (77.2%)	70 (56.9%)	68 (55.3%)	68 (55.3%)	69 (56.1%)	32 (26.0%)
Twice-a-year (n=121)	99 (81.8%)	59 (48.8%)	61 (50.4%)	33 (27.3%)	57 (47.1%)	27 (22.3%)

Table 4. Overall trend of *Ascaris* and *Trichuris* infection rates SVES, Biñan, Laguna
Days 0 to 374

Treatment group	Parasite	Day 0	Day 7	Day 180	Day 194	Day 360	Day 374
Once-a-year (n=123)	<i>Ascaris</i>	48 (39.0%)	37 (30.1%)	15 (12.2%)	19 (15.4%)	36 (29.3%)	11 (8.9%)
	<i>Trichuris</i>	87 (70.7%)	55 (44.7%)	63 (51.2%)	61 (50.0%)	58 (47.2%)	25 (20.3%)
Twice-a-year (n=121)	<i>Ascaris</i>	52 (43.0%)	25 (20.7%)	12 (9.9%)	6 (5.0%)	22 (18.2%)	5 (4.1%)
	<i>Trichuris</i>	85 (70.2%)	47 (38.8%)	56 (46.3%)	30 (24.8%)	49 (40.5%)	23 (19.0%)

Table 5. Arithmetic and Geometric mean* *Ascaris* and *Trichuris* egg counts over time SVES, Biñan, Laguna Days 0 to 374

Treatment group	Parasite	Day 0	Day 7	Day 180	Day 194	Day 360	Day 374
Once-a-year (n=123)	<i>Ascaris</i>	13000.9 (21.51)	2037.1 (5.50)	1307.7 (1.66)	2290.9 (2.77)	5512.6 (11.81)	1110.9 (0.92)
	<i>Trichuris</i>	1763.6 (116.2)	199.8 (7.3)	413.5 (20.0)	393.6 (18.8)	329.4 (14.6)	50.0 (1.6)
Twice-a-year (n=121)	<i>Ascaris</i>	10634.4 (27.22)	904.3 (2.21)	604.0 (1.10)	114.8 (0.36)	2836.4 (3.14)	54.9 (0.34)
	<i>Trichuris</i>	1975.7 (112.4)	176.3 (5.8)	446.7 (15.5)	85.9 (2.6)	273.1 (9.9)	44.4 (1.4)

*Arithmetic mean (Geometric mean)

Table 6. Overall trend of mean log *Ascaris* and *Trichuris* egg counts SVES, Biñan, Laguna Days 0 to 374

Treatment group	Parasite	Day 0	Day 7	Day 180	Day 194	Day 360	Day 374
Once-a-year (n=123)	<i>Ascaris</i>	3.11	1.87	0.98	1.33	2.55	0.65
	<i>Trichuris</i>	4.76	2.11	3.04	2.99	2.75	0.96
Twice-a-year (n=121)	<i>Ascaris</i>	3.34	1.16	0.74	0.31	1.42	0.29
	<i>Trichuris</i>	4.73	1.92	2.80	1.28	2.39	0.88

the once yearly group is significantly different from that in the twice yearly group. The change in the mean log egg counts from Day 0 to Day 194 in the twice yearly group is significantly higher compared to that in the once yearly group. The mean log egg count increased from Day 7 to Day 194 while the mean log egg count decreased in the twice yearly group. The mean log egg counts from Day 194 to Day 374 dropped in both treatment schedules, but the decrease was greater in the once yearly group (Tables 5 and 6).

DISCUSSION

This study has illustrated the development and testing of a model for school-based control of intestinal helminths utilizing existing school infrastructure in the delivery of mass treatment and health education. Crucial to the success of such an undertaking was the partnership that was forged between the project team and the schoolteachers under the guidance of school officials, among them, the District Supervisor, the School Principal, and the Department of Education (DepEd) Medical Officer. The project team initiated the building of ties with the local government officials. Although the municipal and barangay officials were represented in major events, the fruits of the relationship among such officials remained to be seen up to the time of closing of the project.

Acceptability of the mass treatment concept was shown by this study to be excellent which gives promise that teachers, parents and children may subscribe to and actually participate in mass deworming programs in the school. The necessary ingredients were proven to be adequate information dissemination and health education that aimed to correct misconceptions which led to unwise decisions in the past. Regular feedback of results and progress of the mass deworming campaign to the teachers and parents also may have contributed to sustaining interest in the program.

Possible areas of focus in terms of health education and promotion may have to be considered when initiating mass deworming programs in the school. For schoolteachers, their important role in the mass treatment process and health education must be emphasized. For parents, attempts to correct misinformation should be made in order to make possible an enlightened decision to allow their children to participate. For pupils, the ease of taking deworming drugs, their pleasant taste and the wonders of eliminating worms from their system are important issues to consider. In all, basic information on intestinal helminths, their modes of transmission, clinical manifestations, complications, treatment and control will be prerequisites of a good understanding of the problem and a wise decision making.

Morbidity is directly related to worm burden. The greater the number of worms in an infected person, the greater will be the morbidity caused by the worm. Helminth infection and disease adversely affect child growth and development, nutritional status and cognitive capacity. These effects have also been shown to be proportionally associated with worm burden.

The first objective of a control program is to reduce morbidity. This is done by reducing the proportion of heavily infected individuals in the target population. Heavily infected individuals suffer most of the clinical consequences of the infections and are the major sources of infection for the rest of the community, although their proportion in the community may be small. Lightly infected individuals have minimal health consequences.

Although a majority of schoolchildren monitored in this study had light to moderate intensities of infection, infection rates were nonetheless high which meant that the proportion of infected individuals or reservoirs was high. This could probably be explained by long-standing high transmission due to high prevalence of infections in a background of poor environmental sanitation. There was a need therefore to aim for reductions of worm prevalence through mass treatment which was the subject of this research and intervention initiative.

Chemotherapy, health education and improvements in sanitation are the major components of an intestinal helminth control program. Chemotherapy is aimed at reducing worm burden and decreasing transmission. Health education is aimed at encouraging healthy behavior. Lastly, improvements in sanitation are aimed at reducing soil or water contamination.

Mass chemotherapy was the primary component of this project. Mass treatment is recommended in areas where more than 50% of the population are infected with soil-transmitted helminths. Mebendazole is one of only a few drugs that are recommended by WHO for treatment. These safe and effective drugs have been used widely in the past several years. Mebendazole was given as mass or targeted treatment aiming to cover the highest percentage of target individuals like school children, one of the main high-risk groups in the community. Targeted treatment is the strategy which is recommended in areas where prevalence is high and where intensity is noted to be light. Such an area is typified by San Vicente Elementary School.

Crucial to mass or targeted treatment will be issues of coverage and frequency. The higher the coverage, the higher is the likelihood of being able to eliminate more worms, thereby reducing as much morbidity as possible. Although the efficacy of the measure is higher if the whole target population is treated simultaneously, there may be a need to determine the ideal or minimum coverage that will result in a significant reduction of worm burden. Frequency is another important issue related to chemotherapy. Mass treatment given two to three times per year may be necessary in areas where high intensities of infections exist. In this study, the benefits of targeted mass treatment given two times a year has been shown to be superior over once-a-year treatment. In the future, attention may be given to operational research that will help to determine ideal treatment intervals, frequency and end point of treatment as well to explore age-targeting and other related issues.

Health education may have proven to be an important component of this study. In areas where targeted or mass treatment will be implemented, submission to treatment and monitoring will be necessary to ensure the success of the control

program. The community preparation through meetings with school and local officials as well as with parents and guardians may have proven to be beneficial in attaining high consent rates and treatment coverage. The schoolteachers proved to be essential partners in this undertaking. From securing consent from parents and guardians, sharing important information that would correct misconceptions, helping with administration and inventory of drugs as well as recording treatment accomplishments per class, the schoolteachers surely provided invaluable services that helped to ensure desirable outcomes. Their innate creativity in the teaching-learning process should be maximized to help in the delivery of important messages that in turn will lead to right decision making and submission to treatment.

Among the components of an intestinal helminth control program, improvements in sanitation may be the most difficult to accomplish since poor socioeconomic conditions may be a limiting factor in achieving such improvements. Families, however, can be important resources that may help improve sanitation and reinforce school education activities. Improved sanitation, including proper disposal of human waste, provision of safe water supplies, and personal and food hygiene greatly contributes to reduction of diseases spread through human feces.

Control programs are designed to reduce worm burdens. Children will become reinfected, but they will have much less worms for longer periods throughout the year if periodic mass treatment will be continued. In the short-term and long-term, periodic mass treatment will have a positive effect on their health.

School-based intestinal helminth control programs show enough promise for reducing morbidity and transmission. To date, a number of countries have initiated such programs that have offered opportunities for improvement of the quality of life of school children. Results of a number of operational researches have shown advancement in knowledge in terms of the type of interventions to be administered, the frequency of application of intervention, the logistic and financial support required, and the collaborations and partnerships that lead to successful implementation and sustainability of the intestinal helminth control program.

The school system in developing countries may provide an efficient means of reducing health problems that are caused by helminth infections. Schools may be able to help in the delivery of interventions with potentially sustainable results. In most communities, there are more schools than health centers and more teachers than nurses. Teachers may play an important role as health educators and as facilitator of community actions to improve sanitary conditions.

Helminth control interventions in schools may benefit the entire community. Children with heavy worm infections are more likely to contaminate the environment, thus increasing the risk of transmission to others. Effective worm control efforts in the schools can help reduce the spread of helminth infections within the community. Indeed, repeated treatments involving the most heavily infected like the pre-school and school-age children have helped to lower the prevalence of soil-transmitted helminth infections in the whole community. In addition, health education provided to the children on intestinal helminths may

also serve as a means to inform families and other community members about ways to reduce helminth infections and prevent reinfections.

This study, probably the first well-monitored school-based intestinal helminth control program in the Philippines, has attempted to document the impact of this program in terms of standard indicators. The results of this undertaking will certainly help in guiding current and future practice as well as future applications and research. It is most beneficial to document milestones and lessons learned including problems encountered and possible approaches to these.

In a study area with an epidemiologic situation like SVES, twice yearly deworming has been shown to be superior to once yearly deworming. The questions that remained to be answered are: in the future, how frequent and up to when? Monitoring may help to answer these two important issues that need to be settled. The project team recommends limited monitoring as recommended by the WHO that will consider selection of from 40 to 50 pupils in the third grade to be monitored during pre-treatment periods over a few years. The parasitologic status of this indicator group of pupils will provide a "window" through which policy- and decision-makers will be able to appreciate progress of the mass deworming program. Significantly lowered infection rates and intensities of infection may signal the need to decrease the frequency of mass deworming to once a year, and later to shift to selective treatment.

The WHO sees monitoring as an integral part of control programs themselves. This will be essential in ensuring that programs are run effectively and efficiently by health and school authorities and that maximal benefit is attained by infected individuals, their families and their communities (WHO, 1999).

ACKNOWLEDGEMENTS

The members of the project team wish to express their sincerest gratitude to Johnson & Johnson (Philippines), Inc. and Janssen Pharmaceutica for providing the resources that made this important study possible. Special thanks to the Department of Education Division of Schools Laguna, District of Biñan for allowing the project to be conducted in San Vicente Elementary School (SVES), Biñan, Laguna. The support and cooperation of the schoolteachers from SVES under the leadership of Mrs Serenidad Ledesma, Principal; and Ms Romana Espinosa, District Supervisor, are deeply acknowledged. Finally, the members of the project team express their appreciation for the assistance provided by Mr John Mark Galang, Ms Paulyn Jean Acacio and Mr Mac Gerald Cueto.

REFERENCES

- Albonico M., P.G. Smith, E. Ercole, H.M. Chwaya, K.S. Alawi, and L. Savioli. 1994. A randomized controlled trial comparing mebendazole 500 mg and albendazole 400 mg against *Ascaris*, *Trichuris* and hookworms: 4 and 6 months follow-up. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 88:585-589.

- Bundy D A P., A. Hall, G.F. Medley, L. Savioli. 1992. Evaluating measures to control intestinal parasitic infections. *World Health Statistics Quarterly*, 45:168-179.
- Drummond, MF *et al.* 1990. *Methods for the Economic Evaluation of Health Programmes*. Oxford University Press, New York.
- Nokes, C *et al.* 1992. Moderate to heavy infections of *Trichuris trichiura* affect cognitive function in Jamaican school children. *Parasitology*, 104:539-547.
- SAS-Stat Version 6, 4th Edition, Vol. 2. 1990 SAS Institute Corporation, Cary, North Carolina.
- Savioli L., D. Bundy, and A. Tomkins. 1992. Intestinal parasitic infections: a soluble public health problem. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 86:353-354.
- Stata Reference Manual, Release 6, Vol. 4, 1999. Stata Press, Stata Corporation, Texas
- WHO. 1987. *Prevention and Control of Intestinal Parasitic Infections. Report of a WHO Expert Committee*. Geneva, World Health Organization, 1987 (WHO Technical Report Series No 749)
- WHO. 1994. *Bench Aids for the Diagnosis of Intestinal Parasites*. Geneva, World Health Organization
- WHO. 1995. *Physical Status: The Use and Interpretation of Anthropometry. Report of the WHO Expert Committee*. Geneva, World Health Organization.
- WHO. 1996. *Report of the WHO Informal Consultation on the Use of Chemotherapy for the Control of Morbidity due to Soil-Transmitted Nematodes in Humans (WHC/CTD:SIP/96.2)*. Geneva, World Health Organization.

DETECTION OF CHIKUNGUNYA VIRUS FROM SERA OF DENGUE-SUSPECTED PATIENTS IN THE PHILIPPINES

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ABSTRACT

Due to the similar symptoms with dengue fever (DF) and dengue hemorrhagic fever (DHF), Chikungunya (CHIK) virus infection has been diagnosed as DF or DHF. In this study, we examined more than 300 serum samples collected from dengue suspected patients in San Lazaro Hospital and St. Luke's Medical Center. Reverse transcriptase-polymerase chain reaction (RT-PCR) and antigen sandwich enzyme-linked immunosorbent assay (ELISA) were applied for CHIK virus detection. IgM-indirect immunofluorescence antibody test (IgM-IFA), IgM capture ELISA and IgG indirect ELISA were applied for detection of serological evidence of CHIK virus infection. Although we could not detect any Chikungunya virus by RT-PCR, 4.1% of the examined samples showed high titer for Chikungunya virus by antigen sandwich ELISA. By

IgM-FA, 15.3% was positive for CHIK virus. Thirteen point seven percentages showed positive for CHIK virus by IgM-capture ELISA. Eighteen point five percentages showed positive for CHIK virus by IgG indirect ELISA.

Key words: Chikungunya (CHIK) virus, dengue virus, dengue fever (DF), dengue hemorrhagic fever (DHF), RT-PCR, antigen sandwich ELISA, IgM-IFA, IgM capture ELISA, IgG indirect ELISA, Philippines

INTRODUCTION

Chikungunya (CHIK) virus belongs to genus *alphavirus* in the family *Togaviridae*. CHIK virus infection is one of the mosquito-borne diseases. It is transmitted by mosquito, *Aedes aegypti* and *Aedes furcifer-taylori* in Africa. India and southeast Asia including the Philippines (Hayes *et al.*, 1986). Due to the similar symptoms with dengue fever (DF) and dengue hemorrhagic fever (DHF), CHIK virus infection has been diagnosed as DF or DHF (WHO, 1997). CHIK virus infection sometimes caused outbreak (Carey *et al.*, 1969; Thakruea *et al.*, 1997) and also disappeared 7 to 8 years to several decades (Burke *et al.*, 1985; Pavri, 1986).

Previously, Basaca-Savilla reported that 6.94% of 55 clinically diagnosed influenza cases had HI antibody for CHIK virus and 1.5% of 67 children aged 0 to 14 years had HI antibody for CHIK virus in the Philippines (1966). Three cases of CHIK virus infection was diagnosed by IgM capture ELISA; the CHIK virus was also isolated from one of the patients in June and November 1985 and in January 1986 in the Philippines (Hayes *et al.*, 1986).

The aim of our study was to clarify the impact of CHIK virus infection among suspected DF/DHF patients in the Philippines. We examined more than 300 serum samples that were collected from dengue suspected patients in San Lazaro Hospital and St. Luke's Medical Center. Reverse transcriptase-polymerase chain reaction (RT-PCR) and antigen sandwich enzyme-linked immunosorbent assay (ELISA) were used for CHIK virus detection. IgM-indirect immunofluorescence antibody test (IgM-IFA), IgM capture ELISA and IgG indirect ELISA was applied for detection of serological evidence.

PATIENTS AND METHODS

Patients

All patients had been admitted to the San Lazaro Hospital or St. Luke's Medical Center as suspected DF or DHF patients. The clinical condition was graded by medical staff according to World Health Organization guidelines (WHO, 1986).

Virus and cells

C6/36 *Aedes albopictus* cells (Igarashi, 1978) were infected with CHIK virus (Kenyan Strain). CHIK virus was plaque purified three times using baby hamster kidney (BHK-21) cells.

RT-PCR

Serum separated from blood sample was inoculated to C6/36 cells then incubated 7 days for dengue virus and CHIK virus detection. Viral RNA was extracted from 100 µl of infected culture fluid (ICF) of C6/36 cells by 300 µl of Trizol-LS (Gibco BRL, Grand Island, N.Y.) and 80 µl of chloroform. The mixture was centrifuged for 10 min at 14,000 x g, the aqueous phase was mixed with equal volume of 2-propanol, centrifuged for 15 min at 14,000 x g for 15 min for RNA precipitation. The RNA precipitate was washed once with 75% ethanol, and re-suspended in 20 µl of RNase-free water. The complementary DNA (cDNA) synthesis was performed for 30 min at 37 °C in 1.5 ml tube containing 20 µl of RNA template, 200 units of reverse transcriptase (SuperScript™ II RT, Gibco-BRL), 50 µM of Random hexamer (Takara, Kyoto, Japan), RNase inhibitor (40 unit, 5 Prime-3 Prime, Inc. Boulder, CO), 1 x conc. reaction buffer and 0.2 mM of dNTP (Takara) in a 40 µl reaction volume. Oligonucleotide primers (CHIK/NS1/S and chik/ns1/c) for PCR were chosen from aligned nucleotide sequences of the nonstructural protein 1 (NS1) gene of CHIK and O'Nyong-nyong (ONN) viruses (Hasebe, personal communication). The PCR was performed in 0.2 ml thin-walled tube containing 5 µl of cDNA template, 1.25 units of Taq DNA polymerase (Amersham Pharmacia biotech, Arlington Heights, IL), 1 x conc. reaction buffer and 0.2 mM of dNTP (Takara), 1 µM of sense primer and 1 µM of antisense primer in a 50 µl reaction volume. The condition for PCR was at 94 °C for 3 min followed by incubation at 94 °C for 1 min, 54 °C for 1 min 30 sec, 72 °C 2 min for 25 cycles in a model 2400 thermocycler (Perkin-Elmer Corp., Norwalk, CT). The last elongation step at 72 °C was increased 5 min to ensure complete extension of the PCR products.

Antigen sandwich ELISA

The antigen sandwich ELISA procedure selected was similar to that reported by Thant et al. (1995) with the following modifications. The 96-well flat bottom micro plate (Nalge Nunc International, Roskilde, Denmark) was coated overnight at 4 °C with 100 µl of capture antibody (256 ELISA units). The capture antibody was prepared from CHIK virus hyperimmunized rabbit. Each well was inactivated with 100 µl of Blockace (Yukijirushi, Japan) for 60 min at 37 °C, washed 3 times with PBS-0.05% Tween 20 for 3 min each and drained. After emptying and washing the plate, 100 µl of sample ICF and positive standard antigen were added in each of duplicate wells and incubated 60 min at 37 °C. Positive standard antigen (80 ELISA units) was prepared by inoculation of CHIK virus to BHK-21

cells in 5 days incubation at 37 °C. After the plate was washed, 100 µl of detection antibody (2000 ELISA units) was added in each well, then incubated for 60min at 37 °C. The detection antibody was selected from anti-CHIK virus IgG high titer human sera by CHIK virus IgG indirect ELISA. The plate was washed, and then reacted with 100 µl of horseradish peroxidase (HRPO)-conjugated anti-Human IgG goat serum (1:3000 dilution, Zymed Laboratories, Inc., So. San Francisco, CA). After 60 min incubation at 37 °C, the plate was washed, and 100 µl of substrate solution containing o-phenylenediamine dihydrochloride (OPD) and 0.02% hydrogen peroxide added. After 60 min incubation at room temperature in the dark, the reaction was stopped by adding 100 µl of 1N hydrochloric acid to each well. The optical density (OD) of the sample ICF of antigen sandwich ELISA were measured at 492 nm by an ELISA reader (SpectraMAX190, Molecular Devices Co., Sunnyvale, CA) exceeded positive standard antigen of that were considered as high titer.

IgM-IFA

The IgM-IFA procedure selected was similar to that reported by Henchal et al. (1982) with following modifications. Uninfected and infected BHK-21 cells mixed were at 2:3 ratio, then spotted on 8 well fluorescent antibody slides (ICN Biomedicals, Inc., Aurora, OH) 10µl of cell suspension in each well. Before staining, each slide was inactivated with Blockace (Yukijirushi, Sapporo, Japan) for 60 min at 37 °C, washed 3 time with PBS for 5min each and drained. Ten µl of the test and control sera diluted 1:10 with PBS-2% normal goat serum (Dako A/S, Denmark) were added to each well and incubated for 60 min at 37 °C in a humid chamber. After washing the slides as described above, the cells were reacted with 10 µl of FITC-conjugated anti-human IgM (µ chain specific) goat serum (MBL, Nagoya, Japan) diluted 1:800 with PBS-2% normal goat serum and incubated for 60min at 37 °C in humid chamber. After washing the slides as described above, mounted with FluoroGuard™ Antifade Reagent (BioRad, Hercules, CA) and sealed with nail polish. The slides were viewed with 20 x or 40 x objectives using a fluorescent microscope Axiolab (Carl Zeiss, Germany).

IgM capture ELISA

The IgM capture ELISA procedure used was similar to that reported by Bundo and Igarashi (1985) with following modifications. The 96-well flat bottom micro plate (Nalge Nunc International) was coated with 100 µl of goat anti-human IgM (µ-chain specific, 5 FC units, Cappel/ICN Pharmaceuticals, Inc. Aurora, OH) overnight at 4 °C. Each well was inactivated with 100 µl of Blockace (Yukijirushi) for 60 min at 37 °C, washed 3 times with PBS-0.05% Tween for 3 min each and drained. After emptying and washing the plate, 100 µl of the test and control sera diluted 1:100 with Blockace containing 10% serum bovine CADET (ICN Biomedicals, Inc.) were added into each duplicate wells and incubated for 60 min

at 37 °C. The wells on the plate were washed and 100 µl of CHIK virus antigen (64 ELISA units, prepared in C6/36 cells) was added and incubated for 60 min at 37 °C. After washing, the wells were reacted with 100 µl of CHIK virus hyperimmunized rabbit serum (262 ELISA units). The plate was incubated for 60 min at 37 °C, washed, and then reacted with 100 µl of HRPO-conjugated anti-rabbit IgG rat serum (1:1000 dilution, Zymed Laboratories, Inc.). After 60 min incubation at 37 °C, the plate was washed, and added 100 µl of substrate solution containing OPD and 0.02% hydrogen peroxide. After 60 min incubation at room temperature in the dark, the reaction was stopped by adding 100 µl of 1N hydrochloric acid. The OD at 492 nm was measured by an ELISA reader. A positive : negative (P:N) ratio was obtained by dividing the OD₄₉₂ of the test specimen by OD₄₉₂ of the negative standard. The specimen showing P:N ratio greater than or equal to 2.0 was considered positive, provided the standard positive specimen definitely showed positive result. Negative standard serum was selected from CHIK IgM IFA and CHIK IgG indirect ELISA double negative samples.

IgG Indirect ELISA

The IgG indirect ELISA procedure selected was similar to that reported by Bundo et al. (1981) with following modifications. The 96-well flat bottom micro plate (Nalge Nunc International) was coated with 100 µl of CHIK virus antigen (20 µg/ml) overnight at 4 °C. CHIK virus antigen was prepared by ultracentrifugation using Sucrose gradient. Each well was inactivated with 100 µl of Blockace (Yukijirushi) for 60 min at 37 °C, washed 3 times with PBS-0.05% Tween 20 for 3 min each and drained. After emptying and washing the plate, 100 µl of the test sera diluted 1: 100 with Blockace containing 10% serum Bovine CADET (ICN Biomedicals, Inc.) and two fold serially diluted positive standard serum were added into each duplicate wells and incubated for 60 min at 37 °C. The plate was washed, and then reacted with 100 µl of HRPO-conjugated anti-Human IgG goat serum (1:3000 dilution, Zymed Laboratories, Inc.). After 60 min incubation at 37 °C, the plate was washed, and added 100 µl of substrate solution containing OPD and 0.02% hydrogen peroxide. After 60 min incubation at room temperature in the dark, the reaction was stopped by adding 100 µl of 1N hydrochloric acid. The OD at 492 nm was measured by an ELISA reader. The titers of the samples were determined by comparison with serially diluted positive standard. The titer greater than or equal to 100 was considered as positive.

RESULTS AND DISCUSSION

RT-PCR

No positive sample was found in 315 samples examined by CHIK RT-PCR using CHIK NS1 primers. We also examined 44 of cDNA samples for PCR using Toga-alphavirus consensus primers (M2W and cM3W) which can widely amplify

alphaviruses (Pfeffer et al., 1997), also could not find any positive sample. The isolation of arboviruses requires several times of blind passages in suckling mice or in cell lines (Pfeffer et al., 1997). Therefore, in case of low virus titer due to slow virus growth or low adaptation to C6/36 cells, it might be difficult to detect the presence of the virus by RT-PCR.

Antigen sandwich ELISA

Six hundred forty ICF samples were examined by CHIK antigen ELISA to detect the existences of CHIK virus protein. The OD of 26 ICF samples showed greater than or equal to that of positive standard ICF. As a threshold value of antigen sandwich ELISA, other researchers used much lower criteria such as a mean absorbance of 50 wells for supernatant from uninfected C6/36 mosquito cells or BHK-21 cells (Greiser-Wilke et al., 1991) or more than a two fold ELISA-OD above that of the negative control (Pandey et al., 1999). Table 1 shows the increase of high titer samples in rainy season in the Philippines, 1999. Therefore, there is a possibility that CHIK virus infection occurred from August to December in 1999.

IgM-IFA

Five hundred ninety serum samples were examined by IgM-IFA to detect the existence of IgM against CHIK virus. Ninety (15.3%) were positive (Table 2). Using the same samples, 302 (51.2%) were only positive for dengue, 44 (7.5%) were double positive for dengue and CHIK by either RT-PCR or IgM capture ELISA (Matias et al., 2000).

IgM capture ELISA

Three hundred forty three serum samples were examined with IgM-IFA. Forty seven (13.7%) were found to be positive (Table 2). Using the same samples, 157 (45.8%) were only positive for dengue, 19 (5.5%) were double positive for dengue and CHIK by either RT-PCR or IgM capture ELISA (Matias et al., 2000). To determine P:N ratio, we arbitrarily chose negative standard serum among CHIK IgM-IFA and CHIK IgG indirect ELISA double negative samples. Therefore, there is a possibility of existence of false positives and false negatives in the above described results. Further experiments for selection of negative standard such as hemagglutination inhibition test or some other serological test will be required. Although the results of IgM-IFA and IgM capture ELISA were similar (Table 2), the association between these two tests was not statistically significant (Chi-square (χ^2) value: 0.47, degrees of freedom (df): 1, p value: $0.40 < p < 0.50$) due to unknown reason.

Table 1. Monthly trend of CHIK virus appearance in 1999 by CHIK antigen sandwich ELISA.

Month	Total	<0.2*	0.2~0.5*	0.6~0.9*	1.0~1.3*	1.3<*
January	59	59	0	0	0	0
February	32	32	0	0	0	0
March	17	16	1	0	0	0
April	33	33	0	0	0	0
May	44	43	1	0	0	0
June	70	68	2	0	0	0
July	62	58	4	0	0	0
August	127	40	64	13	10	0
September	92	53	18	12	9	0
October	37	25	8	3	0	1
November	50	31	11	3	2	3
December	17	11	5	0	0	1
Total	640	469	114	31	21	5

*—Titer of sample OD492/ positive standard OD492 was indicated.

Table 2. The results of serological examinations for CHIK virus infection.

Type of examination	Positive	Negative	Total
IgM-IFA*	90 (15.3%)	00 (84.7%)	590 (100%)
IgM capture ELISA*	47 (13.7%)	296 (86.3%)	343 (100%)
IgG indirect ELISA	91 (18.5%)	401 (81.5%)	492 (100%)

*--The association between these two tests was not statistically significant.

Chi-square (χ^2) value: 0.47, degrees of freedom (df): 1, p value: 0.40<p<0.50.

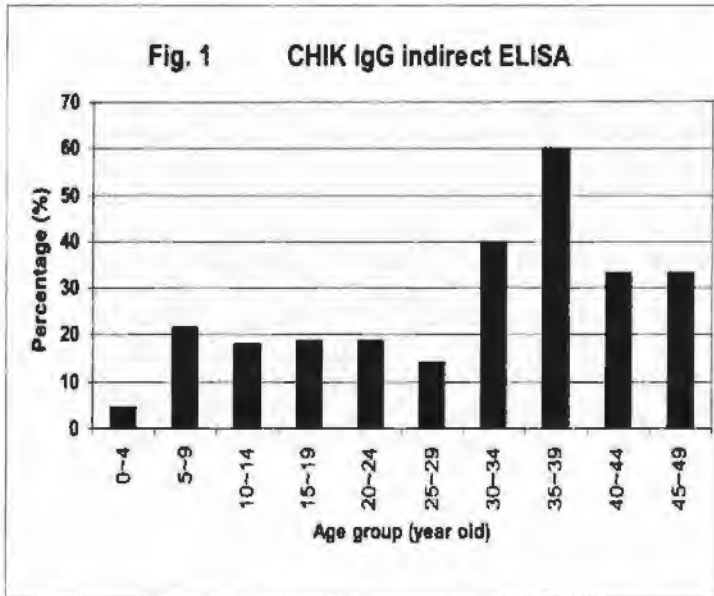
IgG indirect ELISA

To detect the existence of IgG against CHIK virus, 492 serum samples were examined by IgG indirect ELISA. Ninety one (18.5 %) were positive (Table 2). Fig. 1 shows that IgG positive ratio was constantly around 20% except for 0 to 4 and 25 to 29 year old groups. Aside from antigen sandwich ELISA, examinations for antigen detection such as RT-PCR, plaque assay, one-day old mice inoculation did not show any clear positive result (data not shown). As a another approach, blind passages in C6/36 or other cell lines (BHK-21, Vero), hemagglutination test of ICF and IFA for antigen detection using serum sample inoculated cell lines might be worthwhile to perform.

The results of IgM-IFA and IgM capture ELISA indicated that at least 7 to 8 % of DF/DHF suspected patients was only positive for CHIK virus. The results of antigen ELISA and IgG indirect ELISA also support the existence of CHIK virus infection in the Philippines. Therefore, careful and continuous monitoring of appearance of CHIK virus infection throughout the year and every year will be important to determine whether it only appeared during the rainy season of 1999 or not. If CHIK virus infection constantly appears every year in the Philippines, the introduction of vaccination for CHIK virus might be one of the effective solutions. In the U.S.A., researchers have already developed a live attenuated vaccine strain (CHIK 181/clone 25) for CHIK virus infection (Levitt et al., 1986). As part of the safety tests of the CHIK virus vaccine, CHIK 181/clone 25, Turell and Malinoski examined the transmissibility of the vaccine virus from vaccinated monkeys to a second vertebrate host by mosquito (1992). They also examined the possibility of its reversal to a more virulent form, not only in the original immunized vertebrate host, but also in an arthropod vector (1992). Fortunately unlike DF/DHF, immune enhancement phenomenon has never been reported in CHIK virus infection. Therefore the introduction of CHIK virus vaccine might be much more acceptable to the society where CHIK virus infection and DF/DHF coexist, than that of the vaccine for DF/DHF at this time.

ACKNOWLEDGEMENTS

We thank all the staff in the Research and Biotechnology Division, St. Luke's Medical Center for their technical assistance. The work was supported by St. Luke's Medical Center grants to dengue research project (95-001) and Chikungunya research project (00-008). This study was partially supported by the budget of the Department of Virology, Institute of Tropical Medicine, Nagasaki University by Grant in Aid for Scientific Research Nu. 07459098 from the Ministry of Education, Science, Sports and Culture of Japan.



LITERATURE CITED

- Basaca-Sevilla, V. (1966). Recent virological studies of Philippine haemorrhagic fever. *Bulletin of the World Health Organization*, 35, 58-59.
- Bundo, K., Matsuo, S. and Igarashi, A. (1981). Enzyme-linked immunosorbent assay (ELISA) on Japanese encephalitis virus. II. Antibody levels in the patient sera. *Trop. Med.*, 23, 135-148.
- Bundo, K. and Igarashi, A. (1985). Antibody-capture ELISA for detection of immunoglobulin M antibodies in sera from Japanese encephalitis and dengue hemorrhagic fever patients. *J. Virol. Methods*, 11, 15-22.
- Burke, D.S., Nisalak, A. and Nimmannitya, S. (1985). Disappearance of Chikungunya virus from Bangkok. *Trans. R. Soc. Trop. Med. Hyg.*, 79, 419-420.
- Carey, D.E., Myers, R. M., DeRanitz, C. M., Jadhav, M. and Reuben, R. (1969). The 1964 Chikungunya epidemic at Vellore, South India, including observations on concurrent dengue. *Trans. R. Soc. Trop. Med. Hyg.*, 63 (4), 434-445.
- Greiser-Wilke, I., Moennig, V., Kaaden, O.-R. & Shope, R. E. (1991). Detection of Alphaviruses in a genus-specific antigen capture enzyme immunoassay using monoclonal antibodies. *J. Clin. Microbiol.*, 29 (1), 131-137.
- Hayes, C.G., O'Rourke, T. & Sarr, A. (1986). Chikungunya fever among U.S. Peace Corps volunteers-Republic of the Philippines. *M.M.W.R.*, 35 (36), 573-574.
- Henchal, E. A., Gentry, M. K., McCown, J. M. & Brandt, W. E. (1982). Dengue virus-specific and Flavivirus group determinants indicated with monoclonal antibodies by indirect immunofluorescence. *Am. J. Trop. Med. Hyg.*, 31 (4), 830-836.
- Igarashi, A. (1978). Isolation of a Singh's *Aedes albopictus* cell clone sensitive to dengue and chikungunya viruses. *J. gen. Virol.*, 40, 531-544.
- Leviitt, N. H., Ramsburg, H. H., Hasty, S. E., Repik, P. M., Cole, F. E. Jr. & Lupton, H. W. (1986). Development of an attenuated strain of chikungunya virus for use in vaccine production. *Vaccine*, 4, 157-162.

- Matias, R. R. & dengue study group. (2000). 22nd Annual Scientific Meeting, National Academy of Science and Technology, Philippines.
- Pandey, B. D., Karamanos, N., Cropp, B., Takagi, M., Tsuda, Y., Ichinose, A. & Igarashi, A. (1999). Identification of a flavivirus isolated from mosquitoes in Chiang Mai Thailand. *South-east Asian J. Trop. Med. Public Health*, 30 (1), 161-165.
- Pavri, K. (1986). Disappearance of chikungunya virus from India and South East Asia, *Trans. R. Soc. Trop. Med. Hyg.*, 80, 491.
- Pfeffer, M., Probstler, B., Kinney, R. M. & Kaaden, O.-R. (1997). Genus-specific detection of Alphaviruses by a semi-nested reverse transcription-polymerase chain reaction. *Am. J. Trop. Med. Hyg.*, 57 (6), 709-718
- Thaikrua, L., Charearnsook, O., Reanphumkamkit, S., Dissomboon, P., Phunjan, R., Ratchbud, S., Kounsang, Y. & Duranapiyawong, D. (1997). Chikungunya in Thailand: A re-emerging disease? *Southeast Asian J. Trop. Med. Public Health*, 28 (2), 359-364.
- Thant, K.-Z., Aye, K.-M. & Igarashi, A. (1995). Antigen production of dengue type 4 virus strains in *Aedes albopictus* clone C6/36 and Vero cell cultures. *Trop. Med.*, 37, 115-121.
- Turell, M. J., & Malinoski, F. J. (1992). Limited potential for mosquito transmission of a live attenuated chikungunya virus vaccine. *Am. J. Trop. Med. Hyg.*, 47 (1), 98-103.
- WHO (1986). In: *Dengue Haemorrhagic Fever: Diagnosis, Treatment and Control*. Geneva: World Health Organization.
- WHO (1997). In: *Dengue Haemorrhagic Fever. Diagnosis, Treatment, Prevention and Control*. Geneva: World Health Organization, 2nd edition, 13-23.

HUMAN RESETTLEMENT AS AN INTERVENTION IN COMMUNITY DEVELOPMENT

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ABSTRACT

This study focused on the Bagong Buhay resettlement in Nueva Ecija settled by displaced families from Pampanga and Zambales as a result of the Mt. Pinatubo eruption in June 1991. It analyzed the complexities and attendant characteristics of the resettlement process, its outcome and viability, the factors affecting such outcomes/viability, and the implications that may be useful in future resettlement planning, implementation, monitoring and evaluation.

The resettlement of Mt. Pinatubo victims as in the case of Bagong Buhay proved to be an important and powerful mechanism through which the displaced were assisted to rebuild their life anew. Results of the study revealed that resettlement was a complex, stressful, and often difficult process. The settlers had to adapt to a new and unfamiliar environment and at the same time create productive enterprises and new social organization to cope with the new situation.

The implications of the study point that resettlement must not be approached simply as a movement of the displaced to a new land but must be holistically approached as a development opportunity to mobilize the resources of the state, different government organization (GOs)/nongovernment organizations (NGOs), and the settlers themselves who are the main actors in the pursuit of development.

Keywords: resettlement, community development

INTRODUCTION

Resettlements have become part and parcel of the development process and pose major challenges to governments trying to promote strategies for economic growth and social change.

Resettlements caused by development projects (i.e. dam construction) as well as disasters continue to be of great concern to development planners and project designers. In the case of resettlements due to disaster, the Mt. Pinatubo experience in the Philippines brings to fore a more complex and painful type of resettlement. The Mt. Pinatubo eruption had caused adverse impact in terms of loss of life, social and economic disruption, and damage to property as well as the environment. The extent of damage caused by the volcanic eruption had forcibly led to massive relocation of the affected families as thousands abandoned their homes and moved to resettlement areas sponsored by the government.

In such a case, the task of re-creating a new and viable community becomes enormous as the people are faced with great uncertainties. The displaced are also faced with the task of developing entirely new production system as well as a coherent and viable patterns of community organization in an unfamiliar environment.

For these reasons, development projects that caused displacement of people from their own environment should be avoided or minimized or planned with utmost care. Since natural disasters, social disruption, and the implementation of development projects will continue to accompany future technological, social and economic change, further improvements in resettlement policies and strategies in planning, implementing, monitoring and evaluation and in the social science research on resettlement remains imperative. Likewise, while it is certain that more lands will be settled in the next decades, there is a need for policy makers and development workers to design effective forms of intervention in the resettlement process.

This research attempted to explore and achieve an understanding of the resettlement process and its effect on the displaced population and the social, economic, and environmental consequences of resettlement. The increasing concern for environmental sustainability requires that the consequences of resettlement be critically considered and that future project design includes plans to mitigate the negative environmental consequences associated with resettlement. Certainly there are lessons to be learned which becomes relevant in identifying situational factors likely to facilitate the success of new settlements and policy issues that need to be addressed in planning for future undertakings.

OBJECTIVES OF THE STUDY

This study was committed to meet the following objectives:

1. To describe and assess the process followed in establishing a resettlement community;
2. To assess the implications of the policies and strategies invoked by GOs and NGOs in the process of establishing a resettlement community;
3. To analyze the settlers' response to their resettlement with particular reference

- to their coping mechanisms as well as their receptiveness to development opportunities;
- 4. To determine the positive and negative outcomes of the resettlement process and factors affecting such outcomes;
- 5. To find out problems and critical issues in resettling the displaced people; and
- 6. To develop an alternative framework useful for the systematic planning, implementation, monitoring, and evaluation of resettlement.

METHODOLOGY

Conceptual Framework

The occupation of a new land by the displaced population and its gradual transformation into a community operating as a social system is a dynamic process shaped and influenced by changing conditions and several factors affecting it in a variety of ways.

The resettlement process encompasses the various stages of a dynamic transformation of a land into a new community commencing with planning, continues through the move and reinstallation of the resettlement area, adaptation to the new environment paving the way for economic and social development and ends with handing over and incorporation. The resettlement process thus indicates that the displaced population passes through development sequences as they move away from their old community and adapt to their new environment. It also indicates the settlers' coping mechanisms or the kind of response exhibited to their new situation, the environment as well as the opportunities for development.

The resettlement process necessitates the involvement of many actors with the inherent roles and functions in close partnership with one another, citing in particular the GOs, NGOs, and the settlers themselves. Resettlement building is always a major and complex feat of human creativity, receptiveness, and ability to adapt to and control natural environment (McMillan et al, 1992). The key factor in the resettlement process is its social actor - the settler himself or the population group at work. The settlers thus, take center stage not only because they are the direct beneficiaries of resettlement as a development intervention but also because their involvement is fundamental in the success or failure of the outcome of the resettlement process. The resettlement process is conditioned and determined by the roles and actions of the government with particular reference to its policies and strategies zeroing in on project design, resource mobilization, project planning, implementation, monitoring, and evaluation. Complementing this is the role of the NGOs with a particular task to play given its familiarity with the settler-beneficiaries and excellent position to directly link and collaborate with both the people and the government as well. It is put forward that the interface and collaboration between

the GO-NGO-Settlers will influence the nature and outcome of the resettlement process.

Resettlement as a process is in itself a process of change with the outcome either positive or negative. The outcome either social or economic at the individual level is influenced by a variety of socio-economic, communication related, and psychological factors. The outcome at the community level may be positive or negative focusing on land tenure, socio-economic and institutional development and environmental impacts. The outcome is also influenced by several factors such as the resource base of the community, budget allocation, scale of resettlement, and settlement management.

The viability of resettlement as a development intervention becomes the final outcome on the basis of the socio-economic and environmental consequences.

The hypothesized conceptual framework is presented in Fig. 1.

Research Instrument/Data Collection

This study was conducted in Bagong Buhay resettlement in Palayan, Nueva Ecija, Philippines involving 182 respondents.

Primary and secondary data were gathered in the conduct of the study. Personal interview was conducted to gather the primary data using an interview schedule involving the household heads as the main respondents of the study.

A participant observation technique was used to observe the settlement and its production patterns, non-farm activities, topography and climate in the area, patterns and manifestation of kinship and community support, manifestations of deforestation and soil erosion as well as the conservation practices employed, evidences of abandonment, and other activities and behavior which may be deemed useful and important to the study.

Primary and secondary data were also gathered from key informants particularly project staff from GOs/NGOs involved in the resettlement area using a questionnaire. Project documents, reports, and minutes of agency meetings were used in the collection of secondary data.

An Assessment and Development Classification Guide developed by the Department of Agrarian Reform with the indicators and point system based on the National Economic and Development Authority (NEDA) standards were used and validated to determine the level of development of the resettlement community focusing on land tenure status, socio-economics, and institutional development.

An environmental impact assessment (EIA) was also undertaken to determine and assess the impacts of resettlement. A group of experts from Central Luzon State University together with the researcher undertook the EIA using an interaction or Leopold matrix. The matrix listed activities relevant to resettlement along a horizontal axis and existing environmental characteristics along a vertical axis. The interaction matrix identified and evaluated impacts on the basis of interaction between activity and the subsequent impacts on the environmental setting.

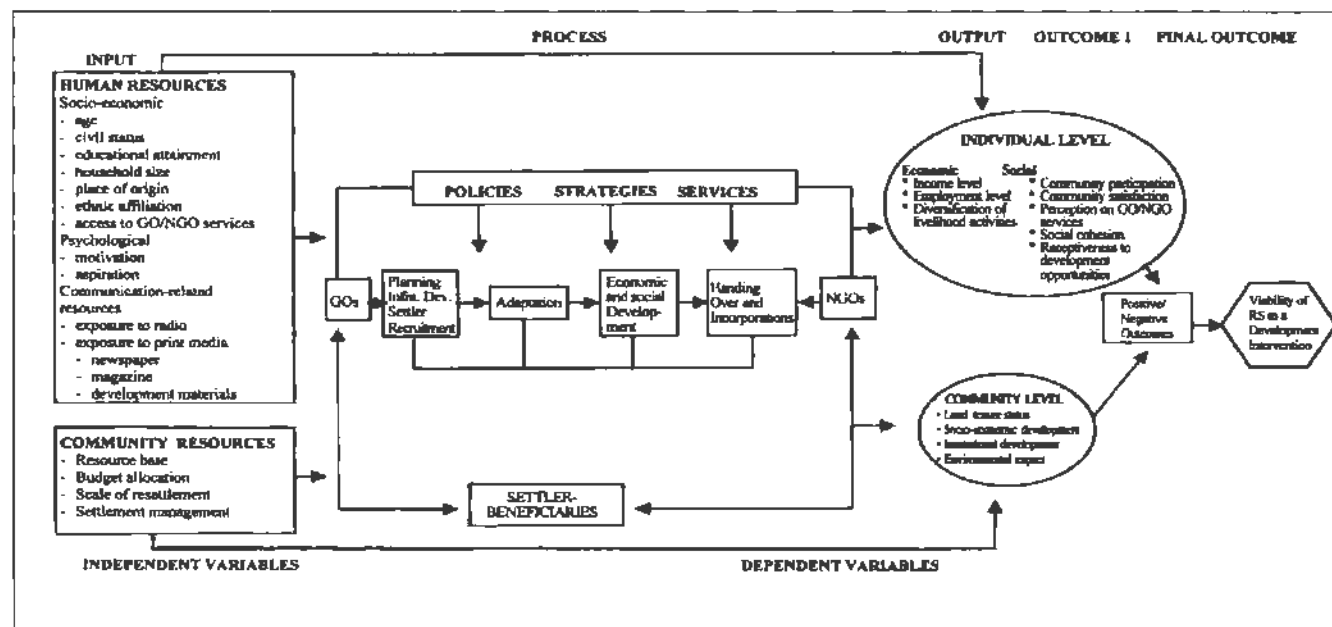


Figure 1. Conceptual of the resettlement process showing the outcomes and hypothesized relationships between the independent and dependent variables.

Data Analysis

This study addressed both the area of content (through quantifiable indicators) and the process as well.

The viability of resettlement as a development intervention was determined both at the individual and community level.

The quantifiable data were processed and analyzed using the Statistical Package for the Social Sciences (SPSSx). Descriptive statistics using the mean, range, percentage, and standard deviation were used to summarize the descriptive data. The Pearson Product Moment Correlation was used to determine relationships of the independent and dependent variables. As correlation merely shows the direction of the relationships, multiple regression analysis was utilized to determine the extent to which the variables can explain changes in the outcome of the resettlement process. The multiple regression analysis was also utilized to determine how the viability of resettlement could be predicted from the economic social outcome of resettlement.

The resettlement process was analyzed in the context of the components and stages of resettlement and the corollary events activities involved. Contextual analysis traced back the events that happened, defined the environment, ascertained the development stages and described the actual state of affairs, described the interaction of people/GOs/NGOs, stating in detail the features and characteristics of the resettlement community and exposing the problems, strengths and weaknesses as well.

RESULTS AND DISCUSSION

The Settlers and the Settlement

The 200-hectare Bagong Buhay resettlement is nestled on the foothills of the Sierra Madre range and forms part of the Fort Magsaysay military reserve in Nueva Ecija. A greater portion of the resettlement was forested (89.30 ha) with 20 ha used for residential purposes while 16.25 ha were utilized in the establishment of schools, chapel, parks, market, community building, and roads. The area intended for agriculture purposes was limited to a minimal 8.5 ha.

The Iglesia ni Cristo (INC) organized its own resettlement program in an effort to help its brethren displaced by the Mt. Pinatubo eruption. As they abandoned their place of origin, the victims moved to Bagong Buhay taking the challenge of rebuilding their lives and establishing a new community.

After four years since the mass movement of the settlers on February 20, 1992, Bagong Buhay evolved as a community with the people sharing a common territory, enjoying a sense of belongingness, brotherhood and unity and the resilience to live life to the fullest and strive to attain their common needs and aspirations.

The selected socio-economic characteristics of the respondents revealed a mean age of 43.38 years with the age structure reflecting that majority of them

belonged to the working age (15 years and above). This indicates a potential source of harness able human resource for development.

Majority of the respondents were married (87.9%) indicating that most of the settlers moved in the resettlement site with their families. The average household size was 4.6.

With regards to access to credit, majority (72.0%) availed of loans to undertake livelihood activities. Access to GOs/NGO services in the form of organizational support, extension services, production promotion projects, education, health, and recreation was also high with 86.8 percent of the respondents availing of the above services.

The level of motivation of the respondents showed an overall mean rating of 4.45 reflecting that as a whole the respondents were highly motivated. This indicates the respondents' high regard towards their work, pursuing it to fruitful conclusion and achieving the desired goal. The respondents also had a generally high level of aspiration to achieve a better life for them and their children.

The communication-related factors revealed that the only means of broadcast media available in the resettlement was radio owned by 67.0 percent of the respondents. More than half of those who owned radio, listened to development programs, mostly agricultural in nature aired by stations DZRJ, DZXO, DZMM, and DWNE.

Exposure to print media was relatively high with 66.4 percent and 87.9 percent of the respondents, having exposure to newspapers and magazine, respectively. Exposure to development materials was very low however, with 8.7.4 percent of the respondents having no exposure at all.

The Resettlement Process

The resettlement process (Fig. 2) in the context of Bagong Buhay followed a series of stages reflective of the nature and complexities of the process as well as the response made by the settlers as they adapt to their new environment. Stage I (Planning/Site Acquisition) and Stage II (Screening/Selection of Settlers and Infrastructure Development) sets in motion the resettlement process with the different GOs/NGOs and the settlers themselves, actively involved in the planning process and the decisions concerning settlement management and the productive use of resources. The adaptation period (Stage III) was a difficult and stressful phase as the settlers were faced with the uncertainties of what lies ahead in their new environment. The period was characterized by a security-oriented, conservative attitude of the settlers as their priority focused on meeting their basic needs. They were cautious, taking very little risk and favor continuity over rapid change. This stage came to an end when the settler-families learned the ropes of adaptation, begin to feel comfortable and at home with their new environment.

The economic and social development phase (Stage IV) was characteristic of the settler families moving from a risk averse stance to a settled population ready to take risks. They were not only concerned in meeting their subsistence

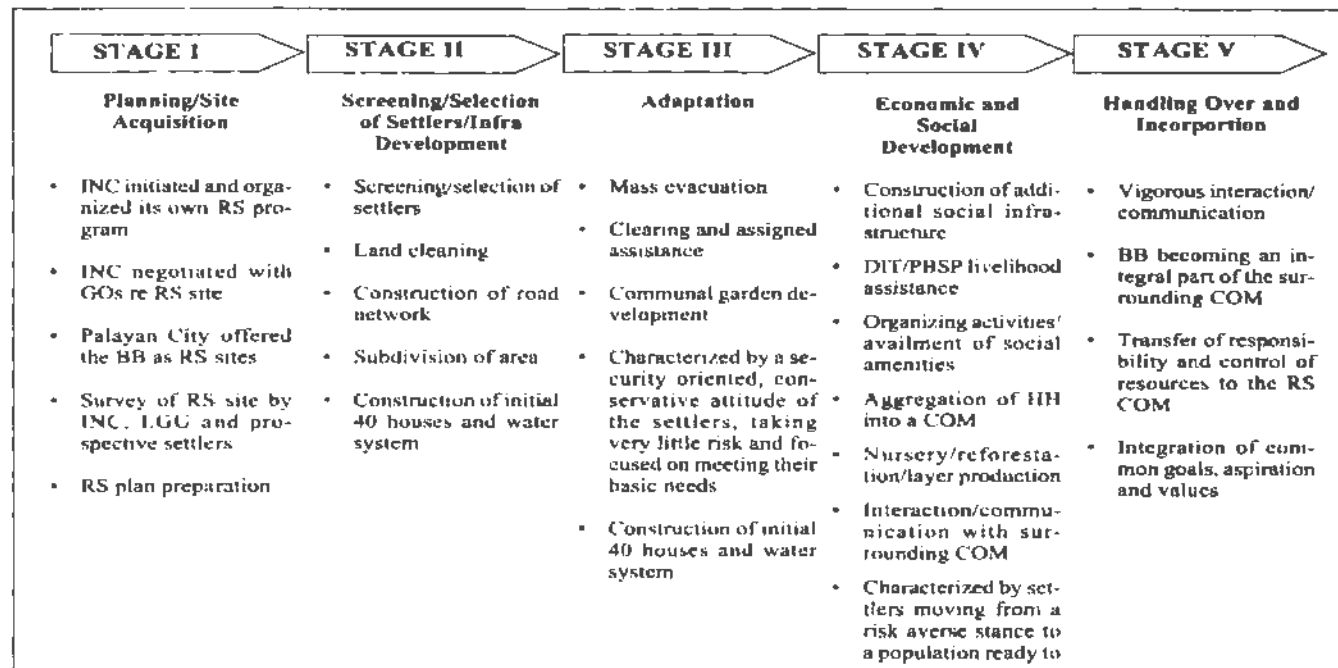


Figure 2. The resettlement process in Bagong Buhay

needs but also acting on an array of livelihood activities to diversify their source of income. It is to be noted however, that with a generally low level of diversification of livelihood activities, farm/non-farm enterprise development remained a thrust to be pursued to enable the settlers to have a sustained source of income. This stage was also associated with the settlers taking advantage of the social infrastructure present in the resettlement put in place through the concerted efforts of several GOs/NGOs providing assistance in the site. This stage also highlighted the evolution of the aggregates of households into a community as the settlers started to organize, undertake community activities together and felt the sense of brotherhood and unity in a territory they can call their own.

The last stage of the process, handing over and incorporation, necessitated the transfer of responsibilities and control over decision-making process and resources to the settlers themselves and other line/government agencies involved. Handing over proved to be a smooth and gradual process where the task to assume full responsibility was motivated by their commitment and reliance on their own capability to restore to normalcy their disrupted lives. Incorporation was evidenced by the integration of Bagong Buhay to the surrounding communities where they maintain social, economic and spiritual contact.

The length of each stage of the resettlement process varied and the sequence reflected the manner by which several actors interact and showed results of the settler's adjustments/responses in their new ecological, social, and economic milieu. Taking cognizant of the different stages of the resettlement process will guide planners to anticipate problems and formulate appropriate measures.

Coping Mechanisms

For the settlers of Bagong Buhay, moving to a new place to live necessarily involved fundamental changes and responses. Over the years, they resorted to several coping mechanisms in order to survive in an unfamiliar and often difficult environment. The settlers struggled hard to diversify their income to meet their basic needs. The support of the family played an important role to cope up with the living condition in the area as spouses and the children helped to ease the economic burden of the family. Sharing of responsibilities and interdependence became focused as husbands and wives enjoyed equality in making decisions on matters pertaining to the family. The findings indicate that the degree to which the family members helped one another becomes relevant as they cope with their daily lives in the resettlement. Family bond remained strong with responsibility and commitment taking prominence in their family life.

The settlers of Bagong Buhay also relied on community support as evidenced by borrowing money/land and seeking advice from friends and relatives and community leaders and relying too on "sari-sari" store credit for their food needs. The kind of support exhibited by relatives was a manifestation of the traditional reciprocal obligations and expectations among kin relations in rural communities

The support from friends and community leaders were also manifestations of mutual sharing and helpfulness in times of need.

The GOs/NGO support on the other hand, opened windows to stimulate economic activities in Bagong Buhay and for the settlers to take advantage of the social amenities present such as schools, rural health unit, and other community buildings as well as recreational facilities. The spirit of "bayanihan" reinforced collective living as settlers shared labor, resources, and time as well. Home gardening proved reliable as the planting of vegetables, root crops, and fruit trees in their garden provided food and some cash for their everyday need.

The religious support became prominent as the settlers were saddled with the burdens of day to day living in the resettlement site. Being all INC, the church provided remarkable social function: cohesiveness, discipline, persevering faith and sacrifice. The church to them provided solace and a sense of security, provided meaning to their existence and the reassurance and hope for a better life in the future.

Policies and Strategies/institutional Linkages

The resettlement outcome was conditioned and influenced by the policies/strategies invoked by several GOs/NGO assisting the resettlement area. The policy initiative of the local government of Palayan City centered on the development of infrastructures and administrative integration while the Department of Social Welfare and Development (DSWD) had long adopted its social rehabilitation and relief policy. The Department of Trade and Industry (DTI) focused its policy initiative in livelihood generation and the Philippine Business for Social Progress (PBSP) centered on organizing and capability building. The role of the Mount Pinatubo Commission in the resettlement effort was most profound on the provision of financial resources and decentralizing of planning and implementation tasks to the GOs and forging a partnership with the NGO in program implementation. The linkage among the actors in the resettlement involved a process of interaction aimed at assisting the community towards development. The linkage (Fig. 3) highlighted the significance of collective action and cooperative efforts resulting into clearer division of tasks among the GOs/NGO and the settlers, thereby generating an integrated community development.

Economic Outcome

The economic outcome of resettlement (Table 1) at the individual level pointed to mean annual income from all sources posted at P54,700.00. Comparing the monthly household income to the 1994 subsistence threshold placed at P3,011.00 revealed that 40.6 percent of the households had incomes falling below the subsistence threshold which was more than the subsistence incidence at the national level (18.2%) and on rural areas (25.6%).

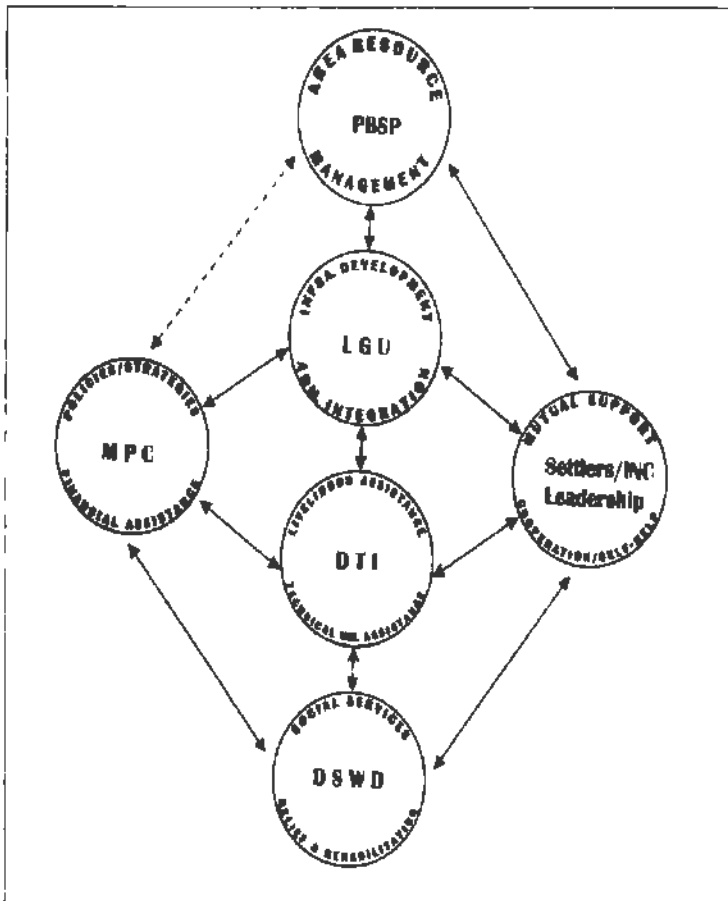


Figure 3. Linkages between and among the several actors in Bagong Buhay.

The estimated poverty incidence became more widespread with 63.7 percent of families having incomes falling below the poverty threshold. The result was much greater than the poverty incidence at the national level and in rural areas, placed at 35.7 and 47.1 percent, respectively. The results were indicative of the settler-families living marginally and that poverty remained a crucial concern which demanded urgent action.

The level of employment of the majority was moderate with 50 to 70 percent of the adults eligible for work were gainfully employed. The unemployment rate of 38.9 percent was considerably high compared to the 1995 unemployment rate at the national and regional level (Central Luzon) placed at 12.89 and 12.84

Table 1. Economic outcome of resettlement.

Item	Frequency/Value	Percent (%)
Level of income		
Range : P9,000 - 336,000		
Average : P54,700		
Poverty incidence		63.70
Subsistence incidence		40.60
Level of employment		
Total labor force	501	61.10
Employed	306	38.90
Unemployed	195	
Diversification of livelihood activities		
High	1	0.50
Moderate	4	2.20
Low	104	57.20
No diversification	73	40.10

percent, respectively. The employment outcome also showed a proportion of older citizens (12.62%) and women (25.56%) maintaining working capability and showed potential both for self-reliance and contribution to their families and community. The occupational distribution indicated a relatively high (46.07%) employment in constructed-related occupations indicating a change in occupational distribution of the settlers, transforming them into construction workers as a result of a shift in occupation from previous work as farmers, agricultural workers, fishermen, factory workers and as salaried workers. The change further indicated the kind of response made by the settlers as the new avenues for employment in the surrounding environment have their own demands for the skills needed.

The diversification of livelihood activities was found wanting with more than half of the respondents (57.2%) having low level of diversification and 40.1 percent having no diversification at all. This is indicative of the inadequate avenues or opportunities in the resettlement site to diversify their livelihood activities. The situation however, can be turned into a positive avenue or opportunity for more entrepreneurial thrust in the resettlement that is vital in an effort to enhance the income-earning capability of the settlers.

Social Outcome

The social outcome of resettlement (Table 2) showed a relatively low level of participation particularly because of the low organizational participation with 55.5 percent of the respondents being non-members of any organization or association and with very few among the members holding any organizational

Table 2. Social outcome of resettlement.

Item	Frequency Value	Percent (%)
Level of community participation		
High	7	3.80
Moderate	24	13.20
Low	151	83.00
Receptiveness to development opportunities		
High	8	4.40
Moderate	87	47.80
Low	87	47.80
	Mean Rating	Description
Level of community satisfaction	3.17	Fair
Perception of life condition	2.87	Fair
Perception on services provided by		
LGU ¹	2.56	Fair
DTI	2.42	Unsatisfactory
DSWD	2.85	Fair
PBSP	2.97	Fair
Social cohesion	4.40	Moderately High

position. Similarly, organizational involvement in terms of planning and decision-making was limited to the officials of the organization with most members involved only in implementation. This pattern followed the pyramid type of participation where at the apex were the few decision-makers; at the middle base were those involved in monitoring and evaluation and at the lower base were the majority implementors/beneficiaries.

The level of community satisfaction was generally fair with a satisfactory rating placed on community relations, community leadership and physical location. The inadequacy of infrastructures and the bad conditions of the access roads led to an unsatisfactory rating. Investment in small scale industries and employment was practically nil resulting to the unsatisfactory rating of both criteria. The unsatisfactory rating indicates the need for the improvement of infrastructures and the need for investment in rural industries paving the way for more employment opportunities.

The respondents' own perception of life condition was generally fair and reflective of the settlers' capability to recover and fairly adapt to their new and unfamiliar environment. Perception on the services provided by the Palayan City government, DSWD and PBSB was fair with an unsatisfactory rating for the DTI. The fair rating for the LGU could be attributed to the fact that the city government

of Palayan City welcomed them with a place to settle and put in place the initial infrastructure in the area such as roads, housing and electricity. The DSWD assisted the settlers in the initial years of resettlement through the Cash for Work and Food for Work programs plus the establishment of two day care centers. The PBSP vigorously assisted in terms of organizing, providing credit, water source, and technical assistance. The unsatisfactory rating for the DTI could be attributed to the failed implementation of several DTI assisted livelihood projects which put most of the beneficiaries in debt.

The findings revealed that there was no disruption of community social cohesion. The settlers remained capable of continuity of purpose and action through shared work, common interest, cooperative decision-making and family ties.

Receptiveness to development opportunities showed that only 4.4 percent of the respondents were found to have high level of receptiveness. An equal number of the respondents (47.8%) were found to have moderate and low level of receptiveness. The results suggest that the existing opportunities in the resettlement site may not be adequate to create an attitude of greater receptiveness on their part. This may also be an indication that the existing opportunities were not responsive to the immediate needs of the settlers.

Level of Development

The level of development of Bagong Buhay (Table 3) was also determined using the major criteria such as land tenure status, socio-economic development, and institutional development. On the basis of these criteria, Bagong Buhay was assessed as a developing barangay indicating to a certain extent its capability to put in place a land to be settled, the socio-economic infrastructures, and institutions that set in motion the development process.

The environmental impact assessment and evaluation looked into the interaction or interrelation between the major activities in the resettlement and the subsequent impact on the existing environment condition in the resettlement site. The overall assessment revealed a low level of environmental impact indicating that the major activities such as residential/bunkhouse development, land cultivation in areas with high degree of slope, agricultural activities, waste disposal and extraction of additional ground water were not that extensive as yet to cause greater environmental degradation.

Viability of Resettlement

The viability of resettlement as a development intervention was assessed both at the individual and community level (Table 4). Viability refers to the feasibility and effectiveness of resettlement in achieving its stated socio-economic goals. Results at both levels revealed a moderate level of viability indicating that after four years of resettlement, moderate improvements had been made on the socio-economic condition of the settlers. This also suggests that moderate

Table 3. Level of development of the resettlement area.

Assessment Criteria	Points Earned
Land Tenure (20 points)	
Land distribution of untitled lands	15
Socio-economic development (50 points)	
Transportation	3
Housing	2
Education	4
Health	4
Power supply	4
Communication	1
Sports and recreation	4
Economy and livelihood	6
Ecological balance	2
Sub-total	32
Institutional Development (30 points)	
Status of organization/associations	13
Organizational/strengthening activities	3
Presence of development institutions in the area	2
On-going projects/activities	5
Sub-total	23
Total points earned	70
Development classification	Developing

Levels of classification:

80 - 100 pts	= Developed
50 - 79 pts	= Developing
<50 pts	= Underdeveloped

improvements had been made to ease the difficulty of the settlers in adapting to their environment. This could be attributed to the immediate provision of a land to settle, the provision of basic community services, however inadequate they may be and the spiritual concern which enabled the settlers to respond positively even in the most trying situation they were in.

Yet, a lot more needs to be done as the economy and the livelihood of the settlers remained a formidable task to undertake. The level of poverty as indicated by a generally low level of income, high unemployment, and low level of diversification remained a critical concern. It is but imperative that the settler-families be led towards the development of greater livelihood and self-employment

Table 4. Viability of resettlement

Item	Frequency/Value	Percent (%)
Viability at the individual level		
High	24	13.20
Moderate	152	85.50
Low 151	6	13.20
Viability at the community level		Points Earned
ECONOMIC		
Income level		4.53
Employment level		5.84
Diversification of livelihood activities		1.23
SOCIAL		
Community participation		1.42
Community satisfaction		3.00
Perception of life condition		3.00
Perception on GOs/NGO services		3.00
Social cohesion		5.00
Receptiveness to development		2.14
Opportunities		10.00
Level of development		20.00
Environmental impact		10.00
	Total points	59.60
	Classification	Moderately viable
80 - 100 pts High viability 50 - 79 pts Moderate viability < 50 pts Low viability		

opportunities to provide sustainable income sources and eventual attainment of socio-economic condition, however slow and arduous the task may be.

Relationship Between Economic/social Outcome and Viability of Resettlement

Except for community satisfaction, all the economic and social outcomes were found to have significant association with the viability of resettlement at the individual level. The insignificant relationship between community satisfaction and the viability of resettlement indicates that community satisfaction did not in any way influence the viability of resettlement. The significant association with the economic and social outcomes were indications of the importance of the level

of income, employment level, diversification of livelihood activities, community participation, perception of life condition, perception on services provided by GOs/NGO, social cohesion, and receptiveness to development opportunities in determining the viability of resettlement. A critical consideration of these economic/social outcomes must be addressed in an effort to achieve greater effectiveness in developing resettlement programs.

A regression analysis to determine the joint effect of the economic/social outcomes on the viability of resettlement revealed that the combined economic/social outcomes account for 87.14 percent of the variation in the viability of resettlement and that other factors not included in this study must be influencing the remaining 12.21 percent variation in the viability of resettlement. The results establish the linking core characteristics of resettlement to the specific economic/social outcome. The F-test undertaken revealed that the economic/social outcomes were significant predictors of the viability of resettlement at the individual level.

Development Issues/Problems

The study also points to several development issues and problems that must be addressed if current and future resettlements are to benefit larger number of settlers and with due regard to the environment. The identified issues/problems were marginal resource-base, resource conservation, land tenure status, housing rights, indebtedness, impoverishment, dependency, collaborative efforts between INC, GOs/NGO and the beneficiaries, settler homogeneity, maintaining balance between livelihood and infrastructure facilities, lack of greater means to secure access to social services, unsatisfactory transport condition, lack of investment and employment opportunities and poor linkage in the market system. The issues and problems tend to reflect the physical, social, economic, and institutional constraints and/or advantages specific to Bagong Buhay that may influence the planning and implementation of future actions aimed at a long-term and sustainable development of the resettlement community.

A Resettlement Framework in Community Development

A resettlement framework (Fig. 4) in community development is put forward which highlights the lessons learned and findings of the study. The framework is also recommended to be used in the systematic analysis of resettlement particularly in planning, implementation, monitoring, and evaluation. The resettlement as it evolved into a community is theoretically and conceptually viewed as a social system made up of interrelated parts and elements composed of the settlers/grassroots institutions, Gos, and NGOs. Central to the framework are the concepts of participation, self help, interdependence, collective action, goal/task orientation, and cohesion. The resettlement community exists in a dynamic relationship with its environment as it receives various inputs and transforms these inputs through a process into outputs.

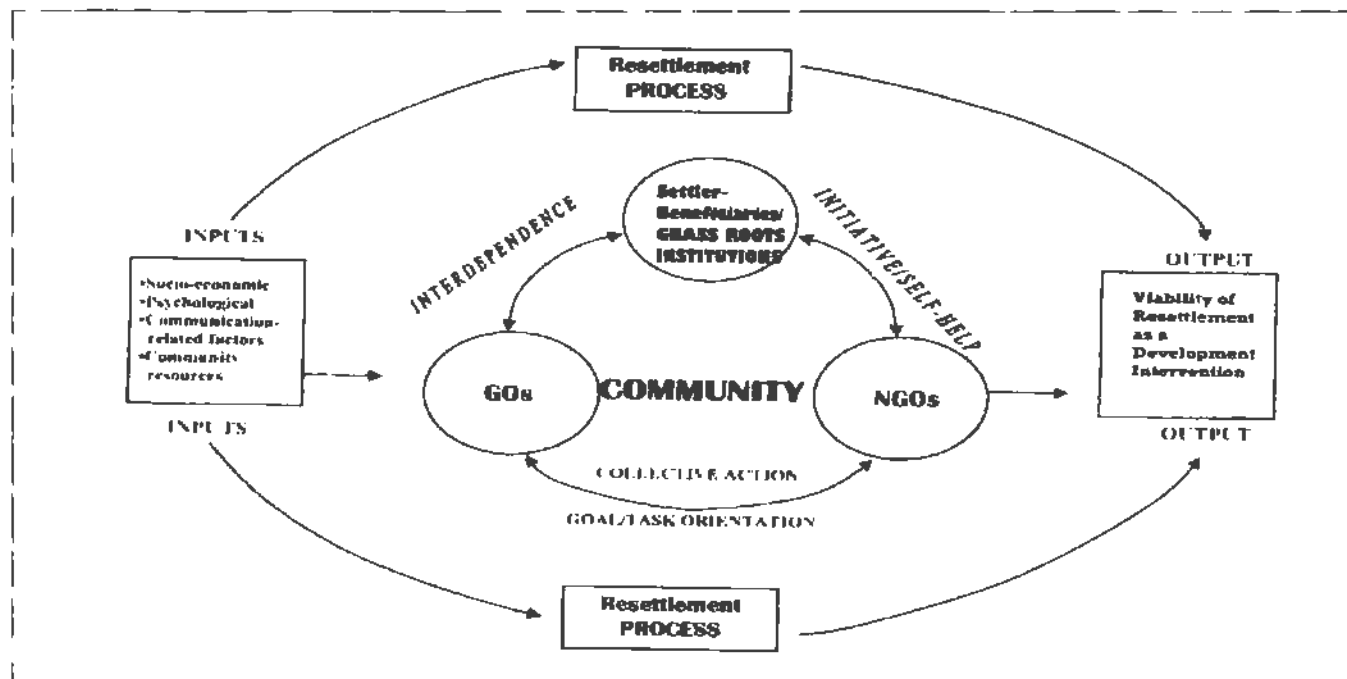


Figure 4. A resettlement framework in community development.

The lessons learned from the research point that the concepts and principles of community development as an approach and field of study had a broadening effect on the resettlement process and the evolution of Bagong Buhay as a new community. The principle of participation elicited settlers' initiative and greater involvement in the decision-making process. This broadened their roles and responsibilities through collective action developing greater reliance on themselves. The concept of goal/task orientation provided the identification and optimum distribution of interdependent tasks between GOs, NGOs, and the local people aimed at generating a capacity for growth and development. The settlers also demonstrated the principles of self-help and mutual aid - indications of their cooperation and willingness to run their own affairs in the long term. Developing a community based on social cohesion where the settlers remain capable of continuity of purpose and action was also shown, having been brought about by religious and ethnic homogeneity.

While the socio-cultural considerations stimulated the birth of a new community in Bagong Buhay, the outcome of the resettlement process point the need for an improvement of the economic condition of the people with the attendant problems and drawbacks that need to be resolved. Despite this however, results indicate that amidst the complexities and difficulties, resettlement as a process provided opportunities for the settlers to build a new productive foundation for life and develop new social relationships as well as a new social structure. Resettlement then must not be treated simply as a mechanism to transfer the displaced to a new land but must be holistically approached as a development opportunity to mobilize the resources of the state, different GOs/NGOs, and the settlers themselves in the pursuit of sustainable development. This therefore calls for changes through improved policy concerns and relevant research and development activities that will prove useful for future resettlement efforts.

REFERENCES

- Cerneja, M.M. 1994. African Population Resettlement in a Global Context. Technical Paper # 227 Washington D.C. World Bank
- _____, 1991. Putting People First: Sociological Variables in RD. 2nd edition. London/Oxford University Press.
- _____, 1968. Involuntary Resettlement in Development Projects. Technical Paper #86. Washington D.C. World Bank.
- Icamina P., 1992. Life after Pinatubo: Confronting crops from fire, mud and ash. *Ceres FAO Review No. 134, Vol. 24 No.2.*
- Mburugu, E.K. 1994. Dislocation of settled communities in the development process: The case of Kiambere hydroelectric project. Technical Paper # 227. Washington D.C. World Bank.
- McMillan, D. Painter and I. Scudder. 1992. Settlement and development in the River Blindness Control Zone. Technical Paper #192. Washington D.C. World Bank.
- Paderanga, C. 1986. A Review of land settlements in the Philippines. Discussion Paper #8615. Diliman, Quezon City: School of Economics, University of the Philippines.
- Searbrook, J. 1991. Victims of Development: Resistance and Alternatives. London/New York: Verso Publishing.

- Scudder, T. 1985. A sociological framework for the analysis of new land settlement. In Ciernia M. (ed.) *Putting People First: Sociological Variables in Rural Development*. London, Oxford University Press.
- Thapa, G.B. and K.E. Weber. 1988. *Land Settlements in Tropical Asia. Prospects for an Alternative Planning Strategy*. Thailand: Asian Institute of Technology, Thailand.

PARTNERING IN AGRICULTURAL EXTENSION

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ABSTRACT

This paper is mainly based on the actual experiences of field personnel of the Agro-Industrial Development Program (AIDP), UPLB-College of Agriculture from 1993 to 1999. The AIDP is a collaborative research and extension program between and among the UPLB-College of Agriculture, the local government units (LGUs), and the local state colleges and universities (SCUs). It is geared to develop a management organization that delivers agricultural extension in the context of devolution, as mandated in the Local Government Code of 1991, and the Agriculture and Fisheries Modernization Act (AFMA).

The program used two types of agricultural extension management organizations for effective delivery of agricultural extension services to the farming population. These are: (1) the province-wide organization, which is operating in Oriental Mindoro and Marinduque, and (2) the municipal-level organization, which is operating in seven towns of Laguna and a town in Cebu. These organizations are composed of the different stakeholders, particularly the LGUs and SCUs.

In the process of building up the partnership between the different levels of LGUs and the local SCUs, a number of facilitating and constraining factors were identified. The facilitating factors were: (1) joint planning and consultation among different stakeholders; (2) clear identification of roles by the LGUs; (3) shared commitment of the different partners; (4) development emphasis of the LGU; (5) initiative of the people and strong farmers organizations; and, (6) social networks of individuals in the organizations. On the other hand, the following were the constraining factors: (1) political conflict; (2) insufficiency of financial and manpower capability to share in

the partnership; (3) lack of commitment in terms of priority; and, (4) absence of clear cut role and responsibilities

Key words: partnership, agricultural extension, devolution, stakeholders, local government units (LGUs), state colleges and universities (SCUs), empowerment

THE CONTEXT OF AGRO-INDUSTRIAL DEVELOPMENT PROGRAM (AIDP I)

Farming today is becoming very complex. Farmers now need to know the technological options they have and the skills necessary to make appropriate decisions. In the past, the Department of Agriculture was the agency mandated to extend these technological options to farmers. This function of the Department has been devolved to the local government units (LGUs) since the enactment of the Local Government Code of 1991. This in effect empowers the provincial, municipal and barangay governments to set their own priorities for agriculture. While empowerment of LGUs is good, it nevertheless gives rise to one difficulty: the lack of coordination and synchronization of programs between LGU's provincial and municipal levels. The law is also silent on the relationship between the Office of the Governor, through its Provincial Agriculturist Office (PAO), and the Office of the Mayor through its Municipal Agriculturist Office (MAO). Because of this, provincial agricultural development programs cannot be implemented at the municipal levels unless approved by municipal executives. The same holds true in implementing national programs. All this points to the need for an organization that would put the LGUs into a single management.

Even if they are put into one organization only, the LGUs nevertheless lack agricultural information to disseminate. They have no formal links with knowledge centers, since they have been traditionally isolated from these institutions. One way to correct LGUs' institutional isolation from knowledge centers is to devise an organization that would pave the way for collaboration between the LGUs and local SCUs. LGUs and SCUs enhance their ability to meet farmers' information needs by working hand-in-hand in mutually acceptable arrangements that do not compromise their institutional goals and visions. This was the premise of the first phase of Agro-Industrial Development Program of the College of Agriculture in its partnership arrangements with the LGUs and local SCUs from 1993 to 1999. The AIDP wanted to find out how exactly this process would proceed.

This paper argues that AIDP I is more than an extension program of the College of Agriculture. It attempts to develop an organization that delivers agricultural extension in the context of devolution, Agriculture and Fisheries Modernization Act and General Agreement on Tariffs and Trade. This paper discusses the processes involved in evolving organization founded on partnership that binds the different levels of LGUs, local SCUs, and other stakeholders in agriculture. It also discusses the other unique features of the program, such as participatory planning, institutionalization of monitoring and evaluation for every

project site, procedures of resource mobilization, and institution building and strengthening. But before proceeding to these issues, let us highlight first the theoretical basis of partnership in coming up with an accountable and sustainable agricultural extension delivery system.

Partnership among stakeholders has become today's buzzword and a favorite subject of development advocates. There are several reasons for its advocacy. In agricultural extension, duplication of efforts of different service agencies is commonly observed despite these agencies' inadequate manpower and financial resources. If all of these agencies could work together, duplication would be solved. They might also be able to create greater impact to their beneficiaries than before, since partnership would equally mean greater pooling of resources, skills and expertise. Yet, despite its potentials, partnership, is little understood. It could either refer to the way the public and private actors match their means and competencies in providing social services to their clients or the way partners assume ownership of the services they provide (Jalal as cited by Cornwall, et al. 2000). The latter definition is, however, more complex as the owners of services are not only limited to service provision but also the end users. Jalal terms this phenomenon as "responsible partnership" (Jalal as cited by Cornwall et al. 2000). Gibbon furthers this definition to include what Cadbury is advocating as the equal sharing of power among the partners and Fowler's advocacy of "understood and mutually enabling, interdependent interaction with shared intentions" (Fowler as cited by Cornwall et al. 2000).

Partnership, therefore, is an extremely complex strategy. It attempts to combine and coordinate people, technology, job tasks and other resources to achieve effectively a common or shared goal. According to Swiss Commission for Research Partnership with Developing Countries, the establishment of partnerships is founded on the following basic principles: (1) deciding on the objectives together, (2) building up of mutual trust, (3) sharing of information, (4) developing of networks, (5) sharing of responsibility, (6) creating transparency, (7) monitoring and evaluating collaboration, (8) disseminating the results, (9) applying the results, (10) sharing of gains equitably, and (11) building on the achievements.

Establishing partnerships basically requires continuing dialogue and exchange of experiences among all those involved, including members of the local community. Discussing common problems together can motivate all partners to cooperate actively. The best division of tasks and responsibilities, based on varying strengths of partners, offers the best chance of producing and using synergy, and that all those involved - right up to the end-user - will really benefit from partnerships. Like any kind of cooperative enterprise, partnership in agricultural extension must always be oriented towards a particular goal in a specific setting.

Despite the potentials of partnership, Bell et al (1999) indicate that there are disadvantages associated with its use. Firstly, coordinating many people to work together is not easy. Decisions are not easy to reach in this arrangement because there are many minds working at the same time. This is even magnified when

each of the parties involved has its own interests to pursue. There is also the problem of communication, commitment as well as the credibility of partners. Sometimes ethical problems emerge, affecting an otherwise effective functioning of partnership. Summarily, it is a costly exercise in terms of time, effort and even money. But despite all these negatives, AIDP I believes there is no other way to deliver agricultural extension services to farmers except through partnerships.

AGRICULTURAL EXTENSION ORGANIZATIONS IN AIDP SITES

The program is able to come up with two types of agricultural extension organizations: province-wide and (2) municipal-level organizations. The province-wide organization of agricultural extension is operating in Oriental Mindoro and Marinduque; the municipal-level organization in seven towns of Laguna and one town in Cebu.

PROCESSES INVOLVED IN COMING UP WITH AGRICULTURAL EXTENSION DELIVERY SYSTEM

Initiation stage. The manner by which the idea of partnership was introduced to partners by UPLBCA/AIDP depended on the circumstances of encounter. In Oriental Mindoro, this idea of partnering was put across to the governor of the province only after it had sought the assistance of UPLBCA in developing and implementing its rural and development programs for the province. At that time, it was easy for the UPLBCA/AIDP to introduce such an idea since there was already a proposal for partnership from the Center for Rural Organization and Support Services (CROSS), an NGO operating in Bansud. In the same way, the birth of partnership among the province of Marinduque, the Marinduque State College (MSC) and UPLBCA/AIDP came about because of the initiative of the provincial government under Governor Carreon. Elliot (1999, personal observation) refutes this, however. She claims instead that the academic people started such a partnership. At first, it was President Javier (UP President, 1993-1999) who first recognized the need of the province to be assisted by UPLB. President Javier recognized this after he had gone to the province to look after his relatives. Dr Roberto E. Coronel, erstwhile head of National Genetic Resources Laboratory of Institute of Plant Breeding, also realized this when he was asked to make an agroeconomic system and information for the formulation of sustainable agricultural development. At the same time, President Monterey of Marinduque State College (MSC) convinced the UPLBCA to include the province as an AIDP site. In Laguna, where the initiatives from the provincial executives were lacking, partnerships originated either from the efforts of local officials of a number of municipalities and from UPLB's commitment to reach out, first, to its surrounding areas before any other area. For example, the partnership of the municipality of Rizal with Laguna State Polytechnic College (LSPC) and UPLBCA/AIDP came about after one of its councilors made representation to the UPLBCA Dean to include Rizal

as an AIDP area (Gesmundo, 1999: personal observation). The same is true for Cavinti. Introducing partnership idea to executives of Nagcarlan, Luisiana, Liliw, Magdalena, Los Banos, Sta. Rosa and Calamba, came very naturally, since these towns had been sites of UPLBCA's agricultural extension activities.

Signing of MOA. A memorandum of agreement among various stakeholders will legitimize the organization of agricultural extension. Signing the MOA is integral to the program. Although signing is not a guarantee that the partners will do their share, the experiences of some AIDP-covered provinces and municipalities showed that this served as a binding force among partners to render their responsibilities/commitments to the different AIDP project sites. Signing of MOA among Oriental Mindoro partners was at two levels. One, at the provincial level, was in February 1996 at the Development Academy of the Philippines (DAP) in Cavite. The other, the municipal level was held in Oriental Mindoro in October 1996. In Marinduque, signing at the provincial level was held in September 1996, followed by the municipal levels.

In 1995, MOAs were simultaneously signed in each of the AIDP-covered municipalities in Laguna, except for Rizal which had its signing in April 1996.

Participatory planning. One of the ingredients of a successful partnering system is a project plan to which it can be applied. Nevertheless, the conception of a project plan has to undergo a long and tedious process of consultations and assessments of the area's potential for development as well as stakeholder's commitments. The process involves conducting planning workshops for stakeholders.

Planning workshops. The workshops covered provincial and municipal levels. For the Oriental Mindoro partnerships, a series of workshops was conducted at UPLB and in the province in 1996.

One major workshop output was the drafting of AIDP Oriental Mindoro indicative plan, which was used as a reference and justification of the provincial budget office on annual program/projects' financial support. Marinduque conducted a similar workshop in the province in 1996, which was followed later on by a consolidation workshop at UPLB.

In Laguna, participatory planning workshops were conducted in Nagcarlan, Liliw, Magdalena, Luisiana, Sta. Rosa and Calamba, to devise their respective Municipal Agricultural Development Plans (MADP). While the rest of the AIDP-covered Laguna municipalities focused their activities on their own MADPs, Rizal, being a neophyte in the program, started its planning at the barangay level based on the concept of Community Development Process (CDP). Barangay level plans were later integrated to form the MADP.

Resource Mobilization. Smooth project implementation in each AIDP area depended very much on equal sharing of resources among stakeholders. Budgetary problem was common among government institutions and some LGUs, with the exception perhaps of Oriental Mindoro. Indeed the provincial and municipal governments of Oriental Mindoro were able to release P3,970,000 and P1,806,875, respectively, to support identified projects under AIDP. Barangay governments

likewise, gave counterpart funds of P40,000 each. Such government agencies as the Technical Education and Skills Development Authority (TESDA), Office of the Southern Cultural Community (OSCC) and the Department of Agriculture (DA) also gave their support as well as other government organizations (GOs) and peoples organizations (POs), such as the Plan International of Mindoro which gave P184,000 and Farmers Association of Victoria with P50,000. These funds were released to support projects in Oriental Mindoro towns, including rice, cutflower, vegetable and fruit, livestock and forest trees productions. Plant nurseries, plant pest clinic and agricultural breeding stations were established serving as support facilities for the production of forest trees seedlings, fruits and vegetables.

While AIDP-Mindoro was enjoying financial support from the partnership, AIDP-Laguna, specifically Nagcarlan, Liliw, Sta. Rosa and Los Baños did not have such opportunity due largely to stakeholders' failure to deliver the resources expected from them. Consequently, only a few tangible projects were implemented, including a cooperative in Magdalena and a municipal nursery in Luisiana. There were other accomplished activities though they did not need financial assistance; these included training on swine production, anthurium production, asexual propagation of plants, kimchi and tomato sauce processing.

The mobilization for internal budgetary support took place not only in the provinces and municipalities but also in barangays as it did in Rizal, Laguna. In preparing their agricultural plans, several barangays in Rizal took a substantial amount from their local development funds to support AIDP identified livelihood projects. In Magdalena, local officials were able to mobilize some resources to provide credit assistance to deserving farmers for their crop or livestock production projects.

Monitoring and evaluation. Monitoring and evaluation, inherent components of the implementation of project activities, formed part of the projects' regular operation. Evaluation determined how far or short an activity or undertaking went and how much more would be done to accomplish what was set out earlier.

In Mindoro, project monitoring was made through project visitations, on-site reviews, MAOs regular monthly meetings and annual reviews. This was usually participated by the different stakeholders in the partnership.

In Marinduque, monitoring and evaluation was made through project reviews and monthly management committee meetings.

In Laguna, an annual project review for the overall accomplishments for a given year was usually conducted. Likewise, a mid-year review on project updating is done in all project areas to track down the progress of the project for the first half of the year. This exercise revealed some limitations/problems in project implementation. Solutions or remedial measures were then given to ensure smooth implementation of projects.

Institution building and strengthening. Putting together the stakeholders of agricultural extension into one organization is by far the best option to strengthen them. The first MOA between the LGUs and UPLB provided the former an

opportunity to get familiar with the various units of the university, which could give them other services and technical support. In Magdalena, Laguna, the first IPM training on rice and corn informed the participants on available varietal trials on rice, in collaboration with PhilRice, and corn with IPB. The AIDP project staff likewise facilitated the LGU's linkage with the College of Veterinary Medicine and the Philippine Carabao Center, for some solutions to Magdalena farmers' disease problems in carabao. In Luisiana, the AIDP project was followed up by LGU's arrangements with other government agencies offering development assistance, notably the Philippine Coconut Authority (PCA), Southern Luzon Polytechnic College (SLPC) and National Economic Development Authority (NEDA).

AIDP scored yet another achievement and that is by building up LGUs' leadership capability to put together the various service agencies on agricultural development under one organization. This strategy not only avoids duplication of functions among these agencies but also enables them to realize their complementary roles vis-à-vis other agencies. This likewise allows them to maximize use of their resources. This is well exemplified in Oriental Mindoro when the Provincial Governor's Office, through the Office of the Provincial Agriculturist, was able to solicit the assistance of DTI, TESDA, DOST, DA, PCA and other agencies to work closely with them in developing agriculture in the province.

In Marinduque, implementation of projects/activities began in 1997 with a series of trainings/seminars on crops and livestock production among agricultural technicians, farmers, and NGOs, in coordination with the provincial government, Marinduque State College (MSC) and other related government agencies. Training on mushroom production and fruit processing in UPLB soon followed.

In recognizing the importance of organized groups, associations or cooperatives, AIDP exerted all efforts to facilitate their establishment in areas where there is none and when need is indicated. In Magdalena, the MASIKAP Cooperative was established, which is now engaged in running a store to sell production inputs to farmer-members.

LESSONS LEARNED FROM AIDP IMPLEMENTATION

The success and failure of partnerships in different AIDP areas vary. While some areas had gainful experience from partnerships, other areas had so far been unfortunate to enjoy their benefits. Some facilitating and constraining factors in partnership have been identified as follows:

A. Facilitating factors

Joint planning and consultation among stakeholders to ensure representation of all interests

The presence of LGU officials, DA staff, local SCUs and farmers evinced participation of stakeholders even during the pre-planning phase of the program. Likewise, projects contained in the MADP were a product of thorough planning at

the barangay and municipal levels as in the case of Mindoro and Laguna, particularly in Rizal town.

Clear identification of roles by the LGU

The first implementing year of AIDP projects in all AIDP areas can be regarded as UPLB led. Project implementation eventually changed gears; this time with LGU calling the shots in most AIDP areas. In Mindoro and Laguna, specifically in Rizal, Magdalena and Luisiana, LGU partners considered AIDP the umbrella of all their agricultural activities.

The Mindoro partnerships (provincial, municipal and barangay) demonstrated their commitment with the considerable amount they had released to the program in support of the area's different projects.

Of considerable value, too, is Rizal LGU's allocation of P50,000 for Year II of MADP project implementation. In Magdalena LGU provided the farmers through the MASIKAP cooperative P300,000 to carry out their own business.

Shared commitment of the different partners

This was made manifest in Mindoro and Laguna partnerships as mentioned previously. MinSCAT in Mindoro, MSC in Marinduque and LSPC in Laguna, although hampered by lack of material and human resources, participated in the program from its initiation to implementation stages.

Development emphasis of the LGU

MADPs' implementation in different municipalities has varying degrees of accomplishment because of the natural variations in the development foci of local officials. Some gave priority to agriculture while others were busy on infrastructure concerns.

Oriental Mindoro is a perfect example of an LGU whose local executives showed strong emphasis on agriculture, considering the substantial amount they gave to the program. Likewise, Rizal in Laguna has also been a very supportive partner with their budget realigned for the implementation of AIDP projects.

People's initiatives and strong farmers' organizations

People's initiatives contributed largely to the smooth program implementation as Rizal clearly demonstrated. At their own initiative Rizal requested immediately training on project proposal preparation and packaging to hasten up submission of their proposals to the municipal government office for funding. Farmers felt the need for such training right after preliminary discussions with them on project concepts. Consequently, priority proposals were prepared, submitted and approved by the Rizal municipal government.

Social networks of individuals in the organizations

It is commendable that in Marinduque establishing partnerships between UPLB, MSC and LGUs was facilitated by personal networks of people in these organizations. This confirms that contacts influence decisions more regarding inter-institutional coordination than generalized institutional relationships.

B. Constraining Factors

Political conflict

This particular problem was very much apparent in Nagcarlan, Laguna, wherein the MAO who was supposed to be working closely with AIDP, could not relate well with the mayor on AIDP matters because of conflicting politics with the mayor. As a consequence, no tangible projects were established in the area for the past five years.

In a similar vein, Zara (1999, personal observation) reported that signing of MOA for AIDP-Batangas could not take off because of political conflict between the government's executive and legislative branches. The governor wanted to enter into a partnership, but majority of the Sangguniang Panlalawigan (SP) members barred him so because of some provisions in the MOA with regard the province's financial obligation to the project. In fact, the SP only allocated an amount of P5.00 as the budget of the office of the provincial governor for the year 1999.

Insufficiency of financial and manpower capabilities to share in the partnership

Oriental Mindoro's MinSCAT, Marinduque's MSC and Laguna's LSPC were willing to participate in the program, but were constrained since they could not complement in the partnerships with their scarce resources. Time was another stumbling block; their staff were overloaded with teaching units. Garcia (1998) reported that insufficiency of financial and manpower capabilities were not only due to lack of these resources per se. Rather, these were outcomes of policies that do not allow SCUs to exercise some flexibility in their tasks besides their primary function of teaching.

Lack of priority commitments

This problem was prevalent in Laguna, specifically in Nagcarlan. Liliw and some urban agriculture areas where municipalities focused their priority on high impact projects.

Absence of clear-cut roles and responsibilities

Some AIDP areas claimed that there was clear identification of roles among different partners. Banatiao (1998, personal communication) claims otherwise, saying that in the case of the Urban Agriculture Project (UAP), the MOA that supposedly binds the partnership contains general statements of responsibilities by partners. There are no implementing guidelines either; hence, the SCU is at a loss. Banatiao (1999, personal observation) further adds that there is no stipulation in the MOA specific to the leadership role of the project. More often than not, partners look up to or rather depend on UPLB on what to do.

CONCLUSION

This paper has argued that AIDP I is more than an extension program of the College of Agriculture. It attempts to develop an organization for agricultural extension delivery system that binds the different levels of LGUs and local SCUs for effective delivery of extension services to the farming population. This paper has also discussed other features of AIDP I, such as participatory planning, resource mobilization, monitoring and evaluation of projects, and institution building and strengthening. In building up partnerships between all levels of LGUs and local SCUs, a number of facilitating and constraining factors have been identified.

LITERATURE CITED

- Bell, M. A., E. Edmunds, C. Chung and S. Marin. 1999. New Sectoral Partnerships. A paper presented during the IBC's 4th International Rice Conference 1999, Cebu Island, Philippines, 28-29 October 1999.
- Cornwall, A., H. Lucas and K. Pasteur. 2000. Introduction: Accountability through Participation. IDS Bulletin 31(1): 1-13.
- Garcia, Juliana G. 1998. Latitudes and Limits of SCU's participation in Agro-Industrial Development. A paper presented during the AIDP-PITAS Lecture Series, November 20, 1998, Operations Room, UPLB.
- Swiss Commission for Research Partnership with Developing Countries. 1998. Guidelines for Research Partnership with Developing Countries.

THE SOCIAL REPRODUCTION OF THE MEDICAL PROFESSION: THE CASE OF THE UNIVERSITY OF THE PHILIPPINES-PHILIPPINE GENERAL HOSPITAL MEDICAL CENTER

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ABSTRACT

Among the members of the health team, physicians are accorded the highest prestige. Societies depend on them for their rational authority on matters related to health and disease. For this reason, medical centers are always expected to implement a curriculum that would train and prepare the most competent physicians.

This paper describes the long and tedious process of the final year of clinical internship of the country's top medical center, the University of the Philippines-Philippine General Hospital Medical Center (UP-PGH). In the process, it was able to describe the various traditions that are socially reproduced in the course of training. It was also able to explain the different social structures involved in the process of socially reproducing the future medical professionals.

It is a descriptive study as it gathered qualitative data from direct observations of interns and clerks during their rotation in UP-PGH. Key informant interviews, survey questionnaire and review of secondary data were also done. The study was started in 1997 and completed in 1999.

Key Words: Social reproduction, physicians, official curriculum, operational curriculum, hidden curriculum, medical education, clinical internship, uniform elite, professional socialization of doctors

INTRODUCTION

Among the members of the health team, physicians are accorded the highest prestige. Helpless patients and their relatives glorify them during the most difficult stage of their illness. Across cultures and generations, physicians are esteemed for their rational authority and professional competence especially when it comes to issues on health.

Considering the preeminence that societies give to physicians, the institutions society commissions to train these professionals are likewise regarded highly. To most modern societies, the medical centers play the most important part in the training of future physicians.

In their efforts to ensure that society will have a sufficient supply of future doctors, medical centers also become instruments in preserving the very existence of a highly prestigious group of health professionals. These concerns prompted the researcher to investigate the curriculum of medicine aiming to come up with an explanation on how doctors are being socialized to a highly respected profession. The study focused on the terminal year of medical education, that is, the clinical training program where most of doctoring is learned (Spart et al., 1988).

Research Objectives

In the Philippines, the titles of The State University and that of Premier Medical School have always been associated with the University of the Philippines College of Medicine (UPCM) and the attached Philippine General Hospital (PGH). This complex, since managed by the University of the Philippines System, is also known as the University of the Philippines-Philippine General Hospital Medical Center (UP-PGH).

Since 1913, the UPCM has held the distinction of being the first Asian Class A member of the Association of American Medical Colleges (Estrada, 1979). Such honor places UPCM side by side with other medical schools not only in the United States but also in the entire Western Hemisphere. Its products have been consistent topnotchers in the national licensure examinations since the test was instituted (Records of the Professional Regulation Commission and the UPCM). Its graduates have become distinguished members of various faculties of medicine, government servants and advocates of various causes.

This paper studied the clinical training program of UP-PGH. It also determined the various traditions that are socially reproduced in the course of the clinical training. In the process it also explained how the different social structures involved in the training interact with each other in socially reproducing the future medical professionals.

Research Framework

Medical education is a professional socialization experience that involves not only the acquisition of knowledge and skills but also and perhaps more importantly, the acquisition of attitudes, values and ethics (Knight in Wolf et al, 1989). This socialization is operationalized in the form of the medical education's three types of curricula: the official, operational and the hidden.

The formal curriculum includes those proposals that are formally approved by such bodies as the state and local boards of university councils (McNeil, 1990). This might be a collection of ideals suggested by governments, non-government

and special interest groups as to what should constitute the goals and objectives, the content and the structure of the program of study. The operational curriculum refers to what actually goes on in the classroom. Literature attests to discrepancies between what teachers say the hidden curriculum is and what they actually do (Taba, 1962). The last one refers to the hidden curriculum that is about the unofficial instructional influences that either weaken or strengthen the manifest goals. They are the knowledge, attitudes and skills that students learn from but which their teachers and the school itself may not consciously teach.

At the medical centers, the official, operational and hidden curricula all take place in one highly specialized physical environment, a self-contained social universe. In this milieu are manifest the various norms and traditions that are responsible for the social reproduction of those who seek to be formally part of it. Giddens (1984) explains how the process of social reproduction takes place through his theory of structuration. He wrote that the "rules and resources drawn upon in the production and reproduction of social action are at the same time the means of system reproduction". Rules are methodical procedures of social interactions (Giddens, 1984). In this study, rules pertain to the three types of curricula just mentioned. The second variable refers to resources. They are distinguished into allocative and authoritative types. The former refers to the capabilities that enable actors to generate command over objects, goods or material phenomena. They include raw materials, land, etc. Authoritative resources refer to the types of transformative capacity generating command over other persons or actors. In this study, medical knowledge, clinical competence, interns, clerks, their resident physicians, etc. are some examples of these authoritative resources. In Giddens' theory of social structuration (1984), these rules and resources (also called structures) are recursively involved in institutions: the more enduring features of social life. The structures of social system are those with institutionalized features, giving solidity across time and space; they are "circuit switches" underlying observed conditions of system reproduction.

Methodology

This is a descriptive research design. Secondary data including the official curriculum of UP-PGII, the clinical clerkship and internship training programs bulletins, annual reports, and other bulletins were used. Collection of secondary data was done from 1996 to 1997.

Primary data came from 3 sources. Participant observation was done with 4 blocks of interns and 1 block of clerks who rotated at PGH's four core clinical departments namely Obstetrics-Gynecology, Pediatrics, Surgery and Medicine. This was done for 54 days from May to July 1997. All students from the five blocks, their resident physicians and their faculty consultants were also asked to accomplish a survey questionnaire. Response rate from students was 25 percent, 19.35 percent for the residents and 25.27 percent for the consultants. A key infor-

ment interview was also conducted in the course of the participant observation. Selected patients and their watchers (*bantays*) who were under the care of key informants were likewise interviewed. Data were analyzed both qualitatively and quantitatively.

Data showed that for school year 1997-1998, UP-PGH trained a total of 234 interns. This group was divided into 26 blocks of nine students each. Clerks on the other hand, totaled 141. They were grouped into 20 blocks of 7 to 8 students each. The internship program began on May 1, 1997 and ended on April 30, 1998. All teaching hospitals in the country have the same timetable for internship. Clerks on the other hand, began on June 18, 1997 and ended on April 6, 1998.

Findings

The Tradition of Excellence. The curricula of the UP College of Medicine (UPCM) and the PGH have strong built-in mechanisms to make interns master the science and art of medicine. The first among these mechanisms is the *strict selection criteria for admission* to the UPCM and PGH. Only those who have the highest academic credentials and the most promising as determined during the selection interview were admitted to this premier institution. In the case of the post-graduate interns (graduates of other medical schools are at PGH only for internship), only those with the highest academic credentials and promise were admitted. Resident A2 said:

"The ones admitted at PGH are the tops in the batch (from the original medical school). *Magagaling din sila talaga.*"

"The ones admitted at PGH are the tops in the batch of their medical schools. These are their cream of the crop and are expectedly the best in their school."

Another built-in character of UP-PGH that perpetuates the tradition of excellence refers to the very *content of the official curriculum*. All respondents from the groups of medical clerks, interns, residents and consultants acknowledged that UP-PGH's clinical training is a multi-faceted learning experience that equips trainees with a thorough grasp of basic medical knowledge. All these experiences are more than adequate because they are first-hand, hands-on learning encounters. Clinical supervisors reported in the survey that the students:

"are exposed to "virgin" (referring to previously undiagnosed) cases; make plans on their own; actually interact with patients; literally manage patients; get an actual exposure to clinical settings; are provided hands-on experience; are exposed to a fertile learning ground; theoretically should sharpen their problem-detection skills; they get to serve especially charity patients; they develop endurance, resourcefulness and rapport with patients."

Once admitted, *student performance is consistently being monitored by built-in formative and summative evaluations from various raters*. During the first two

years where students learn their basic sciences up to the next two years where they learn their clinical sciences, their knowledge, skills and attitudes pertinent to the practice of medicine build up in preparation for their final year internship.

During internship, students are assigned into groups of 7 to 8 and join the hospital's various service groups. These service groups are the workforce of the medical center and are composed of the consultant (faculty) as head, the senior residents, junior resident, the interns and then the clerks. While clerks are given partial patient responsibility, interns are given full patient responsibility under the guidance of their clinical supervisors. Both rotate in all clinical departments' outpatient, emergency, intensive care and ward sections, including other specialized areas like the nursery, the operating and recovery rooms. In the course of these rotations, they repeatedly perform patient interview and history taking, accomplish written assignments like progress notes, report of laboratory results, journal reports and endorsement reports; they are also expected to give direct patient care including executing basic clinical procedures, monitor vital signs and counseling. Furthermore, they are also expected to be present during medical audits, special case conferences, grand rounds with the department's most prestigious clinicians and various academic and co-curricular exercises of the hospital like, e. g. medical missions, blood letting, etc.

The sheer volume of patients already guarantees that interns and clerks are exposed to as many clinical materials as possible. Even Postgraduate interns (PGIs) acknowledged this. The following statement given by Intern B1 shows this comparative advantage:

'Like 'yong matangkad, he's from Medical School X (referring to a PGI). And then he's gonna endorse. At the first endorsement conference *noang bagong pasok siya, merong case na nakasulat sa board: "PNH". Sabi niya, binulang niya: "ano 'yong PNH?" So sabi naming mga taga-L/P, 'Paroxysmal Nocturnal Hemoglobinuria. Have you never heard of that?" (Laughs). Kasi dito sa PGH, araw-araw, may makikita ka; well, not araw-araw, mga every week, may makikita ka. Mind you in his whole medical school, wala pa siyang na-e-encounter! And the funny things is, two days later, na-admittun siya ng PNH!'*

"Like in the case of X, the tall guy. He is a graduate of Medical School X. And then he's gonna endorse. During our first endorsement conference, he noticed "PNH" written on the board. He whispered to us who are graduates of UPCM. "What is PNH?" So we said "Paroxysmal Nocturnal Hemoglobinuria. Have you never heard of that?" (Laughs). Here at PGH, we see cases of PNH almost every week and yet he never encountered it in his medical school! The funny thing was that two days later, he had to admit a patient who was diagnosed of PNH!"

Similarly, Intern D2 from a medical school in Mindanao (a separate island in the far south) excitedly admitted that in his first few weeks of rotation, the facilities at PGH are simply novel. Intern D2 said:

"I never had the experience to do any ABG (arterial blood gas) test back in (Medical School). *Kaya pagdating ko dito, sabi ko sa lahat ng block mates ko, aka na ang gagawa ng lahat ng ABG ninyo!*"

"I never had the experience to do any ABG test back in (Medical School). That's why when I came here, I told all my block mates to let me perform all their ABGs!"

The volume of patients also provides trainees the opportunity to learn from their patients. They are trained to be well rounded in terms of relating to patients and their relatives. In this aspect, the attitudes of students regularly pass through a reality check. Intern A1 vividly explains:

"No one goes through internship or even clerkship without encountering an obnoxious patient, or an impatient patient, or a rude patient. There are a lot of people who are like that. Because I just think that well, that's because they're in pain. Or you think up of excuses such as they are brought up like that. No one taught them that they couldn't say bad words, stuff like that. We just disregard them. You can't let them get into you".

The operational and hidden curricula also contribute strongly to the tradition of excellence although not in the same plane as the official one. During clinical rotations, clerks and interns admit that there are a lot of unpleasant experiences consciously and unconsciously being provided to students. Analysis of this feedback, however, suggests that students manage to transform these unpleasant experiences into real learning encounters and they end up to be better equipped in performing their role.

During their rotation at the wards, the Intensive Care Unit (ICU) and the Emergency Room (ER), clerks and interns join their service groups during the rounds. These would have been the most intimate teaching-learning situation because the whole group is already with their "material" for learning: their patient. However, because there are 20 to 30 patients to be visited in a full-packed ward, rounds become a short-cut teaching-learning encounter. Intern C6 explains:

"*Maganda yong mga sinasabi nila, pag may sinasabi sila! Pero a lot of time, wala eh! (laughs). Nakatayo ka lang don. Sila (the junior and senior residents) lang ang nag-uusap. Di ko maintindihan ang pinagsasabi nila.* Or you try to join in *nga*. But *uutusan ka* to do something. *Mawawala* for a while and you lose the flow."

"They (the senior and junior residents) explain many important things (during the rounds); that is, if they feel like explaining! But a lot of time, they don't! (laughs). We just stand there. They are the only ones talking; I don't understand what they are talking about. Sometimes, I try to participate join in the discussion but they will ask me to run some errands for them; I leave the rounds and then I lose the flow of discussion."

The scout work, which is an integral part of the rotations, was likewise a most trying experience for the clerks and interns. Residents agree with the students that doing this makes them almost like monitoring machine. They exclaimed:

"kayod kalabaw" manual monitoring, do labs, follow-up labs, monitor to death! Too much robotic work! The nurses should do most of them!!!; Monitoring? We learn nothing; so much clerical work; students being treated as technicians; the work is so physical, you don't need to think; very, very tiring; mindless work, pag walang bantay, patay na kami."

"We work hard like the carabao: we do manual monitoring, do laboratory procedures, follow-up the laboratory results, monitor to death! ... If there are no watchers (who they ask to follow-up the laboratory results), we are dead."

The researcher observed how many patients would need monitoring of their vital signs every hour or sometimes even less. On top of these, the regular paper works like their progress reports, analysis of laboratory results, journal reports and preparing oneself for the morning endorsements become daily routines that interns have to reckon with regardless of their physical conditions. A review of secondary data reinforced these observations. These trainees have apparently expressed patient overload and having to accomplish too many tasks in a single duty day many years ago. The following narrative of a former UPCM clerk would be sufficient to capture these observations:

"Many bewail that they are left with so little time to read textbooks or journals. What makes it frustrating is the time spent on nonessentials because of the lack of organization and system. They would point out the time wasted in following up referrals, going to Xray, or rushing to the Central Laboratory to bring specimens. It is our contention that one's learning capacities are also affected by the material and psychologic conditions around him; and the one with a fresh mind can absorb much more from his readings than another whose mind has been dulled by fatigue and lack of sleep."

But the ultimate test of how well this entire system is doing is through getting feedback from patients, the receivers of the health care service. When asked about their impressions about the UP-PGH clerks and interns assigned to them, patients expressed:

"Mukhang gumagaling ako dito; apat na buwan ako sa (name of an established, private hospital in the Metropolitan Area); hindi na nga ako gumaling ang mahul pa ng gastos! Salamat po sa PGH, maganda naman mag-asikaso; basta may konting diperensiya, sabihin mo sa kanila (clerks and interns decked to them). aalagaan agad. Salamat sa PGH. Sa aming mahhirap, libre ang serbisyo. ...Ang mga doktor dito lahat mahabait, approachable..."

"I think I am improving here; I had been confined at (name of established, private hospital in the Metropolitan Area) for four months. It was ironic that I did not get better there but I had to pay so much! I would like to thank the PGH. The clerks and interns here do their job pretty well; I tell them of any trouble with me and they attend to it immediately. Thanks to PGH for free service; all my doctors here are good and approachable."

The collective feedback provided by patients, residents, consultants, clerks and interns interviewed and observed means that given the most difficult and trying clinical training, the trainees learn not just their basic medical knowledge. More importantly, they also acquire mastery of this knowledge to the high degree of satisfaction of both their clinical supervisors and immediate clients, the patients. This further proves that in fact, the experiences provided by the official curriculum are being reinforced and supplemented by the *"pahirap"* (most unpleasant tasks) of the operational and hidden curricula to produce most competent, time-tested physicians. The whole collection of the experiences of these clerks and interns can be equated as just part of the "strict and long period of formal training necessary for the profession." Such experiences can be interpreted as necessary evils in order to ensure that these clerks and interns would have the knowledge and attitudes that will make them pass the strict requirements of their chosen profession. Similar to initiation rites, satisfactory performance during clerkship and internship is apparently UP-PGH's idea of granting its students "rites of passage" to the medical profession.

The Batch Culture The development of a strong, cohesive and functional learning group is also heavily built-up in the UP-PGH curriculum. From first to internship year, students are grouped alphabetically into blocks of 6 to 9 students each. Interns B1 and B6 explain how their groups developed into a solid one whose history can be traced back as early as their first year:

"Siyempre, sanay na kaming magkakasama; since first year kasi dahil sa aming mga apelyido. First year pa lang, group mates ko na sina at Hanggang ngayon (internship) kami pa rin!"

"Of course we are used to each other; we have been together since first year. We are grouped according to our surnames. Since first year, and we have been group mates. Up to now, at internship."

The blocks passed through the normal stages of group development. They went through forming stage because their blocks simply had to be formed because of numerous tasks to be done; then the storming stage where members struggled with each other or came into conflict as they tried to do the task and at the same time adjust to each other's gears (Forsyth, 1983). This was followed by norming stage where the group reached an equilibrium point of setting rules and standards of performance acceptable to all members. Performing stage followed where all members of the blocks do their respective tasks to accomplish the group goals. Blocks develop into a very strong group with a sense of social integration as they pass through good and bad times. They were together during their "first" like their first successful execution of arterial blood gas test, episiotomy, and major assists in the operating room. This strong integration hardly wanes long after graduation. This is inferred from their resident physicians' culture. Most become classmates again during residency training while one out of a block of nine students was engaged or married to a classmate or a block mate! The whole experience shows that the interns practically grew up together both as professionals and persons.

The entire process of group experience of both clerks and interns at UP-PGH indicate that they are being developed as a uniform elite. Giddens (1984) describes this group as one that shares the attributes of having a restricted pattern of recruitment and of forming a relatively tight unity. This type has clear sense of class-consciousness because the members share a common set of professional and social attitudes, beliefs and values all linked to a common occupation or class. Not only did they pass through a rigid recruitment procedure upon entry to the medical school; they are continually being "recruited" in the entire duration of their medical education as their performance is always being tested for any sign of incompetence or disqualification.

The Culture of Coping. The blocks made clinical training and medical school bearable to interns. Together, they take part in various practices that enabled them to cope with a rigid training. Some of these identified were "*gulangan*" - meaning to take advantage of somebody. While blocks are generally functional working groups, there are some that are really difficult to deal with. These "*magulang*" (wise, scheming people) block mates would just disappear and abandon their posts, log-in a different time if they came late, etc. Then there was "hoarding". Interns hoard extra pairs of gloves, syringes, gauze, etc. when they rotate in some generous ward. They used these stuffs when they rotated in areas where such were not readily available. Another statement from Intern B1 clarifies this culture:

"We call this hoarding. The interns are the ones redistributing supplies. It's like in Department X *ang dami-daming* syringes. *Pumunta ka sa Department Y, walang* syringes. *Pog nasa Department Y na kami, imbis na matoxic kami dahil walang syringe, kukuha lang kami sa dinakwat namin!*"

"We call this boarding. The interns are the ones redistributing supplies. It's like in Department X, there are a lot of syringes. Then you go to another Department Y, there is a shortage of syringes. What we do if we are rotating at Department X, we keep a few syringes. So that when our time to rotate at Department Y comes and we would have immediate need of syringes, we have a ready supply of the needed stuff."

Another tradition identified was the *sunugan* (literally to burn as in grilling somebody) ritual. Intern B4 defines it as:

"*Sunugan* is defined in one word: it's revenge! Whoever has brushed you aside differently or whoever pissed you off. You really want the world to know what an asshole this person is- sorry for the language! But it's a way for us to come out na... On a more positive note, it's also a venue to praise people na rin. *So pinupuri rin namin ang mga taong bilib ka o sa mga taong how ka sa kanya* kasi he's really nice or he's really good."

(Words in italics: means the ritual serves also as the occasion when interns praise and thank all those who inspired them and made them feel good).

This ritual was usually held at the last day of internship. During *sunugan*, interns gather together, they bring placards, whistle, snare drums and even karaoke and revisit all the areas where they rotated. They would jeer the persons they want to grill at the top of their voices. They do the same thing to those they want to praise. The tradition was apparently an accepted one because the various departments and their respective personnel showed willingness to accommodate their "visitors".

Sunugan represents a tradition where interns can temporarily set aside their roles and pursue an opportunity for group therapy. It shows that in spite all difficulties they encounter during the training, they are still willing to express their dissent through the use of legitimate and/or generally acceptable means of social control. Even if some sectors get offended, the whole community does not perceive it as destructive. This is in contrast with dissent expressed through violent means that threaten the very existence of society. This may pave the way for deviance and may require another set of rules for control.

Perpetuation of hierarchy and inequality. There is a strong tradition of hierarchy and inequality at UP-PGH among physicians and between medical and non-medical personnel. Interns perceived this as being a prey to an array of superiors. This sentiment was expressed in the graffiti written all over their call room. One graffiti captures it all:

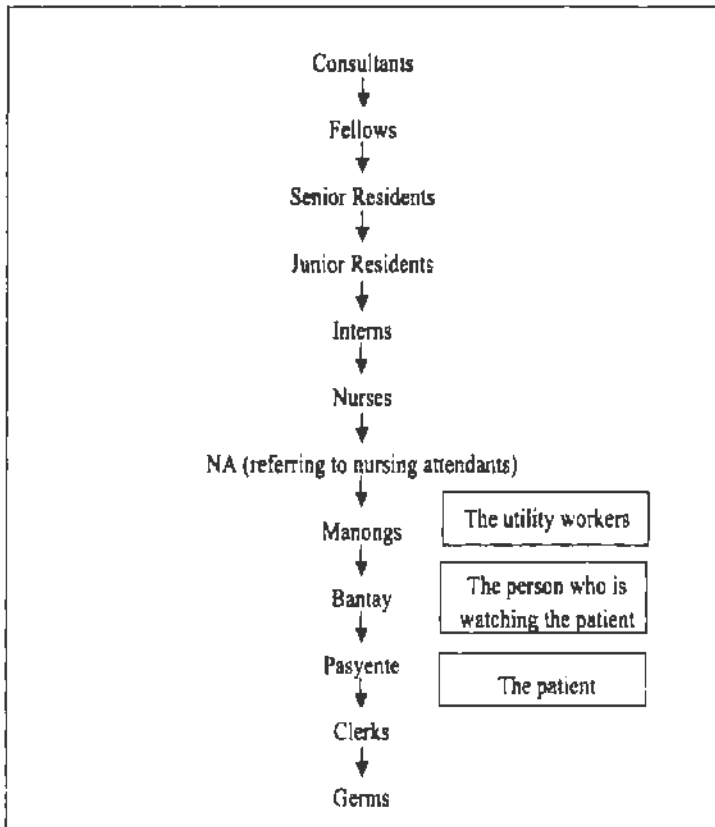


Figure 1. Food Chain in (Name of Ward): by order of harassment

If there is clear hierarchy and inequality among medical professionals, such is even marked between medical and non-medical personnel. This is obviously a source of discomfort between the two groups as revealed in many reminders such as this poster found at the Blood Bank:

"Only Blood Bank personnel are allowed in working areas.
 Others should stay in reception areas including DOCTORS."

Sgd. Blood Bank Head

Similar posters are found in the other common areas where all members of the health team come and go like the entrances and exits of the hospital. The warning specially addresses the doctors who seem to always be able to get their way around to the frustration of those in the other professions.

CONCLUSIONS

This study looked at the formal, operational and hidden curricula of UP-PGH internship program. Findings revealed how these are carried out in the unique learning environment and point out the following conclusions:

1. *Socialization of a uniform elite.* Findings prove that the social reproduction of the medical profession is essentially the reproduction of a uniform type of elite group. This refers to a group of people who have been chosen out of very strict selection procedures. As they undergo a particular experience, these people find themselves forming into a highly cohesive group with very strong feelings of attachment to each other. Not only were the clerks and interns together as a functioning learning group during study sessions and examination periods, they were literally together during their most trying times in medical school. The long hours they spent during their clinical rotations, covering for each other, cheering for one another and just by being there, the groups naturally evolve into one where their sense of unity could stand the test of time.
2. *Competence gap among the professions.* Data also strongly suggest the reasons why among the health professionals, those in the medical profession are accorded the highest esteem. During their medical training especially during clerkship and internship, students are trained to have a strong grasp of medical knowledge as they apply them in various clinical cases. Through oral, written and practical examinations, through continuous reviews of patients' charts and coaching from their seniors, these medical students are socialized to have in their possession the strongest resource that other professionals will never have: that of medical knowledge. While other professions emphasize the learning of "processes", those in the medical profession learn a rigorous scientific discipline as the "content" of their training (Wesibord, 1985). The medical profession therefore develops into a group that has the moral and social integration of a uniform elite, with the power of a professional class based on the acquisition and mastery of medical knowledge. The profession is primarily knowledge-based which evolves into allocative and authoritative resources, generating for the medical personnel command over other people or institutions (Giddens, 1973). Furthermore, the study also showed that the medical profession is highly respected by those from the health-related professions and the non-health personnel, e. g. patients and their relatives. The study supports the classic findings of Freidson (1970) and Parsons (1951) that because physicians possess medical knowledge, there

develops a sort of competence gap which leads to a social stratification not only among those in the field of medicine but moreso, in the general society. Waitzkin and Waterman (1974) summarized this main argument as:

"The differential possession of technical knowledge as one source of professionals' power to shape the action of their clients. In this sense, the "competence gap" between professionals and clients creates a stratified relationship in which professionals hold a super ordinate position and clients occupy a subordinate one".

3. *Preservation of the status quo.* The competence gap that is being inculcated by the medical professions in itself explains the conservative nature of the said profession. The official curriculum maintains that medicine is a profession that can only be practiced by the most competent people so its selection criteria and the entire medical training remain strict, difficult and challenging. To survive the training, medical clerks and interns develop coping mechanisms from both extremes: by simply working as hard as necessary and negatively, by indulge in some acts of cheating and getting even. These identified coping mechanisms are all in the personal level; they do not require major structural adjustments from both the trainees and the medical center. To both, the whole collection of the experiences, pleasant and non-pleasant only serves as their "rites of passage" to the medical profession. The ultimate result is the reproduction of a breed of medical professionals who are exactly the same as their mentors, a social reproduction of the same type of doctors equipped with all the competencies traditionally developed by the medical center, and also with the norms, traditions and values inherent within the profession. The study affirms that the medical profession is one that has built-in capacities to acquire legitimate authority and high occupational prestige; therefore it is expectedly conservative.

ACKNOWLEDGMENTS

This paper would not have been possible without the administrative blessings of the researcher's institution: The University of the Philippines Manila (UPM), the health science campus of the University of the Philippines System. UPM houses both the UP College of Medicine and the Philippine General Hospital. Gratitude is expressed to then Chancellor Perla D. Santos Ocampo, MD and her Vice-Chancellors Dr. Lourdes A. Abadingo (Academic Affairs), Dr. Alejandro De Leon (Planning) and Dr. Napoleon A. Apolinario (Administration). The Dean of the National Teacher Training Center for the Health Professions, Dr. Cristina F. Mencias, facilitated the recommendation for approval of my study leave with pay.

This study would also not have become completed without the able guidance of my dissertation panel composed of Drs. Josefina N. Natividad, Eufracio C. Abaya, Maria Cynthia Rose B. Bautista, Josefina G. Tayag and Milagros D. Ibe. The research grant awarded to this study by the College of Social Sciences and Philosophy, University of the Philippines Diliman is also appreciated.

LITERATURE CITED

- Estrada, H. (1979) *Proceedings of the Thirty Second World Medical Assembly*. Manila: University of the Philippines Press.
- Forsyth, D. 1983. *An Introduction to Group Dynamics*. California: Wadsworth, Inc.
- Freidson, E. 1970. *Profession of Medicine: A Study of the Sociology of Applied Knowledge*. New York: Dodd, Mead and Company.
- Giddens, A. 1973. *The Class Structure of Advanced Societies*. London: Hutchinson University Library.
- Giddens, A. 1984. *The Constitution of Social Life*. Cambridge: Polity Press.
- McNeil, J. 1990. *Curriculum: A Comprehensive Introduction*. Illinois: Scott, Foresman, Little Brown Higher Education, Inc.
- Parsons, T. 1951. *The Social System*. Illinois: The Free Press.
- Sparz, L., et al. 1988. "The Doctor-Patient Relationship During Medical Internship: The Evolution of Dissatisfaction." *Social Science and Medicine*. 26: 11.
- Taba, H. *Curriculum Development: Theory and Practice*. 1962. New York: Harcourt, Brace, Jovanovich, Inc.
- Waitzkin, H. 1991. *The Politics of Modern Encounters*. New Haven: Yale University Press.
- Waitzkin, H. and Waterman, B. 1974. *The Exploitation of Illness in Capitalist Society*. New York: The Bobbs-Merrill, Co., Inc.
- Weisbord, M. 1985. "Why Organization Development Hasn't Worked (So far) in Medical Centers". *Organizational Diagnosis: A Workbook of Theory and Practice Health Care Management Review* 1: 2.
- Wolf, T. M., et al. 1989. "A Retrospective Study of Attitude Change During Medical Education". *Medical Education*. 23: 19-23.

ABSTRACTS OF TECHNICAL PAPERS FOR POSTER PRESENTATION

MATHEMATICAL, PHYSICAL, AND ENGINEERING SCIENCES

1. NONSINGULARITY CONDITIONS FOR TWO CLASSES OF CIRCULANT GRAPHS

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A graph is said to be *singular* if its adjacency matrix $A = A(G)$ is singular, otherwise, G is said to be nonsingular. For example, the complete graph K_n is known to be non-singular for $n \geq 1$, while the cycle graph C_n is singular if and only if n is divisible by 4.

A matrix $A = [a_{ij}]$ is said to be *circulant* if for each $i \geq 2$, the element of row i are obtained by cyclically shifting the elements of the $(i-1)$ -th row one position to the right. Graph G is circulant if its adjacency matrix is circulant.

In this study, we consider two classes of circular graphs. For $n \geq 3$ and $1 \leq r < n$, the graph C_n^r or the r -th power graph of the cycle graph C_n is obtained by forming the edge xy whenever there is a path of length less than or equal to r joining the two vertices x and y . On the other hand, we denote by $C(r, n)$ the circulant graph of order $2n$ formed by adding to the graph C_{2n}^r the edges joining opposite vertices of the cycle graph. Our aim is to determine conditions under which these two classes of graphs will be nonsingular.

Key words: singular/nonsingular graphs, circulant matrix, circulant graphs, r -th power graph, eigenvalues, adjacency matrix, cycle graph, greatest common divisor, least common multiple

2. PULSED 1064 nm Nd-YAG LASER DEPOSITION OF TITANIUM ON SILICON IN AN AMBIENT NITROGEN ENVIRONMENT

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Pulsed laser deposition (PLD) technique was demonstrated for the deposition of titanium nitride (TiN) thin films on Si (100) substrates using 1064 nm excitation. The target material used was titanium (99.5%) and deposition was done under ambient N₂ pressure. Spectroscopic analysis of the plasma plume revealed emission lines due to Ti(I) and N(I), which are the active species that lead to the formation of TiN. Images of the films grown at different laser pulse energies show an increase in the number and size of deposited droplets and clusters with increasing laser pulse energy. A decrease in cluster and droplet size is observed, with an increase in substrate temperature. EDS data show an increase in the Ti peak relative to the Si peak as the ambient N₂ pressure is decreased. An increase in deposition time was found to bring about the growth of large clusters and irregularly shaped structures on the substrate. Post-deposition annealing of the samples enhanced the crystallinity of the deposited thin film.

Key words: pulsed laser deposition, titanium nitride, laser ablation, laser-produced plasma, optical emission, Nd-YAG laser, SEM, XRD, titanium, nitrogen

3. TOURNAMENTS THAT ARE NOT RESIDUALLY GRACEFUL

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A digraph consist of a set of vertices and some directed line segments, culled arcs, joining pairs of vertices. Let m be the number of arcs of a digraph D . Label the vertices of D using distinct values from the set $\{0, 1, 2, \dots, m\}$. If x and y are vertices forming the arc xy , assign to it the value $f(x)-f(y)$, were $f(x)$ and $f(y)$ denote the labels given to the vertices x and y , respectively. If all these values assigned to the arcs are distinct modulo m , and none of them is 0, the labeling is said to be a *graceful labeling* and the digraph is called a *graceful digraph*. This

was the concept introduced by Bloom and Hsu in 1982. They noted that such a concept of labeling has an application to a problem on network addressing.

In 1999, the author introduced a similar labeling as follows. Label the vertices of the digraph using distinct values from the set $\{0, 1, 2, \dots, m\}$. Also compute for the induced arc labels $f(x) \cdot f(y)$. If these values form a complete residue system modulo m , the labeling f is called a *residually graceful labeling* and the digraph is called a *residually graceful digraph*. In an ongoing research, the author has shown that there exist digraphs that are graceful but not residually graceful, and vice versa.

In this paper, we consider a special class of digraphs called tournaments. Consider a round-robin tournament involving n players. Let the players be vertices of a digraph. We form the arc from a vertex to another vertex y if and only if x beats y . The resulting digraph is called a tournament of order n . There are no published results yet on residually graceful digraphs inasmuch as the concept was an original idea of the author was introduced less than a year ago only. The author has obtained some results on graceful and residually graceful paths and circuits.

Examples of tournaments that are residually graceful are given in this paper. Likewise it establishes the existence of some tournaments that are not residually graceful. Sufficient conditions on the order of a tournament for it not to be residually graceful are established.

Key words: digraph, vertex, arc, path, circuit, graceful labeling, residually graceful labeling, tournament.

4. ASSESSMENT OF INFLUENTIAL OBSERVATIONS IN PRINCIPAL FACTOR ANALYSIS

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In the present study, a method for detecting influential observations using iterative principal factor analysis is proposed. To do this, some influence functions $I(x; LL^T)$ and $I(x; \Delta)$ were derived for the common variance matrix $T = LL^T$ and the unique variance matrix Δ , respectively. The main objective here is to investigate the influence of a small change of data on the result of the analysis. To assess the influential observations, some influence measures like the Euclidean norm of $\Delta^{(i)}$ and $T^{(i)}$ were derived which correspond to the theoretical influence

functions for the two components Δ and $T = LL^T$ of the common variance decomposition. The results of the study have shown that the application of the Principal Factor analysis (PFA) to the given data set clearly revealed a two-factor model. Furthermore, the proposed influence function at $\Delta^{(j)}$ showed that the Empirical Influence Curve (EIC) based on the differential coefficient can be used in practice instead of the Sample Influence Curve (SIC) for detecting influential observations in PFA.

Key words: influential observations; influence function; principal factor analysis; perturbation theory of eigenvalue problems; unique variance matrix; common variance matrix

5. INTRODUCTION TO THE JLC STUDY FRAMEWORK

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The importance of computers in the field of High Energy Physics (HEP) is beyond question. Computer simulations have been utilized to guide experiments, predicting the possible outcomes, providing a valid comparison to acquired data based on accepted or proposed physical theories.

In the last 10 years, wide experimental efforts have been poured to detect the last undiscovered particle in the Standard Model – the Higgs boson. Huge experimental projects have been initiated in the United State and Europe to this end. In consonance with this worldwide endeavor the Asian HEP community proposes to build its own: the Joint Linear Collider (JLC). The JLC will be built in Japan.

Here we present a software designed to meet the needs for HELP studies in the JLC: the JLC Study Framework (JSF). We then discuss the processes of event generations, simulation, event reconstruction, data acquisition, and data analysis in JSF using as an example the Standard Model Higgs production process $e^+e^- \rightarrow Z^0 H^0_{SM} \rightarrow q\bar{q}bb$.

Key words: high energy physics, JLC study framework, Higgs boson, computer simulation

6. COMPUTING IN A BEOWULF CLASS COMPUTER SYSTEM

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Current PCs have achieved a performance comparable to the high-end UNIX workstations, at a small fraction of the price. Commodity PCs have become the solution to CPU needs, even for large scale computing in physics. The development of free software has made computations even easier.

The Beowulf class computer system is a type of parallel or distributed system, which consists of interconnected commodity-of-the-shelf (COTS) personal computers working together as a single integrated computing resource. A certain computational task could be divided among the computers to fasten execution of the task.

At MSU-IIT, we built a three-node Beowulf system. Each node has two 350-MHz Pentium II processors. All softwares used are freely available in the Internet. Communication of the results between nodes is done by message passing library Message Passing Interface (MPI), or Parallel Virtual Machine (PVM).

Initial computations show that the performance of the MSU-IIT Beowulf system is 5 to 6 times faster compared to a single processor PC. Since a Beowulf computer system is expandable, installing additional nodes to the existing system increases its computational power.

We can thus build a system with performance comparable to high-end RISC-based computers at a small fraction of the price.

Key words: computing, Beowulf system, computer, message passing interface, parallel virtual machine

7. THE FULL PINCH TECHNIQUE GAUGE INVARIANT HIGGS BOSON SELF-ENERGY

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The Pinch Technique (PT) is an algorithm that renders one-loop gauge and scalar boson self-energies of renormalizable spontaneously broken non-abelian

gauge field theory gauge invariant. In scattering processes at one-loop level, PT unravels self energy contributions from vertex and box diagrams that are otherwise excluded in the conventional manner of computing self energies. In general, by itself, this pinch self-energy contribution is gauge dependent. When combined with the conventional self energy, this PT contribution exactly cancels the gauge dependence of the former rendering the combined self energy gauge invariant. The resulting PT gauge invariant self energy satisfies desirable properties like resummability, unitarity of the S-matrix, and process dependence.

The PT arose from a search for a self consistent scheme for constructing off shell Green's functions which are of utmost importance in cases where the conventional perturbation theory breaks down like in the strongly coupled theory of Quantum Chromodynamics and in the vicinity of resonance in a weakly coupled theory of electroweak interaction in the standard model.

In this work, the S-matrix PT framework inspired by Degrassi and Sirlin is implemented in calculating the full PT contribution to the Higgs boson self energy in the general renormalizable $R\xi$ gauge. The scattering process considered is a four-fermion process with the Higgs boson as intermediate state. The relevant amplitudes reflecting the gauge boson and external fermion interactions are described in terms of matrix elements of Fourier transforms of time-ordered product of current operators. Through successive current contraction with the longitudinal four-momentum found in the propagator of massive vector bosons, Ward identities are triggered. Relevant pinch contributions are then identified upon application of appropriate equal-time commutators of currents.

The results obtained have the following desirable properties. The full Higgs boson PT contribution vanishes on-shell, which is a welcome property if one adheres to the correctness and validity of the Born approximation to the decay width of the Higgs boson. In the 't Hooft-Feynman gauge, the full PT result agrees with Papavassiliou's and Pilafitsis' results. For gauges other than the 't Hooft-Feynman gauge, our results differ only with respect to the UV-quadratically divergent terms of the result whose origin may be traced to the contributions coming from tadpole and seagull graphs, which they omitted in their consideration. The significance of these UV-quadratically divergent terms should not be underestimated though, since they render the non-absorptive part of the Higgs boson self energy gauge independent as well. Finally, our results shows that the full PT gauge invariant Higgs boson self energy evaluated in the framework of the general renormalized R_ξ -gauge is identically equivalent to the full PT Higgs boson self energy 't Hooft-Feynman gauge, a property likewise found in Papavassiliou and Pilafitsis.

Key words: Higgs boson self energy, elementary particle physics, electroweak interactions, non abelian gauge field theory, S-matrix Pinch Technique, gauge invariance, radiative corrections, perturbation theory

8. NEW MEASUREMENT OF MEAN LIFETIME OF ATMOSPHERIC MUONS

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Recent measurements on the mean lifetime of atmospheric muons have been carried out through the months of August to October 1999 at IITHEP Laboratory, Iligan City. A vertical stack of three plastic detectors is utilized to identify cosmic ray muons decaying in a wooden absorber. The method employed is a measurement of the distribution in duration of the time intervals between the stopped cosmic-ray muons in plastic scintillation counters and the detection of the decaying electron in the downward direction.

The standard nuclear physics instrumentation, NIM and CAMAC, are used in this study. The experimental methods and technique are reported. A brief description on the properties and the electro weak decay of muon $\mu \rightarrow e \nu_e \bar{\nu}_\mu$ are also discussed.

Muon decay time distribution curve is shown where a fit of the distribution to the exponential ($-1/\tau_\mu$) yields a mean lifetime $\tau_\mu = 2.176 \pm 0.0429$ μsec , a value, which is significantly in good agreement with internationally accepted μ mean lifetime $\tau_\mu = 2.197 \pm 0.0004$ μsec as presented by the Particle Data Group.

Key words: muon lifetime, NIM, CAMAC

9. MBE GROWTH OF ZnTe/Zn(S, Te) SHORT-PERIOD SUPERLATTICES

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ZnTe/Zn (S, Te) short-period superlattices have been grown by our group on (001) GaAs substrates with very good structural quality. The growth conditions were found to be quite reproducible, leading to a series of samples with periods

between 12 to 29 angstroms. Characterization of the samples with high resolution x-ray diffraction confirmed the high structural quality of the samples showing that all are pseudomorphically grown. The relaxation behavior is strongly influenced by the ZnTe well-width with two critical ZnTe-thicknesses observable.

A substrate temperature of 280°C was chosen in order to avoid Tellurium clustering. ZnS was deposited from a compound source allowing the growth rate of ZnS at four different substrate-temperatures to be measured. Our group found from laser interferometric oscillations that the growth rate decreased from one Å/sec at 150°C to 0.8 Å/sec at 220°C. No ZnS growth could be observed at 240°C. Zn(S, Te) barriers with low Te content were grown instead of ZnS barriers.

A 200 nm-thick GaAs buffer was grown on (001) GaAs substrate in a III-V epitaxy chamber after oxygen desorption. The sample was then transferred under ultra-high vacuum to a II-VI changer. A 20 nm-thick ZnSe buffer was deposited followed by a ZnTe/Zn(S, Te) superlattice structure. A series of lattice-matched samples with intended periods of 1, 18, 24, and 30 angstroms were grown. The numbers of periods were between 120 and 200 leading to a total layer thickness of between 200 nm and 400 nm.

The superlattices were grown under Zn-rich conditions. Zn, Se, and Te were evaporated from elemental sources while ZnS was used in its compound form. A growth temperature of 280°C was chosen for all samples. The shutter opening times were varied to achieve lattice-matched samples with different periods. The beam fluxes were kept constant during the growth of the whole series. To circumvent the immediate relaxation of ZnTe grown on GaAs due to a very high lattice mismatch, the first ZnTe well was made to be only half as thick as the others. This first ZnTe well was then counterstrained by the succeeding Zn(S, Te)-barrier.

All samples were characterized with high resolution x-ray diffractometry (HRXRD). To determine the superlattice period, lattice constant and the strain, Ω -2 θ scans were performed around the (004)- and the asymmetrical (115)-reflections of the GaAs substrate. To distinguish between mosaicity and inhomogeneity, omega scans around the (004)-reflex of the zero-order satellite of the superlattice were carried out using a (200) four-crystal monochromator with an x-ray mirror on the incident side and a three reflection (220) Ge analyzer-crystal on the exit side of the diffractometer.

The results showed that short-period ZnTe/Zn(Z,Te) superlattices can be grown on GaAs substrates with high structural quality and reproducibility. The ZnTe well thickness is the crucial factor for growing samples of perfect crystalline quality. Moreover, the relaxation behavior is influenced by the amount of the average strain in the sample and the incorporation of nitrogen.

Key words: MBE, ZnTe/Zn(S,Te), short-period superlattice, substrate-temperature, ZnSe buffer, lattice-matched, relaxation, XRD, ZnTe well

10. ENERGY ANALYSIS OF A "DRAGON KILN" FOR FIRING "VIGAN JARS"

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The firing of ceramic products involves the partial fusion of the body and as it proceeds, the proportion of the glassy bond increases and the apparent porosity of the fired product becomes progressively lower. In the case of "Vigan Jars", these are fired to vitrify in fuelwood fired "Dragon Kilns" with a length of 30 to 50 meters. The kilns can accommodate different types of ware of various sizes. The thermal consumption.

Results of the evaluation indicate that the kilns consume an average of 30 to 50 cubic meters of fuelwood per firing, the firing cycle takes an average of five (5) days including the preheating phase. The maximum temperature recorded as 1250°C. The uneven heating of the kiln was found to be due the changes in wind directions during the firing process which resulted in cracking and over-firing.

Proper sequencing of the operation with the pre-heating process results in better fuelwood use efficiency. The installation of contraptions can reduce the occurrence of uneven heating. •

Key words: ceramics, Dragon Kilns, energy, fuelwood, Vigan Jars, vitrification.

11. AN INVESTIGATION ON THE POTENTIAL OF HIGHLY ABSORBENT MATERIAL OBTAINED FROM SURPLUS DIAPERS AND SANITARY NAPKINS AS LAHAR CONDITIONER

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Volcanic eruptions such as that of Mt. Pinatubo in 1991 bring about great devastation to the lives of people especially those who rely on the land for their livelihood. In line with the effort of providing assistance to the lahar affected communities, the study was undertaken to explore the possibility of using highly absorbent material such as those obtained from surplus diapers and sanitary napkins as lahar conditioner.

Treatment of lahar containing varying amounts of the absorbent material (e.g., 0.6 kg, 1.2 kg, 1.8 kg, 2.4 kg, 3.0 kg, 3.6 kg) were prepared in three replicates under two experimental set ups – one set was treated with 14-14-14 balanced fertilizer and the other without fertilizer. Controls included pure lahar and pure garden soil. Each pot was planted with five kernels of corn and was watered with a specified amount of tap water daily. Observations on the length of stalk, number of leaves, length of leaves, and other changes in the general appearance of the plants were also noted daily. At the end of the test period, physical and chemical analyses were performed to determine the following: (physical bulk density, water holding capacity, field capacity, permanent wilting point, percent available moisture; (chemical) pH, available phosphorus, organic matter content, total nitrogen, cation exchange capacity).

The results showed that highly absorbent material produced some significant improvement of lahar such as decreased bulk density, increased water holding capacity and improved organic matter content. However, there was no improvement on the permanent wilting point and percent available moisture values. Results from the plant growth experiments show that the smaller the amount of conditioner added to lahar, the more fibrous the root system of the plant became. The addition of fertilizer resulted in healthier plants compared to their counterparts in the non-fertilizer set up.

Key words: Mt. Pinatubo, lahar, garden soil, diapers, sanitary napkins.

12. GENERATION OF NAFIL LOOPS OF SMALL ORDER

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The determination of all non-isomorphic loops of a given order n is one the difficult problems in the theory of loops and quasigroups. This paper deals with the computer generation of loops of small orders $n = 5, 6$, and 7 belonging to the class of NAFILs (non-associative finite invertible loops). This class includes the familiar IP, Moufang, and Bol loops. However, there are many other interesting loops in this class that have not yet been studied as much as the familiar loops.

The NAFILs of orders $n = 5$ and 6 were determined at PUP in 1996 using a Pascal program called ICONSTRUCT. Because of the enormous number of possible loops of order $n = 7$, the distinct NAFILs of this order were determined only in 1999 by a collaborative work with Prof. H. Zhang (Department of Computer Science, University of Iowa, USA) using two software system, SEM and SATO, and a upper computer (with 48 Pentium II400 processors). The results are shown in the table:

Order	5	6	7
Number	1 (CNA)	33 (7A+ 26NA)	2,333 (16A+2333NA)

Where A = Abelian and NA = Non-Abelian. These results have been posted in various Internet discussion forums and they have attracted the attention of loop theorists.

Key words: loops, non-associative, invertible, non-isomorphic, IP, Moufang, Bol Loops, NAFL, quasigroups

13. IMMOBILIZATION OF TOXIC HEAVY METALS FROM ACADEMIC WASTE

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Stabilization/solidification is known as the immobilization of reforming toxic heavy metals to reusable material. Stabilization is a process by which contaminants are fully or partially bound by the addition of supporting media, binders, or other modifiers. Solidification is a process employing additives by which the physical nature of the waste is altered during the process.

The concentrations of cadmium, copper, lead, manganese, mercury, and nickel was established utilizing Atomic Absorption Spectrophotometry (AAS). Varying proportions of waste, cement, fine aggregate, and coarse aggregate were mixed and solidified in metal cylindrical molds. Simulated leaching of toxic heavy metals was conducted and the amount of contaminants was determined using AAS. The compressive strengths of both control and experimental specimens were established utilizing the Universal Testing Machine. Scanning Electron Microscopy (SEM) confirmed the binding mechanism between cement and contaminants.

The SEM result supports the comprehensive strength and leaching data. It further confirmed that cement can immobilize the toxic heavy metals in academic waste. Whether in combination with cement alone, or with cement and aggregates, academic waste in liquid or solid form can be solidified to produce specimens with highly acceptable comprehensive strengths.

Key words: immobilization, solidification, stabilization, leaching, contaminants, concrete, toxic heavy metals, academic waste, comprehensive strength, solidified waste

14. CAUSTIC SODA RECOVERY IN A BOTTLE WASHING PLANT USING MEMBRANE TECHNOLOGY

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The advent of using light containers such as aluminum cans and polyethylene terephthalate (PET) bottles for beverage packing has not at all phased-out the use of glass bottle. Today, glass bottles are still being reused widely by beer and softdrink companies. The reuse of such in the production process entails a thorough cleaning of these returnable bottles before refilling. During the bottle cleaning process, the beverage industry uses a large quantity of water and caustic soda solution for washing and rinsing operations.

When spent caustic soda is finally discharged, the usual practice is to treat it by neutralization using acidic waste streams. The problem with this process is that it produces a solution highly loaded with sodium that will eventually be detrimental to soil quality. Therefore, an effective way of minimizing the amount of caustic soda used and discharged from a processing plant must be found.

Membrane technology can be applied for caustic recovery. This is done by passing the caustic solution through base-stable membranes that can remove some, if not all, types of dissolved and suspended organic contaminants. Particles and colloids with size greater than 0.2 μm can be effectively rejected by microfiltration (MF). On the other hand, nanofiltration (NF) can separate molecules and ions with size less than 10Å. When membrane filtration is employed, the caustic solution may be recovered and the amount used in the process would consequently be lessened. Likewise, the total required volume of process water would be reduced. However, very little information is available on this kind of membrane system application. Only manufacturers of alkali-stable membrane have conducted the few studies done on the feasibility of this membrane application.

This research aimed to determine the performance of MF and NF in caustic soda recovery at pilot-scale level in terms of rejection rate, optimum applied pressure, and permeate quality. The effluent stream from bottle washers of one of the largest softdrink manufacturing plants in Thailand was subjected to membrane filtration using MF and NF. The study revealed that the MF/NF system can purify the caustic soda to a certain extent and can be reused back to the bottle washing units. Within the studied pressure ranges, the optimum applied pressure was determined as 101 kPa for MF and 1,414 kPa for NF with corresponding permeate fluxes of 235 and 15 $\text{L}/\text{m}^2\text{-h}$, respectively. Based on these figures, a large-scale installation was designed for the research site and a financial analysis was conducted for the system.

Key words: caustic soda recovery, microfiltration, nanofiltration, beverage industry, membrane technology

15. A COMPOSITE INDICATOR FOR LAKE WATER QUALITY MONITORING AND ASSESSMENT

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A composite indicator of water quality was formulated in this study to assess the suitability of lake ecosystem for various intended purposes, such as fisheries development. A single value is amenable to the same description and interpretation whoever is doing the assessment and prejudices on certain water quality variables would be eliminated.

The composite water quality indicator (WQI) was formulated by eliciting opinions from a panel of water quality experts the choice of variables, scaling procedures, and weights. The sampling distribution of WQI was estimated using the Monte Carlo simulation, the validity of which was verified for simple cases by showing that simulation and analytical derivation yield the same results.

Ten physicochemical and two biological variables were identified to constitute the composite indicator. The additive model was used in formulating the WQI because of its relative simplicity and sensitivity to extreme values. The WQI, which ranges from 0 (extremely worse condition) to 1 (best condition) may be classified as either very good, moderate, poor, or unaccepted by referring to a tabulated guide.

The WQI was applied to the water quality data for Laguna de Bay. The WQI has a beta distribution, although tests also showed that normality has been attained for sample size $n = 4$ or higher. The standard error of the mean of WQI was $0.0513 / \sqrt{n}$, where n is the number of sampling stations. The reliability of WQI may be evaluated further by constructing confidence intervals about μ_Q .

A test of hypothesis was formulated that would declare a given body of water as suitable for fisheries development if the computed composite indicator is at least 0.50. The power function was plotted for varying sample sizes and values for μ_Q . A graph showing the required sample size for fixed power and tolerance ($0.50 - \mu_Q$) was also constructed which can be used to determine a cost-effective monitoring program. Thus, 25 observations are needed to attain a power of 0.90 when the tolerance level is set at 0.03.

The WQI was also applied in the assessment of bodies of water other than Laguna de Bay. Using the same WQI, results showed that the state of water quality of Taal Lake in Batangas and Tadalak Lake in Laguna were "good" and "moderate," respectively. The ability of the composite WQI to differentiate the state of lake water quality demonstrates the potential applicability of the composite indicator.

16. RADIOLOGICAL ASSESSMENT OF FORMER US BASES: I. CLARK AIR BASE

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Conversion of the former US Air Base at Clark, Pampanga into an economic zone with plans for extensive commercialization of the area requires radiological assessment of Clark Special Economic Zone (CSEZ). The Philippine Nuclear Research Institute, having the capability to measure radiation contamination, initiated a study to perform a radiological surveillance of the former US base in Clark. Natural and anthropogenic radiation measurements in ambient air in 131 km of CSEZ road network is achieved using a computer-based carbome gamma radiation spectrometric system (Eploranium Gr-650) equipped with a global positioning system (GPS).

Natural radioactivity concentrations by carbome gamma spectrometer for potassium (K-40), uranium (U-238), and thorium (Th-232) were measured at 316.44 ± 82.28 , 15.86 ± 3.23 , and 13.08 ± 3.16 Bq/kg sample, respectively with corresponding total absorbed dose rate in air of 26.30 ± 6.68 nGy/h. Total absorbed dose rate in topsoil of natural radionuclides (K-40, U-238, and Th-232) measured by gamma spectrometer (HPGe) is 19-22 nGy/h. These values are within the background concentration vis-à-vis terrestrial absorbed dose rate of the whole country (23 nGy/h).

Evaluation of anthropogenic or man-made source of radiation in air using the carbome gamma spectrometric system indicated man-made sources from Cs-137 gamma radiation is not present within the CSEZ. Measurements of Cs-137 in topsoil showed values (0.98 - 4.64 Bq/kg) lower than the activity range concentration of Cs-137 in the country (<0.09-12.77 Bq/kg dry weight). Analysis of Cs-137 in drinking water from wells including those from Cabcom and DCDC main office showed values lower than the lower limit of detection in HPGe. The results of radioactivity analyses of air, topsoil, and water from the Clark Special

Economic Zone further established that man-made radioactivity (Cs-137) is not present in the former US Base in Clark.

Key words: assesment, US military bases, natural radioactivity, anthropogenic, radionuclides, air gamma dose rate

17. VISCOSITY SENSOR BASED ON A PIEZOELECTRIC QUARTZ CRYSTAL

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Conventional viscosity measurements usually require a sizeable amount of liquid sample (ca. 5 to 20 mL), considerable measurement time, and some specialized personnel skills. The most commonly used devices are the capillary viscometer and the "steel ball" viscometer. In order to simplify viscosity determination, a novel viscosity sensor was developed based on a piezoelectric quartz crystal. This device exploits the effect of the viscosity of a liquid medium on the resonant frequency of a piezoelectric crystal.

In this sensor, one side of the quartz crystal was exposed to about μL of the liquid sample in a fabricated sensor cell. The piezoelectric crystal was driven to vibrate through an oscillation circuit based on a TTL device and the oscillation frequency was measured through a frequency counter. The sensor exhibited a response within 1 second and reached a steady state in 2 minutes. In the presence of liquid sample, the sensor displayed a decreased frequency. The response was highly repeatable even at random sampling procedures. A highly linear relationship was observed between the frequency and the square root of the product of density and viscosity. The response characteristics of this sensor could make it useful for the on-line measurement of viscosity in industrial processes.

Key word: viscosity sensor, piezoelectric crystal, quartz crystal

18. PROCESSABLE pH SENSOR BASED ON CONDUCTING POLYANILINE

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Polyaniline (Pan) is one of the many organic polymers that offer commercial applications. It is being used as coating of films for corrosion protection and as material for electrochromic displays that operate rechargeable batteries and optical devices. We have developed it as a processable pH-sensor that serve as an alternative to glass electrode. The latter has been found impractical in clinical laboratories because of its fragility and large volume requirement of body fluids that are difficult to obtain. A Pan-coated metal wire is robust and could be as small as 0.17 cm². Hence, it could be inserted in vivo for real time pH monitoring. The preparation of the potentiometric pH-sensor involves a straightforward procedure, and is inexpensive, making it highly processable. Absence of an internal filling solution in the sensor allows it to be used in any position – it may be placed vertically, horizontally, or upside down. Electrochemical polymerization was carried out in devising polyaniline-coated pH sensor. The optimum starting compounds and conditions for electrode fabrication are: 0.10 M aniline monomer, 30 mg Bovine Serum Albumin (BSA) dopant, 0.10 M Tris(hydroxymethyl)-aminomethane, at pH 7 30-minute polymerization time without stirring, platinum wire support and 9.34 mA cm² current density.

The sensor showed a sub-Nernstian response of -42.06 mV/pH (0.5), a linearity of 0.9985 and favorable response time of ~ 3 minutes for 3 replicates from pH3 to pH10 at room temperature. The conducting polyaniline-based pH sensor exhibited low hysteresis with -m-5.83 mV/pH, low drift with an RSD ~ <4% high reproducibility with an RSD = ~ <3% and lifetime of > 1.5 months. Through Cyclic Voltammetry, the growth of polyaniline onto a platinum disc was monitored. The voltammogram showed three forms of polyaniline: the reduced (-1.03V), conducting (-1.069V), and oxidized (+0.061 V) states.

The polymer film surface of PAN/BSA sensor was analyzed using X-ray Photoelectron Spectroscopy (XPS) and Scanning Electron Microscopy (SEM). Via XPS, a wide scan spectrum of Pan/BSA was achieved showing the elemental composition C1s, N1s, and O1s. The absence of S2p peak at Eg ~ 168 eV indicates that the BSA dopant exist mostly in the bulk of the polymer film. SEM showed hexagonal-shaped flakes on Pan film on Pt wire under 5000 magnifications. The characteristics of the devised potentiometric pH sensor based on conductive

polyaniline presented are that it has good sensitivity and linearity making it a suitable alternative to the conventional pH glass electrode.

Key words: polyaniline, conducting polymer, sensor, pH, potentiometry, SEM, XPS, BSA dopant, Tris (hydroxymethyl)-amino methane buffer, Nernstian

19. FABRICATION AND CHARACTERIZATION OF CONDUCTING POLYTHIOPHENE AND POLY (3-METHYLTHIOPHENE) MODIFIED SENSORS

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Conducting polymer, an organic material with both electrical and mechanical properties had gained its popularity as potentiometric electrode. This kind of electrode has the advantages of being robust, and with higher mechanical strength. In this study, conducting polymers such as polythiophene (Pyp) and poly(3-methylthiophene) (P3MTp) were developed by electrochemical polymerization. The optimum polymerization conditions for P3MTp monomer, and 30s polymerization time. While the optimized polymerization conditions for PTP were 0.1 M thiophene (Tp) monomer, 10 mL chloroform, Pt solid support, 1 mA current, 0.1 MClO₄ dopant, and 20 s polymerization time.

The PTP-coated [H] sensor exhibited sub-Nernstian response ($335.42 + 3.80$ mV/pH) and good linearity (-0.9925) over hydrogen ion concentrations of 10^{-3} to 10^{-10} M. The 3MTp-based electrode gave a sensitivity response of -47.56 ± 2.51 V/pH with linearity of $-0.0.99775$ towards [H] in concentration range of 10^{-3} to 10^{-10} M. The potentiometric characteristics of the sensor include calibration curve, memory effect, electrode lifetime, hysteresis, electrode drift, and electrode selectivity. Scanning electron microscopy (SEM) was used to study the electrode surface composition was characterized by X-ray photoelectron spectroscopy (XPS).

Key words: polythiophene, poly(3-methylthiophene), conducting polymer, electropolymerization, pH potentiometry, sensor, cyclic voltammetry, XPS, SEM

20. COMPARISON – CONTINUOUS PROCESS OF LYSINE PRODUCTION USING IMMOBILIZED AND FREE CELLS OF *Corynebacterium glutamicum*

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Different gelling agents were used to immobilize viable cells via either alginate or κ -carrageenan gel beads. Based on cell leakage from the gel beads, oxygen and glucose diffusion coefficients and toxicity of the gelling agents, SrCl_2 was found to be the best for the immobilization of microbial cells not only in alginate but also in carrageenan beads. Using Sr -carrageenan gel beads, a lysine concentration of 12.5 g/L was reached in the continuous fermentation with a productivity of 0.75 g lysine/L.h.

To overcome problems of low mass transfer commonly encountered in immobilized aerobic cell fermentation, continuous L-lysine production using free cells in a stirred tank bioreactor coupled with a ceramic membrane was developed. Without ceramic membrane, batch fermentation with *Corynebacterium glutamicum* (wild type) exhibited a volumetric productivity of 0.18 g lysine/L. h. with a maximum lysine concentration of 9.4 g/L. When a ceramic membrane was coupled continuous lysine fermentation process at a dilution rate of 0.6 h^{-1} increased volumetric activity of 1.83 g lysine/L.h. and a maximum concentration of 30.5 g/L were obtained.

Key words: continuous lysine production, *Corynebacterium glutamicum*, free cells, gelling agent- SrCl_2 , immobilized cells

21. DETERMINATION OF ANIONIC SURFACTANTS IN FRESH WATER BODIES IN THE PHILIPPINES BY NEGATIVE ION ELECTROSPRAY IONIZATION-MASS ESPECTROMETRY

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The presence of branched and linear alkylbenzene sulfonates (ABS and LAS, respectively) and cocofatty alcohol sulfates (CFAS) were determined in the

Parig River, Laguna de Bay, and Balatun River (San Pablo City, Laguna using negative ion Electrospray Ionization-Mass Spectrometry. The anionic surfactants were concentrated from the water samples by solid phase extraction (SPE) using a C2 SPE column and introduced into the MS direct injection. CFAS quantification was performed using the ratio of the m/z 237,265 and 293 peak areas against that of m/z 247 of barium per fluorobenzene sulfonate (internal standard). LAS and ABS quantification was performed using the ratio of the m/z 297,311,325,399 and 343 peak areas against that of m/z of the internal standard. The detection limits for both CFAS and ABS/LAS analytes were below 0.5 ppm. Use for formaldehyde as a sample preservative improved the % recovery of CFAS, but did not affect the % recovery of LAS and ABS.

The commercial feedstock of CFAS, ABS, and LAS were analyzed to determine the respective profiles of carbon chain length.

CFAS was not detected in any of the water samples collected between September and November 1999 (below detection limit, <0.48 ppm). The combination of LAS and ABS was detected at around the limit of detection (0.28 ppm), but due to the low level, it was not possible to differentiate LAS and ABS by collision induced dissociation (CID)

Key words: surfactant analysis, LAS, CFAS, ESI-MS

22. STUDIES ON A LECTIN ISOLATED FROM THE SEEDS OF *Dolichos lablab* L. (BATAO)

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Crude extracts from seeds of *Dolichos lablab* L. (batao) indicated lectin activity with hemagglutination assay using human blood types A,B., AB and O. The highest activity was observed with blood type AB. Sugar inhibition assays suggested specificity for mannose and galNAc. The extracts were fractionated by saturated ammonium sulfate and affinity purified through a mannose sepharose column. SDS-PAGE showed bands of 14 and 60 kDa which stained positive to periodic acid Schiff reagent indicating a glycoprotein nature.

The purified lectin showed non specific dependence on cations when tested with Ca^{++} , M^{++} , Mn^{++} , and Zn^{++} . Dialysis against different concentrations of EDTA abrogated lectin activity. Hemagglutination activity was observed to be high at pH 8 to 9.0 and absent at low pH. It is stable up to 60°C.

Purified extracts showed an ability to induce release of superoxide anions in human neutrophils, indicating positive influence in phagocytic cell activity important in cells challenged by infections and tumors. Activity was observed to be dose dependent but not time dependent. The lectin was also observed to induce the release of NO_2 , also indicating positive influence on immune response. It showed minimal ability to induce the release of cytokines, TNFa and IL2 by human mononuclear white blood cells. MTT assays using cell lines A549 a non small cell human lung adenocarcinoma and human breast cell line T47, showed cytotoxic activity especially with the breast cells in a manner almost comparable to the cytotoxicity of taxol which was the positive control used. Moreover, exudates of human mononuclear white blood cells incubated with different concentrations of lectin were cytotoxic to the same cell lines.

Key words: lectin, *Dalichos lablab*, glycoproteins, cytotoxicity, immunodulatory

23. PRODUCTION OF PROTEIN-ENRICHED BANANA PEELINGS FOR ANIMAL FEED INGREDIENT

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High carbohydrate, low protein, and high tannin contents of banana peeling wastes from the food processing company disposal around the country posed several environmental problems for the social health communities. These raw materials could be beneficial to food companies if it will be processed into animal feed. The objective of this study is to convert the high tannin-carbohydrate into more digestible protein feed ingredient using solid state fermentation (SSF) process.

A total of 44 selected fungal strains and isolates were screened for protein enrichment of banana peelings with low tannin and more soluble substances of products. The criteria of selection were the whitish color rapidity of growth in the SSF process, high protein content, and no toxic compounds in the final product. The following microbial strains obtained were *Aspergillus niger* BIOTEC 3104, and its auxotrophs 1031 and 1032B. *A. niger* BIOTEC 3105 and *A. oryzae* BIOTEC 3078 and KBN 616.

Based on the protein, the crude protein (CP) content of dried product from banana peelings fermented with *A. niger* BIOTECH 3104 had increased from 7.24% in raw material to 31.90% in the fermented products after 3 days of SSF process. On the other hand, the water soluble substances increased from 22% in the raw materials to 32% in final product. Feeding toxicity study on mice revealed that 50% substitution of soybean meal by protein-enriched banana peelings in their diets could be formulated without adverse effects on the growth performance of mice during the feeding trial.

Key words: bioconversion, banana peeling wastes, *Aspergillus niger* BIOTECH-314, high protein product, animal feeds

BIOLOGICAL SCIENCES

24. ECOLOGICAL RISK ASSESSMENT FOR HIGH ENVIRONMENTAL QUALITY

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The need to quantify the risk of adverse environmental consequences to human and non-human resources as caused by ecological hazards has generated the development of risk assessment methodology. It is a necessary instrument for a quantitative environmental impact development project. Of the many that are presently existing, most if not all, are so mathematically complicated that they cannot be easily followed and adapted by administrators, scientists, and resource management practitioners. This study represents an attempt to formulate a simple and easily usable treatment of the methodology.

It is the main goal of this study to promote high environmental quality and, to attain this, the following specific objectives are adopted:

1. to evaluate an ecological risk assessment model that measures the probability of occurrences of adverse environmental impacts as caused by ecological stresses and
2. to use this model in assessing the acceptable concentration as the basis of developing mitigating options.

Environmental risk is determined as:

$$ER = (Obs/Exp) \cdot S \quad \text{Eq. 1}$$

Where ER = coefficient of relative environmental risk factor
 Obs = number of affected cases observed in the exposed group
 Exp = number of affected cases expected in the exposed group
 S = ecological stress measured by $C \cdot \Delta T$

- C = concentration in units of mg / cu m
 I = standard intake in cu m
 T = number of days of exposure

The probability of occurrence of adverse environmental impacts is further evaluated as:

$$P = ER \times S \times (\sum ER/n) \quad \text{Eq. 2}$$

- Where P = probability of occurrence of adverse impacts
 R = same as defined in Eq. 1
 S = same as defined in Eq. 1
 n = number of ER rates

The model was tested for prediction of adverse consequences in several studies, such as pesticide use, soil erosion control, mortality rates due to cancer, and morbidity rates due to water pollution. Pesticide use and soil erosion studies used a quasi-experimental design, comparing the exposed and non-exposed groups on the effects of the intervention. The last two studies used secondary data.

The assessments show results that indicate the model as a good predictor of adverse responses which are as good as the standard rates for the general population. The evaluations of acceptable concentrations further are consistent with what are prescribed by public agencies.

The ERA model provides a significant input to decision-making related to developing the most appropriate regulatory actions for human exposures, industrial plant emissions, ambient air and water exposure, and mitigation options formulated to control serious adverse environmental effects.

Key words: ecological risk assessment (ERA), high environmental quality, environmental risk factor, ecological stress, probability, concentration, exposure, time of exposure

25. MULTIPLE SHOOTING IN COTYLEDONARY NODES AND *Agrobacterium*-MEDIATED TRANSFORMATION IN *Pterocarpus indicus* WILLD. (FABACEAE)

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Pterocarpus indicus Willd. (Fabaceae) locally known as narra, is an indigenous tree in Southeast Asia. Its wood gives one of the best materials for furniture making. It is also a good source of red dye, has some medicinal uses, and is one of the multi-purpose trees. However, propagation of the plant by seeds is beset by problems involving their being prone to insect attack and various diseases. Thus, a manageable and efficient method of in vitro propagation is necessary to supplement the conventional method of propagation and to improve its germplasm successfully to genetic engineering.

Germination and survival rates and the response to the formation of axillary shoots in cotyledonary nodes were compared between two provenances of prickly narra. Laguna and Manila provenance seeds germinated at 39.16% after four to seven days and 50.00 after two to five days, respectively. With a survival rate higher in Manila provenance, the total node explants inoculated were 52 while in the Laguna provenance, only 26 were available for use. Murashige and Skoog (MS) and Woody Plant Medium (WPM) supplemented with 0.90 mg/L BA alone or in combination with 0.186 mg/L NAA did not have a remarkable effect on the growth of the excised embryos. However, the formation of axillary shoots on cotyledonary shoots and the quality of seedling growth were both affected by the different concentration and combination of cytokinins added to the MS multiplication medium. Seedling growth in Manila provenance was robust; Laguna seedlings were frail. Cotyledonary nodes from Laguna produced the usual 2 axillary shoots per node while in the Manila provenance, three to seven axillary shoots per node were observed in the following treatments: MS + 2.00 mg/L BA + 2.5 mg/L thidiazuron (TDZ), MS + 2.00 mg BA + 2.5 mg/L zeatin + 2.5 mg TDZ, MS + 2.00 mg/L BA + 5.0 mg/L zeatin, MS + 10.00 mg zeatin, and MS + 10.00 mg/L TDZ. This is the first report on multiple axillary shooting in cotyledonary nodes of narra and its transformation using *Agrobacterium*.

Some tissues were successfully transformed with *A. tumefaciens* LBA 4404 which harbored a binary vector, p81121 (Clontech, Palo Alto, CA) with genes for B-glucuronidase (GUS) and neomycin phosphotransferase (NPTII). The cotyledons were the best tissues that responded to transformation as shown by the appearance of a blue color in the transactions under the treatment of X-GLUC (5-

bromo-4-chloro-3-indolyl- β -D-glucuronide) solution. Further tests with the use of PCR-based technology are needed to supplement the result of the histochemical test for GUS activity.

Key words: cotyledonary nodes, *Agrobacterium*, transformation, *Pterocarpus indicus* Willd., narra, provenance, multiple shooting, axillary shooting, zeatin, thidiazuron.

26. EMBRYONIC DEVELOPMENT OF "HANGA"

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"Hanga" or *Pittosporum resiniferum* Hemsl. is a potential alternative source of energy because of the petroleum-like properties of the oil from the fruit which is comparable to petroleum due to the presence of dihydroterpene and n-heptane (Bacon 1909; Noble 1978). The study was conducted to trace and describe the different stages of development of the embryo from globular to the mature stage. The modified clearing technique using NaOH and chloral hydrate was used. Seeds were removed from the fruits and the embryos in the seeds were isolated. The embryos were measured with a-micrometer eyepiece using a BH-2 Olympus epifluorescent microscope. Morphological and anatomical descriptions were used as criteria for classifying the embryos in different stages. Stage 1 had embryos which were small and globular in shape. In stage 2, the embryos were in the early-heart shape, with cotyledons developing. In stage 3, the embryos were in the mid-heart shape with developed cotyledons. A suspensor, at the base of the embryo was observed. In stage 4, the late-heart-shape, the cleavage between the cotyledons was deeper and the cotyledons more rounded at their tips. The primary tissues (protoderm, procambium, and the ground meristem) were well defined.

One embryo per seed was noted. Approximately 80 to 90% of the seeds dissected showed the presence of an embryo. The presence of an embryo and its developmental stage are not directly related to seed size.

Key words: embryo, suspensor, procambium, dihydroterpene, n-heptane, cotyledons, protoderm, ground meristem Hanga, *Pittosporum resiniferum*

27. THE CULTURE OF *Kappaphycus alvarezii* (Doty) AT THE THREE DIFFERENT WATER LEVELS IN THE MARINE WATERS OF NORTHERN POBLACION, SAN FRANCISCO, CEBU

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Camotes Island is noted for its Camotes Sea, one of the fishing grounds in the Philippines. It is composed of three islands namely: Pacijan Island where the municipality of San Francisco is located; Poro Island, which is divided into two municipalities, Poro and Tudela; and the smallest is Ponson Island where the municipality of Pilar is found. The fishermen in the area are complaining on the decline of their catch. The livelihood of the majority of the inhabitants are fishing and farming and they are becoming poorer because at present they have no alternative livelihood to augment their income.

This study was undertaken to find out what level of water the *Kappaphycus alvarezii* locally known as "gozo" will grow in the marine water of San Francisco, Cebu using the randomized completely block design (RCBD), with the increase of weight as indicator for growth. There were three treatments in the study with three replicates such Treatment 1 represents the surface layer, Treatment 2 represents the middle layer, and Treatment 3 represents the bottom layer. Bamboo rafts were used to hold the plants at the three different levels. Seedling were selected from the midpart of the whole plant up to the top. Plant samples were weighed every 15 days for 45 days.

The area parameters are salinity, 32-39 parts per thousand (ppt); water temperature, 31-32°C; water velocity, 4 to 8 seconds per meter, water depth, 3 meters to 3.75 meters, and water transparency, 3 to 3.5 meters.

Results based on the mean weight of the plant after 45 days show that surface layer has 1,395 grams; middle layer 1,052.50 grams, and the bottom layer is 834.44 grams.

Analysis of Variance (ANOVA) tables shows that there is no significant difference in all the treatments meaning all the levels are suitable for gozo culture.

Key words: *Kappaphycus alvarezii*, Camotes Sea

28. VARIATION IN GINGER (*Zingiber officinale* Rosc.) AND RELATED TAXA USING ISOZYME PATTERNS

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Ginger (*Zingiber officinale* Rosc.), an aromatic herb with an underground rhizome, belongs to the family Zingiberaceae. Related taxa such as *Zingiber zerumbet* (L.) Smith, *Z. purpureum* Rosc. (syn. *Z. cassumunar* Roxb.), *Curcuma* spp., and *Alpinia* spp. have likewise been reported to have some economic and medicinal importance. Earlier works done on *Zingiber* and other members of the ginger family, which largely deal with chemical, taxonomic, and pharmacognostic studies, revealed genetic variation for several morphological characters. However, zingiberaceous plants possess a limited number of morphological features useful in classical taxonomic evaluations, making species level identification of these plants difficult in the absence of the parts, especially floral structures, that bear these key traits. More recent biochemical studies on two genera, *Zingiber* and *Curcuma*, using differences in isozyme patterns have shown interspecific variations for a number of enzyme systems (Ibrahim, 1996); however, such diversity has not been critically evaluated. This project was thus undertaken to determine if there is significant interspecific and intraspecific variation shown through isozyme banding patterns and to ascertain if isozyme analyses could prove useful as taxonomic markers in this group of plants. To do this, protein extracts from mature leaves of 10 accessions of ginger and related taxa (7 *Zingiber*, 2 *Languas*, and 1 *Curcuma*) from at least 3 regions of the Philippines were subjected to electrophoretic techniques using polyacrylamide gels to generate isozyme bonding patterns for 6 enzyme systems: esterase (EST), peroxidase (PRX), acid phosphatase (ACP), glutamate oxaloacetate transaminase (GOT), malate dehydrogenase (MDH), and catalase (CAT). Clustering methods using the Multi-Variate Statistical Package software were performed to analyze the variation in bonding patterns. It was found that EST, PRX, and ACP showed significant interspecific as well as intraspecific variation among the accessions. GOT and MDH revealed interspecific variation but did not substantially delineate between samples of the same species. CAT, on the other hand, exhibited little variation in all the accessions. Clustering of the *Zingiber officinale* samples was observed to be geographically affected, with samples from the north (Luzon) clustering together and those from the south (Visayas and Singapore) forming another group. These results suggest that the use of isozyme banding patterns as taxonomic markers may be possible depending the enzyme systems being used.

Key words: Zingiberaceae, isozyme, interspecific variation, intraspecific variation, electrophoretic techniques, clustering methods

29. CORRELATION OF PANDANUS ALKALOIDS TO THE TAXONOMY OF PANDANACEAE

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Leaves of *P. amaryllifolius*, *P. dubius*, *P. simplex*, *P. laevis*, *P. veitchii*, and *P. variegatus* and the species described as a cross between *P. nobilis* and *P. vidalii* collected from Luzon were detected to contain alkaloids by the Culvenor Fitzgerald field test. *P. tectorius* Sol. On the other hand did not show a reproducible response to the alkaloid field-test. *P. laevis*, *P. veitchii*, and *P. variegatus* were all identified to be cultivated varieties of *P. tectorius*.

The first novel alkaloid, pandamarine, from *P. amaryllifolius* of the subgenus *Kurzia* was reported in 1992 by the team from UST RCNS. These were further substantiated by the report of 3 more new alkaloids from the same plant. Works by Garson and Sjaifullah on the same plant obtained from Indonesia reveal more new alkaloids. More recently, this researcher and the group of Prof. N. Aimi of Chiba University isolated more new alkaloids from plant materials obtained from the Philippines and Thailand. *P. dubius* Sprengel, a species classified under the subgenus, *Rykia*, gave similar alkaloids like that from *P. amaryllifolius*. The results establish the basic structure of the *Pandanus* alkaloid to be $C_9H_{11}NO_2$. *P. nobilis* x *P. vidalii* yields vomifolol (blumenol A) suggesting the occurrence of a false positive reaction with the alkaloid-precipitating reagent. No alkaloid was isolated from *P. tectorius* that responded with the field test. The cultivar *P. variegatus* also did not produce any alkaloid despite its initial response to the field test. The detected alkaloids in *P. laevis* and *P. veitchii* are now being studied. True alkaloids were found present in species belonging to the subgenera *Rykia* and *Kurzia*. The subgenus *Pandanus* where *P. nobilis* x *P. vidalii*, *P. variegatus*, and *P. tectorius* belong to was found to give false positive tests for alkaloids. All of these observations proved to be important for the taxonomic classification of these plants. This paper will present the relationship of the alkaloids found to the Pandanaceae taxonomy.

Key words: Pandanaceae, chemotaxonomy, alkaloids, *Pandanus*

30. LITTORAL FISHES FROM A SEAGRASS AREA IN SAMAL ISLAND, DAVAO DEL NORTE, PHILIPPINES

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Monthly fish samplings were conducted in a seagrass coastal area in Samal Island, Davao del Norte, Philippines, from March 1998 to April 1999 to determine the composition and abundance of fish populations associated with seagrass in the area. A customized beach seine was used to collect fish during the day and at night. A total of 159 fish species comprising 43 families were caught. The pipefish, *Syngnathoides biaculeatus*, and the pufferfish, *Canthigaster bennetti*, were dominant both in total counts and biomass during the day, while at night, the dominant fishes were the rabbitfish *Siganus spinus*, *Apogon coccineus*, *A. fraenatus*, *Cheilodipterus macrodon*, and *C. quinquelineatus*. Fish total abundance and biomass were significantly higher during the day than at night. The dominant species based on total counts were mostly carnivores (39.6%), followed by planktivores (33.8%), herbivores (24.2%), and omnivores (2.6%). Fish density in the study area was 0.46 ± 0.0086 ind m⁻² and 3.89 ± 0.7183 g wet wt m⁻².

Key words: seagrass fishes, Samal Island, Davao del Norte, pipefish, pufferfish, rabbitfish, carnivores, planktivores, herbivores, omnivores

31. THE EFFECTS OF SALINITY ON SURVIVAL AND GROWTH OF TILAPIA *Oreochromis niloticus* EXPOSED AT VARIOUS AGES

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Tolerance of tilapia, essentially a freshwater fish, to artificial seawater acclimation based on survival and growth at 1, 11, 21, 31, and 41 days post-hatching was determined. Optimum survival (100%) was observed in fry exposed to seawater very soon after hatching. Survival rates of 56.67, 76.67, 90, and 93.33% were observed for fry acclimated at 11, 21, 31, and 41 days post-hatching, respectively; indicating a trend towards increased tolerance with age. There was no further statistically significant deviation ($P < 0.05$) as compared to survival of non-acclimated control (96.67%) starting 31 days post-hatching. There were no significant effects on the growth of fish surviving through 61 days post-hatching.

Key words: tilapia, artificial seawater, acclimation, fry, hatching

32. REPRODUCTIVE DEVELOPMENT OF THE SUPERMALE (YY) TILAPIA (*Oreochromis niloticus*)

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GENETICALLY MALE TILAPIA PROJECT

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Histogenesis of the reproductive system of supermale (YY) tilapia and XY tilapia reared at the Central Luzon State University was analyzed using the paraffin technique. In the course of development, the primordial germ cells appeared at the same age in YY and XY at 8 days posthatch. These cells which were larger in the YY (1.85 μm) than in the XY male (0.9 μm) later established themselves in the gonadal anlage by days 9 to 22. The lobules appeared earlier in the YY at day 15. Blastema of the reproductive duct appeared in the YY at day 23 and in XY at day 27. By day 79, meiotically active cells were abundant in both groups. By day 95, the YY fish showed mature sperm cells in the fully differentiated testis as compared to day 105 in the XY fish. The supermale consistently demonstrated bigger testis, thicker somatic tissues, more spermatogenic cells, and more advanced developmental stage than XY fish of the same age. Germ cell and nuclear size in the YY and XY fish were not significantly different by statistical analysis although the general trend was bigger spermatogenic cells in the supermale tilapia. Anova (α 0.05) showed significant difference in the size of the testis, spermatocysts, and vas deferens. The study showed that with the same rearing conditions and same age, the larger supermale tilapia has superior reproductive capacity with its larger testis and ducts and faster histogenesis, differentiation, and spermatogenesis.

Key words: supermale (YY) tilapia, *Oreochromis niloticus*, histogenesis, reproductive development

33. MORPHOANATOMY OF *Vivipara costata* Quoy and Gaimard (Mollusca: Viviparidae) DURING EARLY DEVELOPMENT

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The early development of the freshwater gastropod snail, *Vivipara costata* Quoy and Gaimard, was characterized by observing the live embryos in the following series: 1-cell stage, 2-cell stage, 4-cell stage, 8-cell stage, morula stage, blastula stage, gastrula stage, trochophore stage, early veliger stage, veliger at start of torsion, veliger at 90° torsion, veliger at 120° torsion, veliger at post-torsion, and juvenile stage. The trochophore larval stage was subdivided into 3 substages with different sizes and distinctive characteristics.

The fertilized egg contained a small amount of yolk making the embryo transparent until late veliger stage. Cleavage was spiral. Cleavage cavity and polar lobes were absent. The blastula had a wide blastocoele. Gastrulation was by invagination. There was reduced ciliation in the prototroch of trochophore larva and apical tuft was absent. The veliger larva was of the dominant larval type.

The actual age of the embryos was not determined in this study because they were contained within the brood pouch of the mother and so the different stages were categorized based on their morphological features and relative sizes. The derivatives of the three germ layers namely, the ectoderm, mesoderm, and endoderm, were determined by histological sections using the paraffin method.

The anatomical shifting in the positions of some larval organs relative to their point of origin was attributed to torsion during differential growth. These changes included the shifting of: the heart, kidney, and ureter contained in the visceral sac from the right to the left side; the anus from left to right; the mantle cavity from posterior to anterior; and the crossing over of the right and left intestinal ganglia.

Key words: *Vivipara costata*, morphoanatomy, embryogenesis, organogenesis, trochophore, veliger, torsion, viviparous, histology, conchology

34. DIVERSITY OF THE MOLLUSCAN GASTROPODS, *Terebralia sulcata* AND *Cerithidea cingulata* IN TWO MANGROVE AREAS IN CATANDUANES, BICOL REGION

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Typhoons serve as major stresses in the mangrove communities of the island province of Catanduanes, Bicol region. Such disturbances can shift and change the structure of floral and macrofaunal species found in the mudflats of mangrove areas.

From October 21-22, 1998, a super-typhoon damaged the mangrove areas of the southern portion of Catanduanes, particularly in the municipalities of Bato, Virac, and San Andres. In order to understand possible changes in the diversity of bottom dwelling macrofauna, quantitative surveys were conducted from 1998-1999. This was limited to the molluscan gastropods, *Terebralia sulcata* and *Cerithedia cingulata* (Family Potamididae). The quantitative sampling of the two species of molluscan gastropods was done along a series of quadrats established in two study sites. Samples were collected by dividing a square sheet wooden frame of 1-mx 1-m into 4 parts. *T. sulcata* has very unusual abundance towards the seaward fringes, while *C. cingulata* is abundant towards the landward fringes. The former tends to have preference for high salinity seawater compared to the latter which mostly aggregate under Nipa palms and mangrove trees where the salinity is less.

The Panganiban mangrove area in the north is characterized by river-dominated allochthonous setting and low tidal range as evidenced by the narrow tidal changes during the day. Oco river discharge of freshwater and sediments leads to the deposition of terrigenous silts. On the other hand, the mangrove area in Virac shows a composite river and wave-dominated setting. Sto. Domingo-Pajo river provides the freshwater supply and sediments. The mangrove setting in this capital town represents a combination of high wave energy and river discharge. The sand that is debounced by this river is distributed by waves forming sand sheets.

Surveys conducted from July to September, 1998, showed the mean density values of 115.42 in Panganiban and 123.72 in Virac for *T. sulcata*. In January-February, 1999 (2 to 3 months after the super typhoon ravaged the province) field surveys were again conducted in the said locations using permanent transects and quadrats showed significantly lower mean density values of 75.42 in Panganiban and 58.25 in Virac from 100 quadrats. In December, 1999, another field survey was conducted in Virac and very low densities of both *T. sulcata* and *C. cingulata* were obtained. The decreasing density of the molluscan gastropods in consideration could be attributed to constant flooding in the mangrove areas which disturbs the macrofaunal communities in the province. Habitat change involving changing

patterns of coastal landforms and geomorphic processes could explain these changes in the diversity of molluscan gastropods.

Key words: mollusks, gastropods, diversity, density, mangroves, Catanduanes

**35. ELECTRON MICROSCOPE ANALYSIS OF SEXUAL INDUCTION IN A FISSIPAROUS PLANARIAN,
*Dugesia ryukyuensis***

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Minced flatworm, *Bdellocephala brunnea*, was fed to the asexual mixoploid biotype *Dugesia ryukyuensis* (Okinawa-Hiroshima strain) to determine if the asexual worm could be sexualized. Six weeks feeding led to full development of the reproductive organs of *Dugesia*. Ultrastructural observations showed features previously unreported in worms of the same group.

Key words: *Dugesia ryukyuensis*, *Bdellocephala brunnea*, sexualization, asexual worm, mixoploid biotype

**36. GILL PARASITES OF *Terapon jarbua* FORSKAL,
FROM LINGAYEN GULF**

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Terapon jarbua (Forsk.) locally known as "bagoong", inhabits inshore waters and brackishwaters and enter freshwater bodies. It is found practically all over the Philippines where it is utilized for food. Examination of the gills of samples obtained monthly from Lingayen Gulf in La Union Province, from January to December 1998 resulted in the recovery of parasites. A total of 162 specimens

ranging from 6.0 to 21.2 cm were examined. The most prevalent parasite was a monogeneic fluke, the others found were crustaceans. The parasite species recovered, prevalence (%), and intensity of infection (number per infected host) were: *Diplectanum secundum* (Tripathi) (87%, 1-135), *Caligus* sp. (59%, 121) *Caligus pelamydis* (Kroyer) (38%, 1-9), *Neobrachiella chevreuxii* (van Beneden) (8%, 1-7), *Chalimus* larvae (24%, 1-7), and larvae of *Gnathia* sp. (7%, 1-7). For both the monogenean and crustacean (as a group) parasites, no significant seasonal differences in prevalences of infection were observed. However, prevalences of infection were significantly higher with bigger specimens than with smaller one.

Key words: *Terapon jarbua*, Lingayen Gulf, gill parasites, *Diplectanum*, *Caligus*, *Neobrachiella*, *Chalimus*, *Gnathia*, monogenea, crustacea

37. ISOLATION OF INDIGENOUS BACTERIA FOR THE DEVELOPMENT OF PROBIOTICS IN THE BIOCONTROL OF CLINICALLY IMPORTANT PATHOGENS

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Increasing resistance of pathogenic microorganisms to antibiotics is a major public health problem globally. Resistance of pathogenic microorganisms is leading to increased death and illnesses with consequential expenditure. The use of antibiotics for bacterial infections has become ineffective because of the development of bacterial resistance. This has led to the isolation of probiotics that are most likely to inhibit and control the growth of clinically important pathogens.

A total of twenty bacterial isolates were randomly collected from marine environments in Panay Island, Philippines. Inhibitory activities of these bacterial isolates against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus* spp. Were examined using cross streaking procedure and modified sensitivity test, in vitro. Eleven isolates showed a moderate inhibitory activity against *S. aureus*, three against *P. aeruginosa*, and two against *Streptococcus* spp. Two of the twenty isolates, coded MU4 and SU5, showed a strong inhibitory activity against *S. aureus* using the modified sensitivity test while no significant effect was observed against *P. aeruginosa* and *Streptococcus* spp. Characterization of the bacterial strains using morphological classification revealed that strains MU4 and SU5 were gram-positive bacilli and gram-negative bacilli, respectively.

Key words: pathogenic microorganisms, probiotics, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus* spp., cross streaking, antibiotics, gram-positive bacilli, gram-negative bacilli

38. Δ^4 -3-KETOSTEROIDS FROM *Morinda citrifolia* L. AS POTENTIAL INHIBITORS OF *Mycobacterium tuberculosis* H37Rv

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Tuberculosis, a disease once thought to be under control due to the discovery of effective chemotherapeutic agents, has come back with a vengeance claiming the lives of nearly 3 million people in 1995 worldwide. According to WHO, the Philippines is ranked as the fourth country with the highest incidence of TB in 1995. This ancient scourge, which is now a global epidemic, prompted the search for compounds with antitubercular properties. One promising plant source is *Morinda citrifolia* L. (Rubiaceae). Ethnomedical studies in Hawaii, where this plant is called "noni", reported that the locals used a concoction from this plant to treat tuberculosis.

Preliminary in vitro screening conducted on the alcoholic leaf extract of this plant indicated significant inhibitory activity against the virulent *Mycobacterium tuberculosis* H₃₇Rv, the causative agent of TB. Bioassay-guided purification of the hexane-soluble constituents of the alcoholic extract of *M. citrifolia* L. leaves yielded a fraction that exhibited a minimum inhibitory concentration of <2.0 µg/mL against the test organism. Reversed phase HPLC purification of this fraction by isocratic elution using MeOH as mobile phase yielded two Δ^4 -3-ketosteroids. Elucidation of structures was carried out by infrared and nuclear magnetic resonance spectroscopy and mass spectrometry and comparison with known data. These were identified as stigmasta-4,22-dien-3-one and stigmast-4-en-3-one.

Key words: tuberculosis, chemotherapeutic agent, *Morinda citrifolia* L., Rubiaceae, noni, in vitro, *Mycobacterium tuberculosis* H₃₇Rv, Δ^4 -3-ketosteroids, stigmasta-4,22-dien-3-one, stigmast-4-en-3-one

39. PIGMENTED OFFSPRING OF ALBINO MICE: SCREENING FOR GAIN-OF-FUNCTION MUTATIONS IN FIVE EXONS OF THEIR TYROSINASE GENE BY MULTIPLEX PCR-SSCP ANALYSIS

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Albinism is a condition marked by the absence or inability to produce melanin in the body. We previously reported the serendipitous generation of pigmented (agouti) mice from albino parents. Since albinism results from loss-of-function mutations in the gene tyrosinase which encodes the key enzyme that converts phenolic compounds in the body into melanin, was screened for gain-of-function mutations in the tyrosinase gene of these agoutimice. As a rapid and sensitive procedure to detect point mutations in the gene, was simultaneously amplified multiple exons by multiplex PCR and performed single-strand conformational polymorphism (SSCP) analysis. We discuss the conditions for multiplex PCR-SSCP and the applications of this technique to screen for subtle point mutations in genes which cannot be determined from the length of PCR products alone when primers flanking exons are used.

Key words: tyrosinase, melanin, albino, multiplex PCR-SSCP, loss-of-function mutation, gain-of-function mutation

40. *Schefflera odorata* INHIBITOR OF MARK ACTIVATION IN CULTURED AIRWAY SMOOTH MUSCLE CELLS

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Mitogen activated protein kinase (MAPK) is an enzyme belonging to the group of phosphotyrosine kinases. Phosphotyrosine kinases are enzymes that are involved in cell growth and proliferation. These enzyme are activated by phosphorylation. The effect of the leaf extract of *Schefflera odorata* on MAPK activation in airway smooth muscle (ASM) cells was investigated. Thymidine incorporation assay was done to determine if *S. odorata* can inhibit DNA synthesis of ASM cells. Fetal calf serum (FCS) was used to stimulate DNA synthesis in ASM cells. FCS-stimulated DNA synthesis was inhibited by 50% when treated with 100 µg/mL *S. odorata* extract. Almost 100% inhibition of DNA synthesis was observed when the cells were treated with 200 µg/mL. The effect of *Schefflera odorata* on phosphotyrosine kinases in ASM cells was determined by SDS-PAGE and Western Blotting. FCS was used to stimulate the activation of these enzymes. Immunoblot of the ASM cell lysates treated with FCS and *S. odorata* extract showed concentration dependent inhibition of band formation at 205, 112, and 42 kDa. The effect of *S. odorata* on MAPK activation was determined by performing two assays, MAPK SDS-PAGE mobility shift and MAPK peptide assay. In SDS-PAGE mobility shift assay, the activation of MAPK is indicated by its retarded mobility upon SDS-PAGE, as observed in ASM cells treated with FCS. However, this mobility shift was not observed when the cells were treated with FCS and 100 µg/L of *S. odorata*. No band was formed at all when the concentration of *S. odorata* was increased to 200 µg/mL. The result in the MAPK mobility shift assay was confirmed by the other assay, the MAP peptide assay.

Key words: MAPK, ASM cells, cell growth, cell proliferation, phosphotyrosine kinases, thymidine incorporation assay, MAPK SDS-PAGE mobility shift assay, PAK peptide assay, western blotting, immunoblot

41. THE EFFECT OF *Tinospora rumphii* BOERL ON OVARIAN 3 β -HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN RATS

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The antifertility affects of *Tinospora rumphii* Boerl. ("makabuhai") has been demonstrated. In this study, the effect of *Tinospora rumphii* methanol extract from air-dried stems on ovarian 3 β -hydroxysteroid dehydrogenase activity in both virgin and mated (pregnant) rats was investigated. The enzyme is important in the biosynthesis of reproductive hormones. The drug in single doses of 2 μ g/kg, 4 μ g/kg, and 8 μ g/kg was injected into the ovarian bursae at diestrus in virgin rats and at 5th, 9th, and 18th day of pregnancy in mated rats. Spectrophotometric determination of enzyme activity assayed levels of NADH at 4, 8, 12, 24, and 48 h after treatment. Assay results showed significant reduction of enzyme activity within the first 24 hours in all the *Tinospora* treated rats except those treated with the lowest dose. The effect of 8 μ g/kg was comparable to that of 2 μ g/kg 17 α -hydroxy-6 α -methoxyprogesterone acetate (positive control). There was recovery of enzyme activity after 48 hours. Nevertheless, histopathological examination on the ovaries revealed that many follicles in the *Tinospora*-treated rats had become atretic just like in the positive control but *Tinosporaca* used 72% reduction in fertility and some generated fetuses (and not 100% reduction and no degenerated fetuses as in the positive control).

Key words: herbal drug, atretic ovarian follicles, anti-fertility drug, steroid biosynthesis, 3 β -hydroxysteroid dehydrogenase

42. DESIGN OF A QUANTITATIVE BEHAVIORAL TEST FOR HYPERACTIVITY IN MUTANT MICE

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We previously reported the serendipitous generation of pigmented mice from albino parents orally treated with a suspected mutagen, sodium nitrate. The gain-of-function mutation resulted in the birth of agouti as well as black mice in the

otherwise albino population and this mutation was stably transmitted to the germline. The pigmented F_1 agouti mice were further observed to exhibit hyperactive behavior relative to same-age albino mice raising possibility that the hyperactive behavior may be linked to the coat color phenotype. To test this possibility, we developed a quantitative hyperactivity test in conjunction with molecular and genetic studies being done. Here, we describe the experiment set up we developed to test for hyperactive behavior in mice. We show that our device could be used not only to quantify the endurance of the mouse given a particular test situation, but also to determine whether the mouse respects spatial boundaries.

Key words: hyperactive mice, gain-of-function mutation, hyperactivity test, sodium nitrate, pigmented mice

43. HEPATOTOXICITY OF METALDEHYDE

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The hepatotoxicity of the molluscicide metaldehyde on non-target organism such as milkfish was determined through acute exposure. *Chanos chanos* fingerlings were exposed to varying concentrations of Porsnail[®], namely: 3, 30, 60, 150, and 300 mg L⁻¹ and liver samples were processed for paraffin sectioning.

Light microscopy showed hypertrophic hepatocytes in all sublethal treatments. Except for liver sections at 3 mg L⁻¹, all other treatments revealed sections with pyknotic nuclei and chromatin clumping > Cytoplasmic vacuolation and loss of cellular architecture were evident in liver sections of fish exposed to 300 mg L⁻¹.

Key words: hepatotoxicity, Porsnail[®], molluscicide, milkfish, fingerlings.

44. ASSESSMENT OF THE ACUTE TOXICITY OF SURFACTANTS (LAS AND CFAS) USING SELECTED SPECIES OF FISH

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Despite the wide use of surfactants in the Philippines, data on the environmental toxicity of these compounds to tropical organisms is scanty. The majority

of the chronic and sublethal toxicity databases that are available are limited to studies in temperate areas involving only a few commercially important types of surfactants. Hence, the present study aims to determine the acute toxicity of LAS and CFAS on three local freshwater fish species – common carp (*Cyprinus carpio*, Linnaeus 1758), guppy (*Poecilia reticulata*) and tilapia (*Tilapia nilotica*) – under static-renewal conditions (12h-replacement of test solution).

A series of 96-h range-finding tests was performed to determine the critical concentrations. Subsequently, 96-h definitive tests were done in order to determine the 96-h (LC50 (mg/L)). A series of reference toxicity tests with copper sulfate were performed in parallel. Test solutions were analyzed every 12 h to determine actual surfactant concentration using direct injection negative ion Electrospray Ionization-Mass Spectrometry (neg ESI-MS).

The results of the chemical analysis of the test water showed that CFAS was partially degraded after 6 h and is completely degraded after 12 h while LAS concentration remained the same even after 12 h. The rates of change of both LAS and CFAS concentration-response curves were similar and very shallow implying that large decreases in concentration will only bring about small decreases in toxicity. This further implies a possible similarity in the mode of toxic action of the two compounds on the test organisms. The results of the definitive toxicity tests showed that CFAS was found to be 2 to 3 times more toxic than LAS to all three species. Tests with the reference toxicant (copper sulfate) showed that the rank order of sensitivity among the test organisms was as follows: most sensitive was carp, followed by guppy, with tilapia as the least sensitive. In the absence of a standardized toxicity test procedure in the Philippines that can be used for testing in the aquatic environment, the procedure developed here can be used for future monitoring studies.

Key words: surfactant toxicity, LAS, CFAS, toxicity tests, carp, guppy, tilapia

45. GENERAL PROTEIN BANDING PATTERNS OF THE FRESHWATER PRAWN, *Macrobrachium lanceifrons*, COLLECTED FROM VARIOUS SITES IN LAGUNA LAKE

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This study was undertaken to gather an overall molecular fingerprint of the fresh water prawn, *Macrobrachium lanceifrons*, using general protein banding patterns. Prawn samples collected from different sites in Laguna Lake were analyzed through sodium dodecyl sulfate-polyacrylamide gel electrophoresis

(SDS-PAGE). General protein bands showed variation in terms of mobilities, intensities, and individual patterns. However, a general banding pattern may be deciphered from all samples analyzed; groups of cathodal and anodal protein bands are apparently separated in the prawn. Differential expressions, of proteins are reflected in minor individual variations in banding patterns observed.

Key words: freshwater prawn, Laguna Lake, *Macrobrachium lanceifrons*, SDS-PAGE, protein banding pattern, polyacrylamide gel electrophoresis, molecular fingerprint, differential expression, cathodal proteins, anodal proteins

45. PURIFICATION, CHARACTERIZATION, AND ANTIMICROBIAL SPECTRUM OF A BACTERIOCIN FROM FERMENTED SAUCE ISOLATE

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Bacteriocins are antimicrobial compounds that recently captured the interest of various industries because of their great potential of being effective natural preservatives. Some desirable characteristics are wide range of pH and heat stability, tastelessness, digestibility due to their proteinaceous nature. They have already been consumed for a long time in foods such as fermented dairy and meat products. There are reported types that prevent listeriosis and histamine poisoning and some are useful as genetic markers in the food-grade cloning and expression systems. Nisin is the well characterized and the only legally approved bacteriocin as preservative. It is classified as an additive generally regarded as safe (GRAS). Therefore, knowledge of the properties of known and new bacteriocins will help in hastening the acceptability of bacteriocin-treated products and promote their utilization.

In this study, a bacteriocin from *Pediococcus acidilactici* isolated from fermented sausage was extracted employing a current technique of pH-dependent cell adsorption-desorption of proteins. The desired metabolic product was first adsorbed onto the cell surfaces at pH 5.0 and cells removed from the culture medium by centrifugation. It was selectively released at pH 2.0 and dialyzed to remove acid and salts. Purification under served phase-HPLC resulted in a yield up to 5.76×10^5 times.

Mass spectrometry gave a molecular weight of 4699 Dal, a little higher than previously reported pediocins. The bacteriocin was heat tolerant when exposed to pasteurization setting (61.8°C, 30 min and 71.5°C, 15 sec), boiling temperatures at different time intervals and autoclaving conditions. It was also stable under a wide pH range (pH 3 to 9). Well-diffusion assay showed antimicrobial activity against *Listeria monocytogenes*, a Gram-positive food pathogenic bacterium and some lactic acid bacteria such as *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and *Enterococcus faecalis*.

Partial characterization of the bacteriocin showed promising features as possible antimicrobial additive in both processed and minimally processed products.

Key words: bacteriocin, *Pediococcus acidilactici*, pediocin, purification, antimicrobial spectrum, preservative, adsorption-deposition, well-diffusion assay, anti-*Listeria*, food additive

47. DNA FINGERPRINTING OF COBALT-60 GAMMA RADIATION-INDUCED VARIANTS OF FOLIAGE PLANTS USING AFLP-PCR

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DNA fingerprinting of cobalt-60 gamma radiation-induced variants of a foliage plant (*Murraya exotica*) was studied using amplified fragment length polymorphism (AFLP) coupled to polymerase chain reaction (PCR) technology. Development of the AFLP-PCR protocol was established for variants of *Murraya* and *Dracaena* where polymorphisms are observed using specific selective nucleotide primer pairs to produce unique DNA fingerprints electrophoresed in denaturing acrylamide gel. Genomic DNA used in fingerprinting study was extracted from leaves of a stable mutant of *Murraya exotica* at M₁V₈ vegetative stage. AFLP-PCR amplified fragments produce specific DNA fingerprints for each variant plant that could be used to identify gamma radiation-induced polymorphisms. Genetic markers induced by gamma radiation and observed in AFLP DNA fingerprints were documented against morphological changes of the variant foliage and encoded in the database for *Murraya exotica*. Graphic computer database serves as specialized catalogue of variant plants and includes actual photographs of plants showing variations in leaves, flowers, and size and data on DNA fingerprints. Thus, a simplified selection process would be available to plant

breeders and commercial plant exporters which would facilitate mass propagation within a shorter growth period of desired variant plants.

Key words: AFLP, PCR, ornamentals, radiation, DNA fingerprinting

48. MITOCHONDRIAL DNA (mtDNA) POLYMORPHISM IN THE ASIAN HONEYBEE, *Apis cerana* F. IN THE PHILIPPINES

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Mitochondrial nucleotide sequence was used to characterize the intergenic region between cytochrome oxidase I (COI) and cytochrome oxidase II (COII) in the mitochondrial DNA (mtDNA) of the honeybee *Apis cerana* F. from four geographically isolated islands in the Philippines (Palawan, Luzon Highland and Lowland, Visayas, and Mindanao). Sequencing data revealed a 99 base pair non-coding region predominantly composed of adenine (A) and thymine (T). It is characterized by a "stem" sequence where no sequence variation was observed but had 18 variable sites found within the segment generating eight (8) haplotypes. The mitochondrial gene tree based on these haplotypes had a branch with Luzon and two of the Visayas and Mindanao haplotypes and another branch with the Palawan together with the rest of the Visayas and Mindanao haplotypes. The 3' end of the leucine-tRNA gene was also sequenced but the 29 base pair segment was too short to yield enough informative characters to differentiate between haplotypes.

The mtDNA sequences of the Philippine honeybees were also compared to known mtDNA sequences of *Apis cerana* F. from Borneo, Sulawesi, Java, and Sangihe mtDNA sequences. The mitochondrial gene tree obtained showed a branch supporting Palawan haplotype together with Borneo and Java, as well as the Ozamis and Cebu haplotype from the Visayas and Mindanao group. While the Luzon haplotypes were on the other branch with the rest of the Visayas and Mindanao haplotypes. Migration of bees during the early geologic history of the Philippines may have contributed to the gene flow, thus the shared or closely related haplotypes.

Mitochondrial genome polymorphism exists among the Philippine *Apis cerana* F. The sequence diversity supports morphometric studies on Philippine honeybees showing the distinct separation of the Palawan populations but could not, how-

ever, delineate between the Luzon Lowland and Highland region. Furthermore, it showed enormous diversity among the populations from the Visayas and Mindanao regions.

Key words: *Apis cerana* F., honeybees, mitochondrial DNA (mtDNA), intergenic region, cytochrome oxidase I and II, mtDNA sequence, polyacrylamide gel electrophoresis, haplotypes, mitochondrial gene tree, genome polymorphism

49. MECHANISM OF DNA IMMUNIZATION: HOW DNA VACCINES INITIATE IMMUNE RESPONSES

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The purpose of this study is to elucidate the mechanism by which DNA immunization initiates immune responses. We found that the site of DNA inoculation (target site) played different roles in gene gun and intramuscular (IM) immunization of mice. The skin target site (following gene gun), but not the muscle, played a central, but not completely essential, role in initiating antibody and cytotoxic T lymphocyte (CTL) responses. These results indicate that the skin target site, which is rich in bone-marrow derived cells, plays bigger a role than the relatively immune-privileged muscle target site in DNA immunization. However, for both methods of immunization, CTL responses were restricted to antigen presentation by bone marrow-derived cells, not by skin or muscle cells. Furthermore, we found that antigen secretion, which presumably would lead to increased antigen migration by lymphoid tissues and increased antigen uptake by antigen-presenting cells, thus leading to increased antigen presentation in the context of MCH class II, did not enhance antibody responses. We propose a model for the mechanism of initiation of immune responses by DNA immunization based on these results and taking them within the context of results from other investigators in the field. We propose that DNA immunization initiates immune responses primarily by the direct transfection of bone marrow-derived cells that migrate rapidly out of the target site into lymphoid tissues, and that antigen expression by skin cells may be involved in raising maximal responses.

Key words: DNA immunization, DNA vaccines, antigen-presenting cells, antigen secretion, immune response

50. SEQUENCE ANALYSIS OF DNA VACCINE CONSTRUCTS: DETERMINING POTENTIAL RISK FOR HOMOLOGOUS RECOMBINATION WITH THE HUMAN GENOME AND OPTIMIZING CODON USAGE

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DNA immunization is a novel and highly effective means of immunization whereby plasmid DNA (DNA vaccines) encoding for antigens are delivered directly into animals or patients whose cells will then express the antigen. DNA immunization comes with the potential risk of the DNA vaccine integrating into the human genome by homologous recombination, thus possibly causing mutations that may lead to carcinogenesis. In addition, the use of gene sequences from pathogens distantly related to humans, e.g., malaria, may affect antigen expression in the human vaccinee.

This study is designed to analyze, using the BLAST family of programs, the DNA sequences of multi-epitope DNA vaccines against dengue and malaria constructed in our laboratory. The possibility of integration of the various DNA vaccines with the human genome was examined by searching for homologies between the DNA vaccines and known human genome sequences. In addition, the sequences were analyzed with respect to the mouse genome, since the animal model used in our lab is the mouse. We have extended these studies to a DNA vaccine for hog cholera, to be used in swine, to determine whether this veterinary vaccine may integrate with the pig genome. Studies will also be conducted to detect actual integration of DNA vaccines into the genomes of recipient mice.

Finally, we analyzed the sequences used for the malaria DNA vaccines and found that some of the malaria codons in our constructs are rarely used in humans and may therefore hamper efficient antigen expression in human vaccinees. Malaria DNA vaccine constructs are now being designed with optimized human codon usage, which will hopefully enhance the expression, and thus the antigenicity, of these vaccines.

Key words: DNA vaccines, sequence analysis, safety, homologous recombination, codon usage

51. USE OF RIBOTYPING AND RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) TO DIFFERENTIATE STRAINS OF *Burkholderia andropogonis*

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Burkholderia andropogonis causes leaf spots, streaks, and stripes on a wide variety of host plants such as corn, coffee, chick pea, and velvet bean. The strains of *B. andropogonis* are highly similar in phenotypic traits such as cultural, morphological, and physiological characteristics. However, the host range of *B. andropogonis* is exceptionally wide and diverse and *B. andropogonis* has wide geographical distribution. Some workers had attempted to group strains of *B. andropogonis* from different host plants based on pathogenicity and serological properties. However, the observed differences in host specificity were insufficient to warrant establishment of pathovars and no further work has been reported to support establishment of serovars. Knowing the relationship among strains of *B. andropogonis* will help to identify outbreaks, to determine its mode of acquisition among strains of *B. andropogonis* will help to identify outbreaks, to determine its mode of acquisition, and to define preventive measures. Unlike phenotypic properties which are not reliable for strain differentiation due to their low reproducibility and inherent variability, genotypic traits are not affected by the physiological state of the organism. In this study, genotypic methods such as ribotyping and random-amplified polymorphic DNA (RAPD) were used to determine the relationship among 29 strains of *B. andropogonis*. In ribotyping, the chromosomal DNA was digested with either *Sal* I, *Pst* I, or *Xho* I and probed by digoxigenin-labeled 16S rDNA of *B. andropogonis*. Hybrids were detected by chemiluminescence. In RAPD, each of the seven commercially available primers was used in low stringency polymerase chain reaction (annealing at 37°C). The amplification products were electrophoresed on agarose gel. The difference between the pattern generated by ribotyping and RAPD was established visually on the presence or absence of one or several bands. Similarity coefficients for pairwise combination were determined by Dice coefficient and clustered by the unweighted pair group method with arithmetic mean (UPGMA) procedure. All computations were performed using the NTSYS-PC program. Numerical analysis of the ribn patterns generated by *Sal* I, *Pst* I, and *Xho* I produced a phenogram where the strains were divided into 11 clusters at a similarity of 90%. In addition, the presence of *rnn* operons

was revealed in *B. andropogonis*. RAPD analysis with seven primers grouped the strains into 12 clusters at a similarity of 90%. Comparison of the phenograms generated by ribotyping and RAPD revealed that the clusters of strains at 90% similarity were similar for these two methods. However, the relationship between clusters varied between ribotyping and RAPD, producing phenograms with different groups of clusters and different overall structure between these two methods. There was no strict correlation between the clusters and the time when the strains were isolated or the geographical origin of the isolates; between the clusters and original host; and between the clusters and pathogenicity of the strains.

Key words: *Burkholderia andropogonis*, ribotyping, RAPD, genotypic methods

52. CLONING AND CHARACTERIZATION OF THE ACYL CARRIER PROTEIN (ACP) GENE OF THE COCONUT (*Cocos nucifera* L.) ENDOSPERM

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Acyl carrier protein (ACP) is an essential cofactor in the synthesis of fatty acids. We report here the isolation and characterization of the ACP gene from coconut. The coconut endosperm ACP gene was isolated by RT-PCR using degenerate oligonucleotide primers designed specifically for the conserved region of multiply aligned ACP gene sequences from other plant species. The ~200 bp PCR product generated was cloned into a vector. DNA-sequence analysis and Southern and Northern blot analyses were subsequently performed. The results and future prospects for the cloned coconut seed ACP gene are also discussed.

Key words: acyl carrier protein, ACP, cloning, RT-PCR, Southern Blot, Northern Blot, DNA sequence, primer design

53. THE COCONUT GENE PROJECT: PRIMER DESIGN

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The general objective of the coconut gene project is to modify the fatty acid composition of coconut oil by genetic engineering. Genes for key enzymes in the lipid metabolism of coconut will be cloned and sequenced. Final expression of these genes in the coconut will be dependent on successful tissue culture methods already being worked out by the Philippine Coconut Authority (PCA).

Accomplishments so far include outlined steps and procedures that one may follow in designing PCR primers. Both the internet and a software called Vector NTI have been utilized in PCR primer design, a necessary start point in the said project. General guidelines for PCR primer design are hereby discussed.

Key words: vector NTI suite, primer design, lipid metabolism, Polymerase Chain Reaction (PCR), internet, annealing temperature (T_m), % GC, entropy, enthalpy, free energy

AGRICULTURAL SCIENCES

54. POLYMERASE CHAIN REACTION (PCR) AMPLIFICATION OF DNA GENOME SEGMENTS OF BANANA BUNCHY TOP VIRUS (BBTV)

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Banana bunchy top disease (BBTD) is one of the most devastating diseases of bananas caused by banana bunchy top virus (BBTV). The BBTV has a multi-component genome consisting of at least six single-stranded circular DNA components. In this study, the presence of DNA encoding the coat protein (CP) during systemic infection of BBTV in artificially inoculated banana was determined by the polymerase bp product in pseudostem 26 days after inoculation (DAI), in root and corm 31 DAI, and in rolled young leaf, older leaf and in corm 52 DAI. In naturally infected banana plants, BBTV stem loop (SL) region and BBTV DNA component encoding the movement protein (MP) were detected in young leaves. PCR amplification of SL region and MP DNA segment generated a 1000 bp and a 380 bp product, respectively. Consistent amplification of MP DNA segment indicated that MP DNA was present in high concentration. PCR amplification using specific primers provides a useful tool in determining the presence of BBTV DNA components.

Key words: banana, polymerase chain reaction (PCR), banana bunchy top virus (BBTV)

**55. CLONING, CHARACTERIZATION, AND SEQUENCING OF
MATURATION-RELATED cDNAs FROM SUGARCANE
(*Saccharum officinarum* L.)**

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The aims of this study are to generate and clone cDNAs encoding the ACC synthase through Reverse Transcription-Polymerase Chain Reaction, to monitor the level of expression of the gene through northern blot analysis, and to determine the relative number of copies in the genome by southern analysis.

Oligonucleotides (EZ 2 and EZ 4) based on the conserved regions of ACC synthase were synthesized and used in the RT-PCR using cDNA pool from the 13-month-old apical tissue. A 1.2k b fragment was amplified which was cloned in the pGEM-T-Easy vector and later used in the transformation of *E. coli* DH5 α .

Two putative clones coding for maturation genes were identified and partial nucleotide sequences of SACS1 (612 bp) and SACS 2 (712 bp) were determined. Computer search showed no homology of SACS 1 with known ACC synthase but has sequence similarity with a DNA binding protein. SACS 2 does not have sequence sequence similarity with known structural genes in the Genebank due to unreliable DNA sequence containing 15% unknown nucleotide base N.

A low level expression of SACS 1 was detected on the 6th month when stalk elongation starts to slow down and increase until the 13th month when the sucrose accumulated is uniform throughout the stalk. Southern blot analysis suggests the presence of only one copy of SACS 1 in the sugarcane genome.

Key words: cloning, cDNA, *Saccharum*, sugarcane, RT-PCR, ethylene, maturation

**56. MOLECULAR CLONING OF DNA SEGMENTS
OF ABACA BUNCHY TOP VIRUS (ABTV)**

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Abaca bunchy top virus (ABTV) is assumed to be synonymous to banana bunchy top virus (BBTV) but transmission experiments show dissimilarity between the two. For studies aimed to distinguishing differences between the said viruses at the molecular level, cloning of ABTV genome segments is necessary.

Polymerase chain reaction (PCR) and standard cloning procedure were used to produce gene constructs containing ABTV DNA segments. Total nucleic acid extracts were obtained from bunchy top infected abaca plants. The extract DNA was amplified using the stem loop primers SLR and SLL. These primers were designed based on the nucleotide sequence of the conserved stem loop region of BBTV DNA genome. The PCR products were inserted into a plasmid vector and transformed into *Escherichia coli* DH5 α cells. These constructs will be used in differentiating BBTV from ABTV and in the development of transgenic abaca resistant to ABTV.

Key words: abaca, polymerase chain reaction (PCR), cloning, abaca bunchy top virus (ABTV), transformation

57. GENE INTROGRESSION IN THE NON-TUBER FORMING *Solanum*

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Nineteen species of the no-tuber forming *Solanum*, including *Solanum melongena*, eggplant, obtained from the National Plant Genetic Resources Laboratory were used in the hybridization program with the aim of determining the genetic affinity among the species and ultimately transferring desirable genes (resistance to pests and diseases, prolificacy, tolerance to abiotic stresses) from the wild relatives to the cultivated eggplant. A total of 135 cross combinations were made from which only 43 cross combinations developed fruits with very few seeds recovered. The degree of genetic relationship was measured in terms of percentage seed developed and chromosomal behavior of the hybrids during meiosis. Percent seed recovery ranged from 0.0% to 80.0%. Meiotic analysis of the different species and the hybrids gave a chromosome number $2n=24$. The chromosome behavior of the parentals was generally normal. The hybrids on the other hand exhibited partial to complete homology among the chromosomes of the parental species. Laggards and bridges were observed in both parents and hybrids. Pollen fertility of the different species ranged from 77.37% to 98% whereas they hybrids had a range of 9.74% to 30.5%. Backcrossing to the *melongena* parent was done for four generations in cross *aethiopicum* \times *melongena*. Partial morphological characterization of the hybrids showed gene introgression from *S. aethiopicum* to *S. melongena*.

Key words: introgression, *Solanum melongena*, *Solanum aethiopicum*, eggplant

58. SEGREGATION ANALYSIS IN COCONUT USING MOLECULAR MARKERS

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Selected germplasm of coconut was tested for polymorphism using microsatellite, also called simple sequence repeats (SSR), and amplified fragment length polymorphism (AFLP) marker technologies. Results showed that all the dwarf populations analyzed exhibited a high degree of homogeneity while the tall populations revealed a high degree of allelic diversity. Based on the results of the polymorphism survey, SSR, and AFLP primers were selected to test for the segregation pattern of the F₁ population from the cross between Tacunan Green Dwarf and Bago Oshiro Tall. Segregation analysis of F₁ progenies showed a 1:1 Mendelian ratio typical of a backcross population. The implication of the results of the present study in the coconut improvement program will be discussed.

Key words: coconut, *Cocos nucifera*, microsatellite, simple sequence repeats, amplified fragment length polymorphism

59. MOLECULAR TAGGING OF BRUCHID AND *Cercospora* LEAF SPOT RESISTANCE GENES IN MUNGBEAN USING AFLP AND RGA MARKERS

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Two major constraints in mungbean production are grain loss caused by bruchid (*Callosobruchus*) damage during storage and *Cercospora* leaf spot disease. Amplified fragment length polymorphism (AFLP) and resistance gene analog (RGA) primers were used to develop and identify markers associated with resistance genes to bruchid and *Cercospora*. Sixty four selective Eco RI/Mse I primer

pairs obtained from Gibco BRL/Life Tech Analysis II were used to screen for polymorphism using two mapping populations, P7 x TC and NCM 53 x Acc. 25. Thirty (30) of the 64 AFLP primer pairs with good amplification products generated a total 1,520 bands of which 336 bands (21.1%) were polymorphic between P7 and TC and 261 bands (17.2%) between NCM 53 and Acc. 25. Using a P7 near-isogenic line (RP70, putative AFLP markers associated with the introgressed resistance segmen from TC were identified using 17 AFLP primers. For RGA, 19 primer pairs were evaluated of which 10 primers gave polymorphic bands between P7 and TC. The 10 primer pairs generated a total of 268 bands of which 117 bands (43.6%) showed polymorphism between P7 and TC. The 10 RGA primers will be used to determine markers associated with bruchid resistance genes using a near isogenic line of P7.

Key words: mungbean, *Cercospora*, bruchid, *Callosobruchus*, AFLP, resistance gene analog

60. REGENERATION OF TRANSGENIC NEW PLANT TYPE LINES FROM *A. Tumefaciens*-INFECTED IMMATURE INFLORESCENCE

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The increasing population rate and shrinking area devoted to rice production in the Philippines pose a big challenge to plant breeders. Genetic engineering of PhilRice is being conducted to support the existing strategies to increase the yield potential of rice such as the improvement of pest resistance in the high yielding varieties, in the new plant type (NPT) elite lines, and in the cytoplasmic male sterile (CMS) lines used in hybrid rice breeding. The use of the natural vector *Agrobacterium tumefaciens* for gene delivery to Philippine-bred rice varieties necessitates the investigation of the suitable explant for susceptibility to infection. Early studies focused on the ability of different explants to produce embryogenic calli suitable for transformation experiments from 6 inbred lines, 9 NPT lines, AND 6 CMS lines. The young inflorescence and mature embryos equally produced embryogenic calli, but not as good as the immature embryos scans contamination. *A. tumefaciens* strain EHAI O5 (pTOK233) harboring the reporter β -glucuronidase gene and the hygromycin resistance genes were tested on their infectivity to the different explants and genotypes. A few inbreds and CMS lines, and most of the NPT elite lines showed GUS activity as manifested by the blue

precipitate present in the transformed cells after X-gluc staining. Regenerated plants from these transformed calli showed the presence of the GUS gene in PCR analysis. The efficiency in obtaining transgenic plants is relatively higher compared to the regeneration using calli derived from mature seeds.

Key words: genetic engineering, rice, *Oryza sativa*, young inflorescence, *Agrobacterium tumefaciens*, plant regeneration, calli

61. MOLECULAR ANALYSIS OF ON-FARM BIODIVERSITY OF RICE IN THE PHILIPPINES

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On-farm biodiversity of rice in the Philippines was analyzed using 1992 and 1997 survey data and 41 microsatellite markers to determine the popular varieties planted by farmers and their molecular diversity, respectively. Survey data showed that the use of modern varieties was widespread and more diverse in all regions. A total of 30 different varieties were planted in 1992 and 156 varieties in 1997. Among these varieties, ten most popular varieties were identified. These were released mostly between 1975 and 1999, and preferred by about 77% of the farmers. IR 64, a high-yielding variety with good eating quality, was the most widely used, suggesting the responsiveness of rice farmers to consumer preferences. Over time, the adoption pattern and relatedness of varieties changed. When the pattern of adoption was combined with molecular data in determining the genetic diversity (weighted), the diversity index decreased. The increased relatedness of the popular varieties planted in 1997 decreased the on-farm diversity. Among the regions in the Philippines, Mindanao region has the highest on-farm diversity. The pattern of adoption changed on-farm diversity substantially specially in areas where IR 64 was widely adopted like in the Visayas region where most farmers used it in 1992. Overall, the weighted on-farm diversity is lower than the genetic diversity based on molecular data alone (unweighted). Results show the impact of widespread use of a particular variety in the maintenance of biodiversity.

Key words: biodiversity, DNA marker, microsatellite, rice

62. IDENTIFICATION AND CHARACTERIZATION OF ACC SYNTHASE cDNAs EXPRESSED DURING SINTA PAPAYA (*Carica papaya*, L.) FRUIT RIPENING

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The cloning and characterization of cDNAs encoding ACC synthase from papaya hybrid Sinta is described. Total RNA from 80% ripe fruit was subjected to R T-PCR using primers specific for the ACC synthase gene. Five genes with different ECO RI restriction digest patterns and MW range of 1.1 to 1.4 kb were cloned. A cDNA of length 1,206 bp and coding for a 402-amino-acid polypeptide contained the highly conserved region shared by both ACC synthases and aminotransferases. Partial sequencing information indicate that all five genes are highly homologous to one another as well as to three their ripening-related ACC synthase cDNAs isolated from other papaya cultivars. Hybridization studies on northern and southern blots are currently being done to further characterize the five genes. Such information will help elucidate the ripening process undergone by hybrid papaya fruits.

Key words: cloning, cDNA, ACC synthase, *Carica papaya*, fruit ripening, RT-PCR, ethylene, southern blot, northern blot

63. MOLECULAR CLONING OF THE COAT PROTEIN GENE OF PAPAYA RINGSPOT VIRUS (PHILIPPINE ISOLATE)

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Development of constructs containing certain genomic sequences of viruses has been the basic technique used in the study of the organism's genetic organization. One of the strategies in developing disease resistant crops is pathogen-derived resistance against virus infections. An example of which is the production of a transgenic crop expressing the viral coat protein. Crude RNA from leaves infected with a local PRSV isolate was extracted. Complementary DNA (cDNA) was synthesized using reverse transcriptase and the coat protein (CP) gene sequence was amplified by PCR using specific primers. The fragment encoding the CP gene

was inserted into a plasmid vector and transformed into *Escherichia coli* DH5 α . A gene construct containing a part of the CP gene of one isolate is already available for sequencing and cloning on other isolates is still ongoing. The clones will be utilized in PRSV genetic variability studies and in the development of transgenic papaya resistant to ringspot disease.

Key words: papaya, papaya ringspot virus (PRSV), polymerase chain reaction (PCR), pathogen-derived resistance, complementary DNA (cDNA), reverse transcriptase, coat protein, cloning

64. CONTROL OF RIPENING IN PAPAYA BY GENETIC ENGINEERING

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Papaya is an important fruit commodity in the Philippines and in other ASEAN countries. Its greater production and export potential are limited by the papaya ring spot virus (PRSV) problem and relatively short shelf life. This research addresses the latter problem with the objective of developing papaya varieties with delayed ripening trait, thus, longer shelf life.

In climacteric fruits such as papaya, ethylene controls the rate of ripening. One strategy to delay fruit ripening which has been employed in other fruits with success is the genetic manipulation of the plant genes involved in ethylene production during the ripening process.

In this project, we have cloned the ripening-related ACC synthase genes from the Davao Solo (yellow flesh) papaya variety and constructed an anti-sense transformation vector. We have transformed somatic embryos with this gene construct via the biolistic process. Putative transgenic tissues were selected and regenerated into plantlets. These plantlets will be grown in a BL2 greenhouse and their fruit will further be selected for the delayed ripening trait. The development of delayed ripening phenotypes via antisense technology will produce papaya varieties with better postharvest and transport characteristics that will be reflected in fruits of consistent superior quality and therefore better market prices.

Key words: papaya, ripening, transformation, ACC synthase, ethylene

65. DEVELOPMENT OF VEGETABLE IPM PROGRAM IN RICE-BASED CROPPING SYSTEM

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A study was conducted to identify the different insect pests and beneficials at different growth stages of eggplant and stringbeans and identify as well the critical growth stage(s) of the crops against insect infestation.

The occurrence of insect pests and beneficials relative to crop age was determined in both protected and unprotected plots. After mapping the insect pests and beneficials based on three cropping seasons, a strategic insecticide application was formulated and compared with farmers practice and untreated control.

A characteristic pattern of pest population of natural enemy attraction was recorded. In stringbeans, three peaks of infestation or critical periods (window) for crop growth seems attractive to early sucking pests such as leafhopper and aphid. The critical periods were 19-20, 53-54, and 65-75 days after emergence (DAE). In addition, 51-53 and 65-70 days, were critical periods to podborer damage. In eggplant, leafhopper and thrips predominated throughout the crop growth stage and their respective densities were highest at 20-25, 40-45, and 85-90 days after transplanting (DAT). Another significant observation is the apparent relationship of shootborer damage (highest at 67 DAT) and beneficials like spider and coccinellid. The shootborer damage declined at 47 DAT and 81 DAT, the stage which coincided with the peak of spider and coccinellid populations indicating, among others, the probable role of beneficials in shootborer control.

The arthropod population dynamic data obtained were used to formulate a strategic insecticide application to optimize the effect of insecticides on insect pests while minimizing its impact on beneficials.

Efforts to demonstrate the judicious use of appropriate insecticide application based on insect occurrence and monitoring the critical windows showed that strategic applications in stringbeans and eggplant resulted in reduced spray application frequency. This strategy saved three applications in stringbean and six applications in eggplant compared to the farmers' practice.

While at times there were no significant quantitative yield differences between strategic insecticide application and farmers' practice plots, the savings in

pesticide inputs and environmental safety cannot be ignored. It is apparent that the unprotected (unsprayed) plots in most cases yield is much lower than any of the other two treatments.

Further refinements in the strategies used including insecticide x parasitoid complementation are currently being studied. Initial data showed very promising results.

Key words: vegetable IPM, eggplant, stringbeans, rice- based cropping system

66. TRANSPLANTED IRRIGATED LOWLAND RICE PROGRAM AT PHILRICE

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Transplanting is still the predominant method of planting rice. Majority of the farmers in Luzon and Mindanao are transplanting rice in irrigated lowland fields, the most dependable source of rice in the country. Compared to direct seedling, transplanted rice has higher productivity but it is labor intensive. The average yield in irrigated lowland is 3.5 t/ha; but under the Gintong Ani Program, farmers yield average to 4.4 t/ha. With decreasing area devoted to rice and the global liberalization, research and development activities in transplanted regime have to be continued. The program aims to develop farming technologies that will improve and sustain yields in the transplanted irrigated lowland rice and to attain an average yield of 7.5 t/ha and 10 t/ha in multi-location trials by 2002 and 2005, respectively. Specifically, the research agenda will develop and promote location-specific nutrient and pest management technologies, improve and sustain yields of transplanted irrigated lowland rice; and implement technologies that are efficient, practical, and environment-friendly. Major accomplishments and current activities will be presented.

Key words: rice, transplanted rice, irrigated lowland, technology development

67. IMPROVEMENT OF IR64, C4-63G, PSB Rc 4, AND BPI-RI-10 FOR TRANSPLANTED IRRIGATED LOWLANDS

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Four rice varieties, IR64, BPI Ri-10, PSB Rc 14, and C44-63G, are very popular in the Philippines. However, these are not resistant to bacterial leaf blight (BLB), one of the most serious diseases of rice in farmers' fields. With the identification of new genes like *Xa-21* that was not available during their development, these varieties can be improved through incorporation of new important traits like disease resistance. This study aims to improve BPI-Ri-10, IR64, C4-63G, IR546883, and PSB Rc4 through incorporation of *Xa-21* and *xa-5* for BLB resistance.

For four seasons, 146 advanced lines from the crosses involving these popular varieties were evaluated in replicated trials together with the five high yielding parentals. The plants grown in 6 rows plots with 11 hills were clip-inoculated with Maligaya strain of *Xanthomonas oryza* pv. *Oryza* (Kov) at maximum tillering and was scored 19 days after inoculation. All lines were found resistant indicating that they had the introduced *Xa-21* of *xa-5* gene. Across seasons, the highest yield of 7.02 t/ha was obtained from C4- 63G/IRBB21 cross. This line has *Xa-21* gene good for almost all races of BLB in the Philippines. The IR64 progeny with *Xa-21* closely followed with 6.92t/ha, and PSB Rc4 with *xa-5* at 6.84 t/ha. Although BPI-Ri-10 progenies were not among the best ten lines, several lines from this cross have an average of 6 t/ha across four seasons. These elite lines are now in the replicated yield trails and will be advance to multi-location trials.

Key words: rice, bacterial leaf blight, disease resistance, *Xa-12* gene, high yield

68. ANALYSIS OF TECHNICAL EFFICIENCY OF RICE PRODUCTION IN CAMARINES NORTE AND NUEVA ECIJA IN THE PRESENCE OF THE EL NIÑO PHENOMENON

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This study deals mainly on the analysis of technical efficiency of rice farmers in Nueva Ecija and Camarines Norte as affected by weather abnormality such as the El Niño phenomenon. The technical efficiency of rice farmers was determined through a Maximum Likelihood Estimation of non-deterministic frontier production function. The resulting technical efficiency was then regressed with a dummy variable in order to measure the effect of the El Niño phenomenon. Furthermore, it was regressed against some socioeconomic characteristics of farmers to determine the factors affecting efficiency under such circumstances. The production in each province was also regressed against the El Niño dummy variable to seek its effect on yield level. Results of the study showed that the El Niño phenomenon has no significant effect over the technical efficiency of rice farmers in the two provinces. This implies that farmers do not change their cultural and farm management practices even under the presence of the El Niño phenomenon. The results also revealed that socioeconomic characteristics such as age, education, training attended, and tenure status have no significant effect over technical efficiency. This is not due to inherent lack of relationship between the said variables but merely attributed to the relative homogeneity of the respondents. It was also found out that the El Niño phenomenon significantly increased the yield in Camarines Norte during the dry season while significantly reduced the production in both provinces during the wet season. The production enhancing effect was primarily accounted to the increased solar radiation during the dry season while yield-reducing effect was accredited the absence of normal rainfall that the two provinces usually received during such season. The study also determined the inputs significantly increasing production under such weather stress. These significant factors are water, fertilizer, and pesticide during dry season; and labor, fertilizer, and insecticide during the wet season. This implies that with the efficient use of such inputs, the farmers might minimize the yield reduction or maximize the production enhancing effect of the El Niño phenomenon. Finally, it was recommended that results of the study be verified using a more reliable proxy variable for El Niño such as rainfall data.

Key words: El Niño, dry season, wet season, Camarines Norte, Nueva Ecija, rainfall

69. RAPID DEVELOPMENT OF IMPROVED INDICA RICE VARIETY THROUGH ANTHER CULTURE TECHNOLOGY

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The major application of anther culture (AC) technology in rice breeding is to accelerate production of stable, homozygous breeding lines from genetically diverse, heterozygous genotypes. This results in the shortening of the time required for development of new varieties. The technology also offers the possibilities of introducing into plants variability that could be utilized for crop improvement. Anthers from IR64, a commercially released indica rice variety with premium grain and eating quality, were cultured *in vitro*. From a total of 57,549 anthers plated, we regenerated 45 green plants, of which, 19 are doubled haploids. These doubled haploid regenerants yielded a total of 147 doubled haploid breeding lines (DHL). A DHL is defined here as a tiller developed from an AC-derived doubled haploid regenerant. From these DHL, five were selected based on phenotypic acceptability, excellent kernel quality, moderate shatterability, and field resistance to diseases. The yield performance of three of the five lines was evaluated for two seasons, in 1999 dry and wet season, comparing them with the seed-derived (SD) IR64. At this point, the DHLs were at R_2 and R_3 generation, respectively. For two seasons, the AC-derived IR64 DHL matured earlier, and yielded better than the SD IR64. They produced more and comparable productive tillers in dry and wet season, respectively. The AC-derived IR64 plants were taller than the SD IR64 during the dry season, but shorter during the wet season. The data indicated that the SD IR64 during the dry season, but shorter during the wet season. The data indicated that the SD IR64 elongated more during the wet season trial. It can be inferred that SD IR64 is more sensitive to solar radiation than the AC-derived IR64. This needs further study. Based on yield performance, one AC-derived IR64 was selected and elevated to the general yield trial (GYT). Conventionally, it will take at least six years for a breeding line to reach this stage. However, with the AC-derived IR64 selection, it took only three years. Thus, with anther culture technology, varietal improvement is expedited.

Key words: anther culture, doubled haploid line, yield performance, rice breeding

70. COMPARATIVE ANTHR CULTURE RESPONSE OF GENETICALLY DIVERSE AND HIGHLY HETEROZYGOUS INDICA RICE TO 2,4-D AND PAA-ENRICHED MEDIUM

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Anthers from 18 genetically diverse and highly heterozygous F_1 crosses and two inbreds of rice were cultured in callus induction medium, supplemented with either 2,4-dichlorophenoxyacetic acid (2,4-D) or phenylacetic acid (PAA). Anthers from ten crosses formed more calli in 2,4-D-enriched medium, eight in PAA-enriched medium, while two had comparable callus formation in both media. Percent callus formation ranged from 3.19 to 29.56 in 2,4-D-enriched medium, with a mean of 12.28 ± 7.91 . In medium supplemented with PAA, callus formation ranged from 2.95 to 32.85%, with a mean of $12.94 \pm 8.41\%$. Significant differences in callus formation were obtained for genotype, but not for callus induction medium, nor for the interaction between genotype and culture medium. The callused anthers were transferred to various regeneration media to induce shoot and root formation. The green plant regeneration obtained for majority of the genotypes, was less than 1.0%, based on the total anthers plated. From our previous study with inbreds, we showed that PAA can induce direct regeneration in the same callus induction medium, without transfer of callused anthers into regeneration medium. This is one possible advantage of using PAA, as auxin for callus induction, over 2,4-D. We would like to exploit this advantage with our highly heterozygous breeding materials. This one-step anther culture protocol using PAA saves labor, time, laboratory supplies, and chemicals, as far as generating anther culture-derived breeding lines is concerned. Further study with other genotypes will be conducted to establish this point.

Key words: anther culture, indica rice, 2,4-D, PAA callus induction, direct plant regeneration

71. IN VITRO RESPONSE OF ANTHER CULTURE-DERIVED IR64 BREEDING LINES

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Genotype is one of the major factors that determines the in vitro response of rice, and hence, the extent of utilization of in vitro culture in rice improvement. IR64 is one of the indica rice varieties recalcitrant to in vitro culture. In our anther culture (AC) work, we regenerated green plants from 0.08% of the total anthers (57,549 anthers) we cultured from the rice variety IR64. We advanced and evaluated these regenerants, and we selected some promising AC-derived IR64 breeding lines. This study was conducted to determine if the in vitro response was enhanced in the anther AC-derived IR64 breeding lines compared with seed-derived (SD) IR64. We used three explants, viz., young inflorescence, mature seed, and anther to establish the cultures. Our preliminary results indicated comparable callus formation from mature seeds of the AC-derived and SD IR64. Relatively more embryogenic calli were obtained from AC-derived lines. One of the three AC-derived lines regenerated more green plants than the SD IR64. With inflorescence culture, we used callus induction medium supplemented with either 2,4-D or PAA. In culture medium supplemented with PAA, more young spikelets regenerated shoots directly from AC-derived compared with the SD IR64. With 2,4-D, callus formation for AC-derived lines was not enhanced. However, one of the three AC-derived lines evaluated had enhanced green plant regeneration. With anther culture, better response was obtained from AC-derived lines, in terms of callus formation and green plant regeneration. Further studies will be conducted to establish the repeatability and stability of the in vitro responses observed.

Key words: anther culture, seed culture, inflorescence culture, variant

72. RICE VARIETIES AND GRAIN QUALITY ATTRIBUTES PREFERRED IN ADVERSE ENVIRONMENT

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A survey was conducted in adverse rice growing areas to determine farmer choice for varieties and consumer preference for grain quality. The provinces

visited were Cagayan and Camarines Sur for saline-affected areas; Agusan del Norte and Nueva Vizcaya for zinc-deficient areas; and the province of Ifugao for cool-elevated areas. Five municipalities for each province were chosen and three barangays for each municipality were visited. The most popular variety was IR 66 in Cagayan, PSB Rc 10 in Camarines Sur, PSB Rc 18 in Agusan del Norte, BPI R 10 in Nueva Vizcaya, and a native or traditional variety in Ifugao. All of the provinces surveyed showed that the major reasons for choosing the varieties was high yield, except in Ifugao where the highest premium was placed on grain quality. Asked about the grain quality attributes, the consumers indicated that taste was the most important. The other characteristics preferred were aroma, tenderness, and smoothness for cooked rice and maximum height increase and whiteness for raw rice. This information will provide the breeders and biotechnologists a guide in developing rice varieties with good grain quality for the adverse areas.

Key words: rice varieties, grain quality, adverse environment, saline, zinc deficiency, cool-elevated, raw rice, cooked rice, consumer preference, farmer choice

73. MASS SCREENING FOR RICE SEEDLING SALT TOLERANCE AT PHILRICE

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A rapid screening method for rice seedling salt tolerance was established at the Philippine Rice Research Institute in June 1996 to identify and assess the degree of salt tolerance of different lines and varieties. The procedure is an integral component of the project on development of salt tolerant varieties where by only lines rated tolerant and moderately tolerant in the observational nurseries are advanced to succeeding performance evaluation. The technique was a modification of the procedure being used at the International Rice Research Institute (IRRI) using Yoshida nutrient solution. Salt tolerance at electrical conductivity (EC) 12 mS/cm can be recognized after 21 d after sowing or 16 d after salinization.

Sources of materials for testing included the traditional rice variety collection, uniform lines from the breeding program of PhilRice, and introductions from collaborative breeding institutions. As of January 2000, a total of 5331 lines/varieties had been screened in the different nurseries and trials. Out of those screened, 601 (10.9%) and 1536 (27.8%) were rated tolerant and moderately tolerant, respectively.

The procedure is presently being modified using seawater instead of adding NaCl to increase the EC. Seawater samples from four locations showed an average of 42.7 mS/cm EC and 1.85 ppm sodium content. The use of artificial seawater solution will simulate water conditions during salt intrusion where other salts are present.

Key words: electrical conductivity, mass screening, rice, salt tolerance, seawater

74. HUNTING THE RICE TUNGRO RESISTANCE GENE USING BACTERIAL ARTIFICIAL CHROMOSOMES AND RESISTANCE GENE ANALOGUES

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As a step toward engineering resistance to tungro, the most destructive viral disease of rice in Southeast Asia, a map-based cloning approach was initiated to isolate a resistance gene against tungro spherical virus, the primary causal organism. Fine genetic mapping is underway to identify AFLP markers <1.0 cM from the R locus. In parallel, physical mapping was carried out starting with RFLP and RAPD markers at ~ 1.0 cM. Four BAC clones were selected from libraries obtained from the International Rice Research Institute and the University of California, Davis using three anchor markers (C708, CDO456, and CDO783) at the resistance region. During chromosome walking from the anchor BAC clones, seventeen BAC ends were isolated by TAIL-PCR (thermal asymmetric interlaced polymerase chain reaction) that were used to identify 28 new candidate BAC clones in subsequent hybridization. A contig of 14 clones at the C708 locus, a contig of 12 clones at the CDO456 locus, and another contig of 5 clones at the CDO783 locus were localized. Together with IRRI's screening, 36 BAC clones flanking the genes were identified at the target region. The size and arrangement of the tentative contig at the C708 locus were determined through pulse-field gel electrophoresis and cross hybridization. Gene hunting using PCR with RGA (resistance gene analogue) primers was also initiated on the 14 BAC clones. Clone 8P 16 gave major PCR bands with primer pair 1) ptokin 1 and ptokin 2, and 2) XLRR inverse 1 and inverse 2. Clone 16D8 also produced distinct PCR bands with the ptokin 1-ptokin 2 primer pair.

Key words: BAC, RGA, TAIL-PCR, RTVS, AFLP, RFLP, RAPD, physical mapping, chromosome walking, contig

75. PCR-BASED DNA FINGERPRINTING OF ANTHR-CULTURE DERIVED INDICA RICE BREEDING LINES

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Stable breeding lines were obtained from anther culture-derived variants from indica rice variety Wagwag and IR64. For some variants, differences in some agronomic traits were established. Other variants, however, are morphologically and agronomically similar. To genetically differentiate these variants, we subjected them to RAPD (randomly amplified polymorphic DNA) and SSR (simple sequence repeats) analysis. For RAPD analysis, we screened 37 primers. With Wagwag variants, 24 primers are polymorphic and four are monomorphic. The rest of the primers did not amplify. Only nine, those polymorphic primers that amplify in all samples, were scored. With IR64, 11 primers are polymorphic, 4 are monomorphic. Only 10 polymorphic primers were scored. For SSR analysis, we screened 26 and 40 primers for Wagwag and IR64 variants, respectively. Twenty and 16 primers were scored for Wagwag and IR64, respectively. The results of the amplification reactions with the rest of the primers are not scorable. With the polymorphic molecular markers we were able to genetically differentiate the AC-derived variants from one another, and from the seed-derived parental genotype.

Key words: anther culture, RAPD, SSR, indica rice

76. GENETIC TRANSFORMATION OF RICE (*Oryza sativa* L.) USING PIN2 AND GNA GENES FOR INSECT RESISTANCE

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Currently, insect pests are controlled mostly by chemical insecticides which also eliminate the beneficial insects and arthropods in the rice causing pest resurgence and more extensive damage. Introduction of Pin2 and GNA genes in rice is expected to confer tolerance/resistance to lepidopterans (stem borers, leaf folders, cutworms) and homopterans (brown planthopper, green leafhopper) pests, respectively.

Calli induced from sutella of mature seeds and immature embryos of IR72, PSBRc 14, PSBRc28, LX15, Taipei 309, and IR43 served as materials for transformation. Friable and embryogenic types of callus were shown to be excellent material for transformation as reported for japonica rice by Hiei et al. (1994). All the varieties being used produced the same kind of callus. On the other hand, among the varieties, Taipei 309, and LX15 had the highest callus induction rate while PSBRc14 had the lowest.

Embryogenic calli bombarded with pTWA and pubiGNA containing Pin2 and GNA genes, respectively, using the particle inflow gun apparatus were kept in the selection medium for at least one month or until the calli from the control treatment (not bombarded with Pin2 and GNA genes) died. Calli were then transferred into plant regeneration medium.

Plants were regenerated from BASTA^R and hygromycin resistant calli induced from both mature seeds and immature embryos. Molecular analyses and physiological evaluation of the putatively transgenic are presently being undertaken. Preliminary PCR analysis showed positive results.

Key words: rice, transformation, particle bombardment, Pin2, GNA, insect resistance, embryogenic calli, BASTA, hygromycin

77. EFFECT OF MIST-POLISHING ON THE PHYSICOCHEMICAL AND SENSORY PROPERTIES OF RICE

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The general impression on NFA rice is that it is of poor quality. One of the possible methods to eliminate this impression is through mist-polishing. Mist-polishing is a process wherein water, in the form of mist, is sprayed unto the grains during the polishing stage to remove excess dust, bran, and the aleurone layer remaining in the longitudinal groove. Physicochemical analysis and sensory evaluation were conducted using stocked NFA rice, remilled rice, and remilled-mist-polished rice to determine the effect of mist-polishing on rice grain quality. The most distinct difference among the three samples was seen in the physical properties of raw milled rice. The mist-polished sample was significantly superior over the remilled sample in color, gloss, translucency, and general acceptability. The remilled sample was, in turn, significantly superior over the original stock sample. However, in the cooked samples, remilled and mist-polished samples did not differ significantly in sensory and physicochemical properties and aerobic plate count. For these parameters, remilling was enough to improve the quality of the stock sample.

Key words: NFA rice, mist-polishing, remilling, grain quality, physicochemical properties, aerobic plate count, sensory evaluation, gloss translucency, color

78. PHYSICOCHEMICAL PROPERTIES OF IRON FORTIFIED RICE FLOUR

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Rice flour prepared from PSBRc 10 rice variety intended for use in the production of traditional rice-based food products and noodles was fortified with iron by manual mixing for 30 min to 1h. The non-fortified rice flour contained $8.00-9.35 \pm 0.679$ mg Fe/100g rice flour while the treated sample had iron content ranging from 17.71 to 37.50 mg/100g. Analysis of variance (ANOVA) at 5% level of significance revealed that non-fortified rice flour was significantly whiter (75.27 whiteness values) compared to those containing ferrous fumarate (FF) (74.20 whiteness values) and ferrous succinate (FS) (73.43 whiteness values) at concentrations of 30 mg/100g. Bulk density of iron fortified rice flour was significantly higher compared to the control. Setback and breakdown viscosities slightly change with the addition of FF. Ferrous succinate treated rice flour on the other hand showed slight decreases in their peak and final viscosities. Other important properties were not affected which indicates that iron fortified rice flour could find several applications in the rice-based food industry.

Key words: rice flour, noodles, ferrous fumarate, ferrous succinate, whiteness, bulk density, setback, breakdown, peak viscosity, final viscosity

79. RICE NOODLE CHARACTERISTICS AS AFFECTED BY IRON

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Noodles were prepared using rice flour fortified with 10, 20 and 30 mgL⁻¹ 100 ferrous fumarate and ferrous succinate. The process involved soaking, steaming, kneading, extrusion, boiling, and drying. The rice flour (RFN) was characterized in terms of cooking and sensory qualities. The retention of iron at different stages

of noodle production was also assessed. Cooking losses of non-fortified and fortified overall flavor, smoothness, and chewiness of RFN were not affected with the addition of ferrous fumarate. No significant changes were also observed in the overall appearance, overall flavor, and overall texture of RFN enriched with ferrous succinate. A slight difference in the overall appearance of ferrous succinate-treated RFN were noted which was attributed to a slight change in color. Sensory qualities were most acceptable at fortification levels of 20 mg/100g.

Key words: noodles, rice flour, ferrous fumarate, ferrous succinate, cooking quality, sensory quality, cooking losses, overall flavor, overall appearance, overall texture

80. VARIABILITY IN RICE STEM BORER POPULATIONS AND ITS IMPLICATIONS IN THE DEVELOPMENT AND POSSIBLE RELEASE OF BT ENGINEERED RICE

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The Bt gene had been successfully engineered in several crops and it will not be long that its transfer and stable expression in rice will be realized. Engineering one very effective gene in rice does not mean a successful, stable agriculture. There are so many unresolved issues and straightforward answers unanswered. Understanding the complexities in the interactions would mean a better chance in the deployment of the technology in a sustainable manner. To achieve this requires good evaluation for useful *Bt* genes; assessment of the potential for resistance in rice pests, and in this case the stem borer; transformation technology and gene expression; field tests of strategies to delay resistance; germplasm development and distribution. Thus there is a need to understand ecological and genetic interactions between *Bt* toxins, rice and rice pests and natural enemies. The results of this study provide some answers to these needs.

As to the search for the useful *Bt* gene, the result, of the study show that there are some *Bt* genes which could serve as candidates for introduction into the rice genome based on several criteria: lack of genetic homology with toxins of standard strains, high efficacy at killing specific stem borer pests, and unique mode of action. These are *CryIAc* for the striped stem borer and *CryIIa*, *CryIIA*, and *CryIC* for the yellow stem borer. The striped stem borer is very susceptible to *CryIAc* but not with the other two endotoxins, *CryIIA* and *CryIC* which were found to be as equally effective as *CryIA/c* against yellow stem borer. Another impor-

tant finding derived from this study is that those *Bt* endotoxins which has no "knockdown effects" are we effective not by killing but also by avoidance of the insects and inhibition of the growth of the larvae. This information is important to develop strategies in the proper development of the engineered rice.

An understanding of the pest movement based on genetic structure is essential for the logical development of resistance management strategies. The results of this study show geographical variation in response of different rice types and *Bt*-transgenic rice. This only indicates that any *Bt*-transgenic rice will not have the assurance of complete success when deployed in all the local fields where it will be planted. It was shown in this study that resistance to the very toxic *CryIIAc* varied from population to population, thus there is a possibility that populations of these insects have the potential to overcome the effect of the *Bt* toxins expressed in rice. The problem will be compounded with large movements of these resistant insects to adjacent rice fields causing yield losses.

The practices of farmers of heavy pesticide use may contribute to the failure of the *Bt*-transgenic rice. It was found in a local survey of farmers management of rice pests that spraying with insecticides is done whether the rice variety is resistant or not. It is most likely that when *Bt*-rice will be deployed, this will be treated just like other varieties. Considerable effort should be exerted to help farmers realize the proper way of managing rice through education and participatory training in pest management

Key words: *Bacillus thuringiensis*, pest management, variability

**81. RESISTANCE SCREENING OF FARMERS' AND
COMMERCIAL VARIETIES OF EGGPLANT AGAINST
THE LEAFHOPPER, *Amrasca biguttula* (ISHIDA) AND THE
EGGPLANT BORER, *Leucinodes orbonalis* BUENEE**

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Eleven eggplant varieties were screened for resistance to the leafhopper *Amrasca biguttula* (Ishida) at the Central Experiment Station, PhilRice, Muñoz, Nueva Ecija in separate trials. Results in the first trial showed that the farmers' variety, Abar, was tolerant having higher yield than Dumaguete Long Purple (DLP), despite comparably the same number of leafhoppers as the susceptible variety. The relatively thicker leaves and more dense trichomes possibly conferred tolerance to Abar against the leafhopper.

In the second trial, antixenosis or non-preference (oviposition and feeding) was demonstrated by SRO2, a farmers' variety from Ilocos, at the early vegetative and reproductive stages of plant growth. The number of nymph and adult leafhopper was consistently low in all three leaf positions. Leafhoppers preferred to feed on DLP and Lon Violet throughout the sampling period. Damage ratings showed least leaf yellowing and, cupping for SRO2 but advanced damage for Long Violet and DLP. The mechanism of resistance of SRO2 should be further investigated in view of its possible use in eggplant varietal improvement. IPB GSI, an improved IPM leafhopper tolerant line, significantly yielded the highest healthiest, and largest fruits in all four harvesting periods despite the number of leafhoppers present. IPB GSI is a good candidate for leafhopper tolerance. However, more breeding works should be conducted to further raise the level of tolerance.

Key words: eggplant borer, eggplant leafhopper, *Amrasca biguttula*, *Leucinodes orbonalis*, host plant resistance in eggplants

82. EFFECTS OF TEMPERATURE, pH, AND NITROGEN SOURCES ON THE GROWTH AND SPORULATION OF *FUSARIUM* CAUSING WILT AND ROOT ROT OF GARDEN PEA (*Pisum sativum* L.)

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The ability of *Fusarium oxysporum* f.sp. *Pisi*, Schlecht. Snyder and Hansen and *F. solani* f. sp. *Pisi*, Apell and Wollenweber to cause wilt and root rot on Garden Pea are influenced by temperature, pH, and nitrogen sources.

Both species of *Fusarium* grew under a temperature range of 10°C to 30°C in potato dextrose agar (PDA). Scanty mycelia was noted at 10°C. *F. oxysporum* reached the widest colony diameter of 80 mm and a spore count of 99/mL at 30°C, while 88 mm colony diameter and 86 spore count /ml was obtained at the same temperature after 1 week.

In terms of pH, both species of fungi grew at pH range of 3.0 to 8.0. However, the widest colony of 70 mm with the highest spore count of 250/mL of *F. oxysporum* was obtained at pH 5. The same trend was noted on the growth and sporulation of *F. solani*. The widest colony of 69 mm and a spore count of 395/mL were recorded at the same pH (5) after 1 week using potato dextrose agar (PDA). Growth and sporulation of the fungus declined as the pH was raised to neutral (7.0).

In relation to nitrogen sources, both species of *Fusarium* utilized different nitrogen sources: ammonium nitrate, ammonium sulfate, calcium nitrate, potassium nitrate, sodium nitrate, and urea. The heaviest mycelia weight of .17 g of *F. oxysporum* was obtained in potassium and is comparable with oven dry weight of mycelia from the standard media potato dextrose agar (PDA), while the heaviest mycelia dry weight of 18 g of *F. solani* was obtained from ammonium nitrate. On the contrary, the highest spore count of 806/mL was produced in calcium nitrate for *F. oxysporum* while 697 spores/mL was found in potassium nitrate for *F. solani*. Spore counts obtained from calcium nitrate and potassium nitrate were comparable with spores produced in potato dextrose agar after 1 week. Limited growth and sporulation was recorded in ammonium sulfate.

The results obtained from the laboratory experiments clearly demonstrated the effects of temperature, pH, and nitrogen sources on the growth and development of *Fusarium* wilt and root rot. These factors must be considered in any management scheme for the disease under natural conditions.

Key words: Fusarium wilt, root rot, arden pea, temperature, pH, nitrogen, management

83. MANAGEMENT STRATEGIES FOR COTTON FLOWER WEEVIL, *Amorphaidea lata* MOTSCHULSKY

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Flower weevil, *Amorphaidea lata* Motsch., is one of the major insect pests of cotton that provides difficulty among pest managers. Its behavioral characteristics render it unmanageable. The adult feeds and lays its eggs mainly on newly opened flowers. This causes a premature shedding of the young boll. It then spends all its larval stages inside until pupation. If left unchecked, cotton farmers experience yield loss of about 28-30 percent.

The management strategies for flower weevil, *Amorphaidea lata* Motsch., is an orchestration of the different researches done for a decade at the Cotton Development Administration Research and Operation Centers. The components of the management strategies are as follows: (1) dusting flower with ash supplemented by collection and burning of shed young bolls, (2) early planting during the months of August and September, (3) wider row spacing of planting, (4) dense planting, i.e., 100,000 plants/ha, (5) close season planting, (6) planting of trap crop like okra, (7) irrigation management at critical stages of the pests, (8) releases of *Euborellia annulata*, and (9) use of granular systemic insecticides.

Various combinations of these components applied at the right time reduced flower weevil population and subsequently change resulting in increased farmers' yield with income with the added benefits of reducing pollution to the environment.

Key words: *Amorphaidea lata*, young bolls, dense planting, close season, trap crops, *Euborellia annulata*, cotton

84. RICE HULL BURNING: A FARMER'S TECHNOLOGY FOR MANAGEMENT OF RICE ROOT-KNOT NEMATODE IN A RICE-ONION CROPPING SYSTEM

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Rice hull burning (RHB) is a traditional cultural practice of many onion growers in San Jose City, Nueva Ecija, Philippines mainly for weed control and yield increase. The resulting carbonized rice hull is incorporated into the soil during land preparation before transplanting of onion. Studies to evaluate the effect of RHB on rice root-knot nematode, *Meloidogyne graminicola*, and onion yield were conducted in a farmer's field naturally infested by the pathogen. Fifteen cm-thick rice hull was sufficient to reduce the nematode population in the soil. The effect of heat from burning rice hull on the nematodes reached up to 30 cm deep. The effect of deep plowing on nematode population was insignificant in comparison with standard plowing. Increasing thickness of RHB gave a significant contribution to increase of onion yield and production of bulbs of export-quality. Plots that received 30 cm-thick rice hull gave 27% more large bulbs than 15 cm-thick hull and 40% more than no RHB. Thirty 3 cm-thick rice hull gave a yield advantage of 31% over no RHB while 15 cm-thick hull gave a yield advantage of 11%.

Key words: root-knot, *Meloidogyne graminicola*, rice hull burning, management, rice-onion system

85. ARTHROPOD PESTS OF BAMBOOS: TAXONOMY, BIOLOGY, NATURAL ENEMIES, AND HOST PLANT RESISTANCE

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Sixty (60) species bamboo arthropod pests (57 insects, 3 mites) were collected from several species of bamboos in nurseries, and natural stands around the

Philippines. These were identified to genus and/or species levels. These include the following: Bamboo node mealybug-*Antonina* sp. aff. *thaiensis* Takahashi; Bamboo shoot mealybug-*Palmicallor* sp. aff. *Bambusum* Tang; Bamboo leaf mealybug *Paracoccus interceptus* Lit; Bamboo culm mealybug - *Chaetococcus bambusae* (Maskell); Bamboo planthopper - *Purohita* sp.; Bamboo green mites - *Schizotetranychus* sp.; Bamboo culm borer - *Chlorophorus* sp. and Bamboo leaf folder - *Pyrausta* sp. A rodent (*Rattus tanezumi*) and one snail were also observed but not collected. New species, whose description are being prepared include: *Neoclavicoccus* sp. nov. (Pseudococcidae), from Makiling and Palawan, *Bambusaspis* sp. nov. (Asterolecaniidae) from Palawan, *Coccus* sp. nov. (Coccidae) from Palawan and Subic Bay Forest Reserve, *Greenaspis* sp. nov. (Diaspididae) from Mount Makiling, *Odonaspis* sp. nov. (Diaspididae) from Davao and *Kuwanaspis* sp. nov. (Diaspididae) from Laguna.

New records include: *Calitoris cahira* (Moore) and *Thosea* sp., as well as *Doleschallia bisaltide philippinensis*, *Melicodes tenebrosa tenebrosa*, *Protaetia* sp., *Melamitis leda* new host records.

The list of bamboo pests from Gabriel's latest compendium was further updated by incorporating new findings in this project and results from further review of literature. The revised checklist records 110 species, which is 93% higher than Gabriel's 57. However, the inclusion of *Planococcus lilacinus* (Cockerell) in Gabriel's checklist is here regarded as doubtful and most probably constitutes a misidentification. It has never been recovered from any of the bamboo mealybugs collected from all over the country. The species being referred to is most probably *Paracoccus interceptus* Lit.

Key words: bamboo pests, bamboo insects, arthropods

86. CRITICAL PEST LEVEL FOR BOLLWORM, *Helicoverpa armigera* (HUBN.)

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Cotton bollworm is one of the major pests of cotton and different control methods are used to control this pest. The use of insecticides is the most effective strategy but it should only be implemented when necessary, that is when the pest has reached the damaging level. In cotton, the critical pest level (CPL) is used as basis on whether to spray or not. Farmers claim that some of the recommended insecticides against bollworm are no longer effective. It is suspected that one of the possible reasons is the CPL currently used as basis in spraying. Hence, this

study was conducted to determine the CPL for bollworm at vegetative, squaring, flowering, and bolling stages of cotton. It was done at the Research and Development Center, CODA, Batac, Ilocos Norte.

The cage technique was used in the experiment. Nylon mesh cloth cages were used to confine 20 hills of cotton with two plants per hill. Determination of the CPL was done at vegetative (22-42 DAP), squaring (43-63 DAP), flowering (64-84 DAP), and bolling (85-105 DAP) stages. Varying number of larvae were released inside each cage. These were: (a) 0; (b) 1; (c) 2; (d) 3; and (e) 4. Shed squares, flowers and young bolls were collected and classified according to cause of shedding. The number of shed structures due to bollworm damage was recorded. Yield loss due to bollworm was computed based on the shed structures. Cost of control and yield loss were used as basis in determining the CPL for bollworm at different growth stages.

An increase in bollworm density meant an increase in the number of shed reproductive structures, resulting in corresponding decrease in the number of harvested bolls and consequently seedcotton yield. At vegetative (22-42 DAP) and squaring (43-63 DAP) stages, results showed that there is need to spray when the CPL of two bollworms/20 plants was reached. However, at flowering (64-84 DAP) and bolling (85-105 DAP) stages, spraying should be done at the CPL of three and two bollworms/20 plants, respectively.

Results imply that proper timing and judicious use of insecticides are important. It should only be done when the need arises or when the CPL is reached to avoid unnecessary expense.

Key words: critical pest level, bollworm, growth stage, yield loss, control cost

87. INSECTICIDE AND FUNGICIDE EFFECTS OF BETEL, *Piper betle* L. VOLATILE OIL ON SELECTED COTTON PESTS

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The variety and versatility in the biological activity of indigenous plants in the Philippines warrant investigation as potential sources of pesticide materials. With an abundant source and ease in cultivation, these materials can possibly be tapped as substitutes or complements to chemical pesticides, the latter being a necessary evil in agricultural production. A common ingredient in the chewing of tobacco by old people the betel, *Piper betle*, is one such plant worthy of investigation.

The volatile oil from betel, *Piper betle* L. leaves obtained by steam distillation, was yellowish brown with a strong peculiar aromatic odor. Through liquid-solid chromatography and successive elution with organic solvents, namely petroleum ether, and dichloromethane and methanol, the oil yielded three fractions.

Bioassay on selected cotton pests showed that the volatile oil and fractions isolated either by petroleum ether or dichloromethane effectively controlled sucking pests of cotton, notably, *Aphis gossypii* and *Amrasca biguttula*, and acted as ovicide against *Helicoverpa armigera* and *Pectinophora gossypiella*. The oil, however, stimulated *H. armigera* adults to deposit eggs and its larvae to feed on treated substrate, but deterred *P. gossypiella* and *Amarphoidea lata*. Interestingly, its fractions inhibited egg deposition of *H. armigera*, *P. gossypiella*, and *A. lata*, indicating a marked selectivity as well as synergism in insecticide action.

Similar trend in effectiveness a fungicide was observed for the oil and its two fractions against damping-off organisms, *Sclerotium rolfsi*, *Fusarium oxysporum*, and *Rhizoctonium solani*, in that order.

Using GC-EL-MS, the fractions had six and three major components obtained by petroleum ether and dichloromethane, respectively. The components were generally terpenes and sesquiterpenes, notably allylphenol, caryophyllene, eugenol, betelphenol, cineol, cadinene, and menthone.

The pesticide action of *P. betle* volatile oil indicates potential as control agent for cotton pests, although, field evaluation is still necessary, in addition to determining its effect on the natural enemies. Also, with the oil being equally or more effective than its fractions, its prospect for formulation and application in agriculture seems viable; however, with its mammalian toxicity to be established yet.

Key words: insecticide, fungicide, *Piper betle*, volatile oil, *Aphis gossypii*, *Amrasca biguttula*, *Helicoverpa armigera*, *Pectinophora gossypiella*, damping-off organisms

88. *Argemone mexicana* L. (PAPAVERACEAE) PRICKLY POPPY: A NEW NON-QUARANTINE PEST RECORD IN THE PHILIPPINES

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Argemone mexicana L., commonly known as prickly poppy, is an exotic weed that was recently detected in onion growing areas of Bongabon, Nueva

Ecija. Aside from competing with soil nutrients and sunlight with onions, the prickly poppy is also hazardous to farmer due to spines that can prick the legs and arms. This weed is not present during the rainy season; it emerges after rice harvest. Thus it can be surmised that it is dormant during the wet season. In fact this weed is common in drylands.

The description of this exotic weed and its field density are discussed.

Key words: onion, *Argemone mexicana* prickly poppy, Bongabon, drylands, exotic weed, Nueva Ecija

89. DAMAGE ASSESSMENT OF LEPIDOPTEROUS PESTS OF ONION IN NUEVA ECIIJA

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The lepidopterous pests, of onion which are leaf chewers were studied during the 1999 growing season. The pest group in Nueva Ecija includes cutworms, earworms, semi-loopers, and a new lepidopterous pest of onion. The cutworms are the dominant species.

Approximately 20% of red pinoy yield is reduced due to the damage caused by these lepidopterous pests. The sampling protocols and details of the result are discussed.

Key words: lepidopterous pests, cutworms, damage assessment, onion, red pinoy, leaf chewers

90. INSECT PESTS AND NATURAL ENEMIES FROM STORED PRODUCTS IN JAPAN

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Severe infestation by insect pests on stored products shortens shelf life and leads to product deterioration. It is important therefore to identify these insect pests as well as their natural enemies as a step toward the development of control measures against them. Stored product samples such as cereal grains and animal feeds were collected from 15 sites in Kumamoto, Ibaraki, Okinawa, and Hokkaido prefectures in Japan. After isolating the insect pests, predators, and parasites from the samples, their identity was determined based on morphological characteristics. A total of 14 species of insect pests were identified from the samples and majority of them were beetles. *Tribolium castaneum* (red flour beetle) and *Stegobium paniceum* (drugstore beetle) were the most frequently occurring species in the samples. Among the various sources of the samples, Kumamoto Kikuchi Shokuryo and Kashima Forage Company had the most number of insect pest species. The predators found in the samples were *Gnathoncus nannetensis*, *Ar:isolabis maritime*, *Alleocranum biannulipes*, *Carcinops pumila*, pseudoscorpion, spider, beetle, and an unknown, while the parasites included two unknown species and *Venturia canescens*.

Key words: stored products, insect pests, predators, parasites, natural enemies, *Tribolium castaneum*, *Stegobium paniceum*, post-harvest, cereal grains, animal feeds

**91. A SURVEY OF BUTTERFLIES AND SKIPPERS
(LEPIDOPTERA: RHOPALOCERA) FROM
MOUNT BANAHAO DE LUCBAN,
QUEZON PROVINCE, PHILIPPINES**

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A survey of the Rhopalocera of Mount Banahao de Lucban was conducted from July 1997 to December 1999. Four main sampling and observation sites were designated, namely, the Samil River area (700-100 m asl), Manglit-Palola (750-900 m asl), Barod-Palola (700-1100 m asl), and Balicatan (900-1200 m asl). A total of 76 species were identified. These are distributed in nine families namely: Hesperidae, Papilionidae, Satyridae, Amathusiidae, Nymphalidae, Danaidae, Peiridae and Lycaenidae. The best represented family is Pieridae with 14 species. *Troides rhadamantus* Lucas which is included in Appendix II of CITES list can still be found in the mountain particularly around the Samil area, but was sighted only twice throughout the entire study period.

Key words: butterflies, Rhopalocera, *Troides rhadamantus*, Mount Banahao de Lucban

**92. GROWTH AND DEVELOPMENT OF RICE STEMBORER
IN AN ARTIFICIAL DIET**

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In searching for an alternative host for the yellow stemborer eggs for parasitoid studies, a corn borer diet was tested for growth and development of rice stemborer for pupa production and found suitable for rearing. Neonates of stripped stemborer, *Chilo suppressalis* (Walker) were infested on the diet. Parameters such as larval/pupal weights, larval/pupal periods, adult survival, and fecundity were observed. Forty eight percent of the larvae survived, grew, and developed into adults on the corn borer diet. Development period ranged from 34 to 57 days with a mean of 44.34 ± 7.52 days. Mean larval period was 37.41 ± 7.05 days, mean pupal period 7.05 ± 1.54 days, and mean development period peaked at 36 to 45

days. Each female was able to lay an average of three eggmasses with a mean egg count of 92.21 ± 55.17 eggs.

Key words: stemborer, *Chilo suppressalis*, larvae, corn diet, eggmasses

93. BUTTERFLY MANURE: A NOVEL SOURCE OF BIO-ORGANIC FERTILIZER

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Butterfly manure from the larvae of *Danaus chrysippus* L., plain tiger was collected from the Tarlac butterfly breeding site. The main host plant where these larvae feed is *Colotropsis gigantea*. The larvae manure was air dried and analyzed at the Analytical Services and Soil/Plant Test Kit Project of the Department of Soil Science, U.P. Los Baños.

The nitrogen (N) content of butterfly manure at 1.19% approximates that of carabao (1.09%–1.22% a.i.). Its potassium content of 2.14% is higher than that of cattle and almost equal to poultry. The other chemical components of butterfly manure are discussed and compared with other sources of bio-organic fertilizers.

Key words: *Danaus chrysippus*, butterfly manure, bio-organic fertilizer, larvae, poultry, cattle, plain tiger, nitrogen, potassium

94. GRAFTED TOMATO FOR OFF-SEASON PRODUCTION

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An experiment was conducted to determine the efficacy of grafting Apollo and FM-TT-22 tomato plants onto eggplant rootstock using rain shelter and raised bed condition.

Growing tomatoes in the Philippines is usually done in lowland areas during the dry season. During the rainy season, (hot-wet months) particularly in Nueva Ecija, production of tomatoes is concentrated on very small hilly areas making the price almost unaffordable to consumers. This situation offers very high opportunity to off season production through grafting and planting tomatoes in the lowlands.

Grafting tomatoes onto eggplant rootstock may offer resistance to flooding during the rainy season. Upgrade tomatoes when flooded results in wilting and ultimately death of the plants.

One-month seedlings of Apollo and FM TT-22 tomatoes were grafted to the same age of EG-203 eggplant rootstock. The rootstock and the scion were cut just above the first leaf to form a wedge and inserted into opposite ends of a rubber tube. Grafted seedlings were transferred to a chamber with approximately 80-90% relative humidity for one week and later on brought out from the chamber for hardening prior to transplanting.

Results of the experiment indicated that plant survival significantly increased in Apollo but not for FM TT-22. The percent survival from grafted Apollo was 74 percent higher than the non-grafted ones. In terms of the number of fruits, grafted Apollo had ten percent more fruits than the non-grafted plants.

Grafting likewise influenced significantly the weight of fruits per plant and computed yield per hectare. Grafted Apollo significantly produced higher fruit weight than the non-grafted plants. Furthermore, significantly higher yield was obtained from grafted Apollo plants, with mean yields of 15.48 tons per hectare from the grafted plants and 7.45 tons per hectare from the non-grafted. Grafting increased yield by 107 percent. For FM TT-22, yield for both grafted and non-grafted plants were the same.

Key words: grafted tomato, root stock, scion, rainshelter

95. BAMBOO SHOOTS AS SUBSTITUTE VEGETABLE DURING LA NIÑA

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Twenty-one (21) provinces were visited to survey several species of bamboo in natural stands and bamboo plantations. Bayog (*Dendrocalamus merrillianus*) is the predominant bamboo shoot species in Northern and Central Luzon, mostly

kawawang tinik (*Bambusa blumeana*) and bolo or kawawang usina (*Gigantochloa levis*) in Southern Luzon, Kiling (*Bambusa vulgaris* var. *vulgaris*) is predominant in the Bicol regions, giant bamboo (*Dendrocalamus asper*) in Central Mindanao and laak (*Bambusa philippinensis*) in Southern Mindanao.

The predominant bamboo species in an area is usually the one used as food and all bamboo shoots in the market are sold fresh. Each region has its own manner of processing bamboo shoots and Unique shoot delicacies. The presence or absence of bamboo shoots in an area's market place is a good indicator of the presence or absence of natural or cultivated bamboo strands.

Key words: bamboo shoots, La Niña phenomenon

96. FREEZE-DRYING CHARACTERISTICS OF MANGO (*Mangifera indica* L.) PUREE AT THREE LEVELS OF PRE-FREEZING TEMPERATURES

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The problem that besets the Philippine mango industry is the seasonality in production vis-à-vis unstable market situations characterized by a period of supply glut (February-June) resulting in low prices, followed by a longer period of severe undersupply (July-January) with prices in the market rising to exorbitant levels. Although there are processed mangoes available in the market, development of new products such as freeze-dried mango powder, is still necessary in the food industry. Hence, it is important to establish technical information in the freeze drying of mango puree in order to produce high quality mango powder.

The study was conducted to establish the freezing and freeze-drying characteristics of mango puree at pre-freezing temperatures of -40°C and -65°C (using an ultra-low temperature freezer) and -196°C (direct immersion in liquid nitrogen). Aseptically processed mango puree used in the study was pre-frozen at -40°C, -65°C, and -196°C. These samples were freeze-dried at 2 to 3% moisture content (MC) wet basis at a vacuum pressure of 0.005 to 0.001 torr. Parameters studied included freezing behavior, freezing point, critical zone, rate of heat removal, freeze-drying duration, freeze drying rates, and percent power recovery.

Results of the study showed that removal of sensible heat at -40°C, -65°C, and -196°C from the initial product temperature to below its freezing point required at time duration of 42, 35, and 2.87 minutes, respectively. Meanwhile, the time required to remove the latent heat from the sample product were 125, 9, and 6.58 minutes for the different temperatures applied. Using graphical analysis, the critical zone at -40°C, -65°C, -196°C ranges from -0.05°C to -11.48°C.

The study showed that the freezing point for mango puree was -2.42°C . The heat removal rate below and above freezing at -40°C , -65°C , and -196°C were 60.7, 46.6, and 37.5 kJ/h, respectively. This effect was attributed to an increase in surface area of sublimation caused by cracks that developed during freezing as a consequence of thermal shock. Freeze drying of mango puree at pre-freezing temperatures of -40°C , -65°C which required -196°C did not have any marked effect on powder yield. The technical information obtained in the freeze drying of mango puree added to the body of knowledge in the field of food processing. Such information can be applied in the commercial production of freeze-dried mango powder in a pilot scale to determine its economic viability.

Key words: freeze drying, mango puree, ultra low temperature freezing, liquid nitrogen, aseptic processing, freezing point of mango puree, vacuum pressure, freeze-drying rate, freeze-dried mango powder, percent powder recovery

97. ANALYSES OF THE SHELF LIFE OF COMMERCIAL TEMPURA AND SAUCE IN CEBU CITY

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This is a study on the shelf life of commercial tempura and sauce sold by street vendors in Cebu City based on its physicochemical, microbial, and sensory analyses observed under ambient and refrigerated conditions, packed and unpacked.

The newly processed tempura contained physicochemical parameters of 39.02% moisture; 8% fat; 11.05% protein; 3.31% ash; 38.62% carbohydrates; and water activity level (Aw) of 0.98. The bacterial count had reached 1.34×10^8 cfu/g higher than the standard count of 10^2 cfu/g by the Bureau of Food and Drugs (1990). Mold growth and *Staphylococcus aureus* were detected in the product. Based on sensory assessments, the shelf life of unpacked and packed tempura stored at ambient temperature lasted for only four days while samples stored at refrigerated temperatures were still acceptable after eight days of storage.

Tempura sauce had physicochemical parameters of 84.39% moisture; 0.2% fat; 0.81% protein; 2.6% ash; 12% carbohydrates; and 0.99 water activity (A_w) level. Mold growth was evident in the product and bacterial count had reached to 4.20×10^3 cfu/g. Based on sensory assessments, the tempura sauce stored at ambient condition, unpacked and packed, lasted for only four days while those samples stored at refrigerated conditions were still acceptable by the panelists after eight days of storage.

There was a significant mean difference between the different treatments at a 0.05 level of significance using the analysis of variance (ANOVA) and least significant different Tests.

Key words: shelf life, tempura, tempura sauce, physicochemical analysis, microbial analysis, sensory analysis, refrigerated condition, ambient condition

98. CHANGES IN SOIL PROPERTIES ASSOCIATED WITH APPLICATION AND BURNING OF RICE HULLS IN PERI-URBAN VEGETABLE PRODUCTION AREAS IN CENTRAL LUZON

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Peri-urban vegetable production is characterized as a highly intensive agriculture system. Large cities like Metropolitan Manila depends on such system for a year-round supply vegetables. However, year-round vegetable production is beset with a wide range of constraints such as rains or floods, insects, diseases, weeds, and soil-related problems.

One of the major peri-urban areas in Central Luzon is found in San Leonardo, Nueva Ecija. In this site, the cost of weed control ranked next to the cost of insect control due to the prevalence of various weed species in succession. This condition prompted farmers to resort to the application and burning of rice hulls in their fields. Considering the negative effect of burning on the environment, a research study was conducted to identify the impacts of burning rice hulls both on soil and on pak-choi. Pak-choi, also known as pechay, is one of the most common peri-urban vegetables.

Rice hulls were applied at a thickness of one foot and one-half foot each in 16 one by four meter-plots. Four plots of the same dimensions were not applied with rice hulls for comparison. These thickness were equivalent to a weight of 74 and 147 tons/ha of rice hulls, respectively. The rice hulls were burned until a

carbonized rice hull (CRH) was left. After five days, when the CRH had cooled, it was incorporated into the soil through the use of a hand tractor.

Pak-choi seedlings were transplanted into the 20 plots. Plots without CRH were fertilized with 90-30-30 kg NPK/ha while four plots each from the 74 and 147 t/ha rice hulls, applied plots was fertilized with 90-30-30 kg NPK/ha and 45 - 30-30 kg NPK/ha. The field layout for the experiment was randomized complete block design.

Two consecutive cropping of pak-choi were done. During the second cropping, there was no application of rice hulls to evaluate any residual effect that could be detected in terms of weed control and yield. Soil samples were taken before planting and after harvest of the first and second crops for physical and chemical analysis of soil properties that were affected by CRH incorporation. Marketable yield of pak-choi and weed density was measured during the study.

A marked increase in soil organic, exchangeable potassium, and soil porosity was observed due to rice hull burning. The increase in porosity was attributed to the significant reduction in soil bulk density.

Burning of rice hull exerted a very positive effect on weed control during the first crop ranging from 180 percent in one-half foot thickness of application to 600 percent in one foot thickness of application. In the second crop weed control ranged from 100 to 1000 percent. Furthermore, a reduction in the amount of fertilizer to achieve the same yield was observed due to incorporation of CRH. This is applicable during the first and second cropping.

99. REGIONAL ASSESSMENT AND COLLECTION OF AVAILABLE DYE-YIELDING PLANTS IN THE PHILIPPINES

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A total of 65 dye-yielding plant species in seven geographical regions of the Philippines were identified, collected, and documented. This discussion focuses on the collection, plant parts extracted, methods of dye extraction, dye-yield color, and uses and/or applications based on structured and unstructured interviews among local people.

The information gathered will serve as reference among people engaged in dye extraction and proprietors of small-scale industries, handicrafts, handloom weaving industries, fan and mat weaving, abaca weavers, fashion accessories, and costume jewelry available in the country. The information is suitable for local

radio and TV interviews, and is an interesting subject matter among high school students especially those conducting investigatory work.

Key words: dye-yielding, extractive, natural forest, indigenous, resource survey, assessment.

100. *Limnocharis flava* L. BUCHL., and *Salvinia molesta* MITCHELL: POTENTIAL THREATS TO AQUATIC ECOSYSTEM IN LUZON

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Limnocharis flava L. Buchl., locally known as "sandok-sandokan", belongs to family Butomaceae. Introduced from Tropical America, it was first collected in Java in 1870 and is now a native of Tropical Asia. *Salvinia molesta* Mitchell is a floating aquatic fern of South American origin. It has become a serious pest in parts of Asia, Africa, and Australia. Its presence in Iloilo was earlier reported and has spread to Luzon. Local folks call it "giant Azolla". Both weeds have infested lowlands rice paddies, while the latter has started invading swampy areas, irrigation canals, and waterways in Lucban, Quezon threatening nearby towns.

Limnocharis flava has a long triangular petiole crowding at the base of the stout rootstock. Leaves are large, the blade somewhat rounded with a peltate base. Flowers are in umbels with stout peduncles 3 sepals 3 yellow petals. Fruits are round capsules with 14-34 carpels, maturing in 21-24 days after flower opening. Each carpel has 22-123 small, brown to black seeds.

Salvinia molesta as fragile horizontal floating stems with difficult-to-wet hairy leaves. It produces numerous sporocarps, which contain the megasporangia and microsporangia. Growth is rapid and a means of dispersal is through fragmentation of the stem occurring rather easily.

The capacity of *Limnocharis* to produce tremendous numbers of small seeds and to reproduce vegetatively through offshoot production at the tip of the flower stalk make this plant potentially damaging.

Key words: *Limnocharis*, *Salvinia*, *Salvinia molesta*, *Limnocharis flava*

101. MANGROVE COMMUNITY STRUCTURE IN CARMEN, CEBU

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Coastal ecosystems are particularly important to the people as they provide both subsistence and cash-crop fisheries and other benefits, such as wood from mangrove forests. These ecosystems also contain a high biodiversity of animals and plants which can be a source of novel biochemical products and form a basis for valuable eco-tourism industry activities and discovery of new biochemical products. In Carmen, Cebu, mangrove communities are being utilized by the local coastal community into aquaculture farms or reclaimed for human settlements. Like any other coastal areas in the Philippines, these resources are being exploited without logical understanding of their ecology. This may eventually lead to depletion. Thus, the need for the residents of the community who are direct users of the resources to understand and rationalize their use. One objective of the CSCST-CFT, Carmen, Cebu Campus program is to assess the remaining valuable marine resources by conducting a study on the community structure and distribution of mangroves of the coastal areas of Carmen, Cebu. The result of the study will provide the community baseline information about the status of mangrove resources and guide them on how to utilize and maintain the remaining resources wisely.

Carmen, Cebu is located 41 km northeast of Cebu City. Five stations were established for the study namely: Dawis, Luyang, Puente, Cogon, and Poblacion. The mangrove vegetation structures of the five stations were analyzed from June to September 1999 by taking the following measurements: number of individuals per unit area, basal areas, and tree height. The number of individuals per species were counted from a 10 x 10 sq meter-area; 20 to 50% of the trees of each species were measured for their circumference above the primary root using a calibrated tape. Tree height which is the vertical distance between the ground up to the tip of the crown was measured using a calibrated pole. Field testing and identification of other mangrove species outside of the quadrant were also done.

After four months of sampling, the most common mangrove species like *Sonnerati alba* (pagatpat), *Rhizophora stylosu* (bakawan-bankau), and *Avicennia marina* (bungalon) were found in the five stations of Carmen, Cebu. However, the diversity of mangrove species like *Sonneratia alba*, *S. caseolaris*, *Avicennia alba*, *A. lanata*, *marina*, *A. officianalis*, *Rhizophora stylosa*, and *Ceriops tagal* were found mostly in secondary growth in Luyang, Carmen, Cebu. The numerical dominance of *Rhizophora* species is due to its success in colonizing new areas.

One of the reasons for this is attributed to the reproduction strategy of *Rhizophora* species. *Rhizophora* is oviparous, meaning, the propagules germinate and mature in the mother plant before they are released and dispersed. That is one of the reasons why *Rhizophora* species are widely used for mangrove reforestation. Propagules, when laid down in the mud, grow and take root quickly.

**102. THE INFLUENCE OF BODY WEIGHT AND DIET ON
THE AMMONIA EXCRETION OF THE AFRICAN CATFISH,
*Clarias gariepinus***

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Three size groups of African catfish, *Clarias gariepinus*, with mean weights were 124.5 g (adult size group), 5.4 g (juvenile size group), and 1.1 g (fingerling size) were given two types of diets (trash fish and floating pellets) to determine the influence of body weight and diet on ammonia excretion. Highest weight-specific excretion rate ($5.23 \text{ mg NH}_3\text{-N kg}^{-1} \text{ h}^{-1}$) was obtained from juveniles and fingerlings fed with trash fish and lowest ($0.24 \text{ mg NH}_3\text{-N kg}^{-1} \text{ h}^{-1}$) among adults fed with commercial pellets. Regardless of diet given, post-prandial excretion rates at 28°C were generally higher among fingerlings and lowest in adults. Within same size groups, excretion rate was 50-84% higher among test animals fed with trash fish. Significant differences in ammonia excretion in relation to diet stresses the dissimilarities of the protein and amino acid requirements of the test group. The hourly ammonia excretion rate obtained in this study can be used to determine the build-up of ammonia in ponds which could be exported to rivers and inland waters.

Key words: body weight, diet, ammonia excretion, *Clarias gariepinus*, trash fish, commercial pellets, weight specific excretion rates, pollution, water quality, post-prandial excretion rates

103. SEED QUALITY-RESPONSE TO FUNGICIDE TREATMENT OF A LINE AND F_1 HYBRID SEEDS

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IR 58025 A (A line) and F_1 hybrid seeds harvested during the wet season 1999 at the PhilRice-Central Experiment Station were dipped in various concentrations of benomyl to control fungal growth during a germination test. Hybrid seeds were germinated in petri dishes lined with moistened filter paper then stored in a germination room with Temperature range of 28-32°C.

Washing and ten-minute dipping in 5 and 3% benomyl solution resulted in high germination percentage in A line and PSB Rc 27H (F_1) mestizo hybrid, respectively. Germination of hybrid seeds at these fungicide concentrations was characterized by minimal fungal infection.

Fungal growth in A line and PSB Rc 72H (F_1) hybrid seeds washed with tap water was not controlled. A line seeds washed then dipped into 3% benomyl solution exhibited minimal occurrence of fungal growth. Prolonged dipping seeds for 10 minutes in 3% solution significantly controlled fungal growth. On the other hand, five-minute dipping in 3% solution proved very effective in controlling fungal growth in PSB RC 72H (F_1) seed.

Key words: hybrid, mestizo, A line, benomyl, germination, fungal growth, concentration, IR 58025A, PSB Rc 72H, F_1

104. PEDOLOGICAL CHARACTERIZATION AND AGRONOMIC POTENTIALS OF SOILS ASSOCIATED WITH KENNON LIMESTONE

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The importance of understanding the characteristics of soils associated with limestone lies in their extensiveness. Extensive research has not been conducted

on soil-landscape relationships involving limestone in Benguet and in other parts of the country. Thus a study of Kennon limestone is necessary to set the limits of its properties in relation to soil formation and possibly to serve as a tool in predicting properties of soils associated with other limestone formations that exhibit the same characteristics. Several reconnaissance trips were taken across areas where Kennon limestone formation was mapped. After the survey, sites for detailed soil description and sampling were selected. Thus, four landsurface units (interfluvial, fall face, transportationalslope and colluvial footslope) were described and sampled in La Trinidad, Benguet and Green Valley, Baguio City. At each land surface unit, a pit of approximately 1 square meter was excavated up to more or less 2 meters depth for description and sampling. Soil samples taken were prepared for laboratory analysis.

The morphological, physical, chemical and mineralogical properties of soils in four land surface units along the Kennon limestone formation indicated strong correspondence between landscape positions and soil properties. The land surface units studied in La Trinidad, Benguet are the interfluvial, fall face, transportationalslope and colluvial footslope. The same land surface units were replicated in Mt. Sto. Tomas, Green Valley, Baguio City. The soils were classified using the framework of Soil Taxonomy.

The stable nature of the interfluvial in both locations allowed the formations of deep and well-developed profiles. Classified as Typic Haplohumults, the soils show distinct development of umbric epipedons and thick argillic horizons. The soils formed in the fall face of La Trinidad and Green Valley have two different genetic pathways. In La Trinidad, the soil shows distinct umbric epipedon with cambic subsurface diagnostic horizons; hence it is classified as Typic Dystrypepts. In Green Valley the presence of limestone outcrops, which somehow stabilizes the landscape unit, favors the development of mollic epipedon and argillic subsurface layer. The soils are therefore grouped as Typic Argiudolls.

The soil properties of the transportationalslope in La Trinidad are almost identical with those of Green Valley. In both sites, the soil profiles are deep and well developed. Both soils, identified as Typic Hapludalfs, have umbric epipedons and subsurface argillic horizons.

The soil in the colluvial footslope of La Trinidad is more developed than the soil in Green Valley. The soil in the former has a deep and well-developed profile. The surface soil is mollic epipedon and the subsurface horizon is argillic. The soils is Typic Argiudolls. The soil in the latter, on the other hand, has ochric epipedon and cambic horizon in the surface and subsurface layers, respectively. Thus, the soil is classified as Fluventic Dystrypepts.

All soils observed in the different land surface units of La Trinidad and Green Valley, except those that are located in the fall face, are suitable for agricultural uses. The utilization of soils in the fall face is constrained by its steep location. Thus, the soils can be best utilized for forest trees and or fruit-bearing trees.

Key words: Kennon limestone, landsurface units, diagnostic horizons, epipedon, soil classification, soil taxonomy, agro-technology transfer, Haplohumults, Dystropepts, Argiudolls

105. GENETIC DIVERSITY ANALYSIS OF PHILIPPINE MAIZE INBRED LINES USING MICROSATELLITE MARKERS

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The success of a maize hybrid breeding program relies on the systematic evaluation and selection of inbred lines as parents based on their heterotic patterns. Heterotic patterns (HP) can be established using diallele analysis but the process is tedious, time-consuming, and costly, particularly if many lines or populations are involved. Fingerprinting and diversity analysis of inbred lines using molecular markers could reduce the number of lines required for testing and time needed to establish heterotic patterns. Inbred lines developed at IPB-CA-UPLB were analyzed using SSR or microsatellite markers. Sixty maize SSR primers were used to analyze the diversity of the 33 yellow and 47 white inbred lines. NTSYS analysis based on Nei's dissimilarity coefficient revealed clustering of very closely related inbred lines. The results of the study could provide maize breeders relevant information as a guide in selecting potential inbred lines as parents in a hybrid-breeding program.

Key words: maize, inbred line, hybrid, SSR, microsatellite, fingerprinting, heterotic

106. MINERAL CONCENTRATION IN THE BLOOD OF GRAZING GOATS AND SOME FORAGES IN HALAR-LADEN AREAS OF TARLAC, CENTRAL LUZON

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In 1998, about 5% of the country's goat population was raised in Tarlac, one of the provinces hard hit by the eruption of Mt. Pinatubo in 1991. Goats subsist mainly on forage species that grow predominantly or sporadically in lahar-laden areas characterized as sandy, dry, acidic with sulfur content 10 times more than the normal organic or mineral soils, and infertile because of organic carbon and nitrogen. In the absence of concentrate feeding, there is a high possibility that the mineral levels, particularly copper, selenium, and zinc, of the animals are below critical levels, as a result of insufficient minerals from the feedstuffs.

The study was conducted to determine the mineral status of 60 native goats and eight forage species, namely: *Cynodon plectostachyus*, *Pennisetum purpureum*, *Eleusine indica*, *Cynodon dactylon*, *Calopogonium muconoides*, *Centrosema pubescens*, *Leucaena leucocephala*, and *Mimosa pudica* in lahar-affected areas of Concepcion, Tarlac. Forage and blood samples were collected six times from December 1996 to September 1997, and analyzed for calcium, phosphorus, magnesium, sulfur, copper, iron, molybdenum, and zinc using an inductively coupled plasma emission spectrometer, and selenium using fluorometric detection of the 2,3-diaminonaphthalene.

Forage calcium and sulfur were non-limiting. Most forage species had low phosphorous, copper, and selenium, while some species had magnesium and zinc levels lower than the critical limit because of low mineral content and high percolation rate of lahar deposits. Iron and molybdenum were in excess.

The effect of the seasonal variation as well as the direct effect of the feedstuffs from the lahar-laden areas on the mineral status of the grazing goats was observed. More than 20% of the animals had low levels of calcium, copper, zinc, and selenium especially during the dry season possibly due to insufficient amount of these elements and excessive molybdenum and iron in most forages. While the percentage of the animals with low levels of Cu, Zn, and Se decreased during wet season, the percentage of the animals with low levels of Ca increased. The better growth of forage and higher concentration of Cu, Zn, and Se during the wet season could have contributed to this effect. Conversely, calcium in forage was

high, but 47% of the animals had low plasma calcium concentration during the wet season possibly due to low availability of Ca in the forage.

Although no clinical signs of mineral deficiencies were observed, supplemental feeding would be important since the condition of the pasture in lahar-laden areas was not expected to improve in the next years. Intensified use of *L. leucocephala* with better mineral profile would be ideal in order to improve the mineral status of the grazing goats.

Key words: lahar-laden, minerals, goats, forage

107. SELENIUM SUPPLEMENTATION IN GRAZING GOATS: EFFECTS ON BLOOD AND MILK SELENIUM AND GROWTH PERFORMANCE OF KIDS BORN TO DOES RECEIVING SELENIUM-SOLUBLE GLASS-BOLUS

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Philippine goats are traditionally raised under backyard level with negligible concentrate supplementation and feeding is primarily based on available fibrous crop residues or vegetation available in communal pastures. The mineral content of feed resources is generally low; thus, a high possibility that grazing animals suffer from known mineral deficiencies particularly in selenium.

The effectiveness of soluble glass boluses (SGB) to increase Se level has been known in sheep, but only limited literature is available on goats.

This study was conducted to determine the effects of SGB containing selenium on blood and milk Se levels, and growth performance of kids born to does receiving intraruminal SGB. A total of 50 grazing upgraded Philippine goats with mean body weight of 20 kg were evenly divided into two treatment groups; without SGB (Control) and with SGB (Treated). The animal grazed in a Se-deficient pasture in the experimental farm of the Central Luzon State University, without any concentrate supplementation, during the 12-month study. Two boluses in six months intervals were administered. Samples of goats' and kids' blood were collected monthly and analyzed for Se content using fluorometric detection of the 2-3 diamionaphthalene following the procedure of Watkinson.

After three months of SGB administration, the treated group had higher blood and milk Se contents than the control. Blood Se levels among kids born to SGB-treated does reflected maternal treatments, i.e., a two-fold increase in Se level over the untreated group. Significant correlation was noted between blood and milk Se levels in does, and blood Se in kids. No positive response was observed in the birth weight and growth performance of kids suckling milk with relatively higher Se concentration. Results suggest that although SGB administration significantly increased blood as well as milk Se levels of does grazing in Se-deficient pastures, this did not improve birth weight and growth performance of their offspring.

Key words: selenium, Philippines, goats, SGB, blood, milk, growth performance

HEALTH SCIENCES

108. CYCLOSPORIN ANALYSIS IN BLOOD BY AUTOMATED RESERVED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD COUPLED WITH SOLID PHASE EXTRACTION AND SPEED VACUUM EVAPORATION

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Cyclosporin A (CyA) is a cyclic undecapeptide drug used in combating tissue rejection after organ transplant. High cyclosporin doses may lead to nephrotoxicity while a dose below the therapeutic level increases the probability of transplant rejection. A rapid HPLC analysis was developed for the estimation of cyclosporin in blood using a PC 1000 software and autosampler for routine analysis. The mobile phase consisted of acetonitrile: methanol: water (50:30:20) while the analytical column was a C₁₈ column maintained at 75°C with UV detection set at 214 nm. Whole blood samples, spiked with the internal standard cyclosporin D (CyD), was added with protein precipitating agent, centrifuged, and applied to a disposable solid phase C₁₈ column to rapidly extract the CyA and CyD. The extracting solvent was removed by using a speed vacuum apparatus. Average retention times were 8.1 min for CyA and 10.0 min for CyD. Linear calibration curves were obtained from 0-500 mg/mL with average correlation coefficient of 0.995. Calibration standards with increasing concentrations of CyA and fixed concentration of CyD were spiked in blood from healthy volunteers and subjected to the same preparation as CyA-containing blood samples. CyA concentrations in blood samples were determined using internal standard addition method (by area ratio of CyA to CyD) and the obtained calibration curve. This analytical technique is useful in monitoring cyclosporin level in transplant patients.

Key words: cyclosporin, high performance liquid chromatography, reversed-phased, solid phase extraction, speed vacuum, immunosuppressive drug, whole blood, chromatography, internal standard addition method

109. GROWTH PATTERNS AND INFECTIVITY OF A DENGUE-2 VIRUS STRAIN PROPAGATED IN THE HUMAN MYELOMONOCYTIC CELL LINE K562

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Dengue virus is the causative agent of the disease dengue, which is manifested in different degrees of severity. There are 4 serotypes of the virus namely, Dengue 1, 2, 3 and 4. The genomic nucleotide sequences of representative strains of all 4 serotypes have been determined. Recently, Mangada and Igarashi (1998) reported the sequencing of the entire genome of three Dengue 2 virus strains from Thailand. These are ThNH-pll/93, ThNH-28/93 and ThNH-7/9, which were isolated from Thai patients exhibiting dengue fever, dengue haemorrhagic fever, and dengue shock syndrome, respectively. Differences in the secondary structure in the 3 non-coding region, as well as significant amino acid replacements, which could potentially alter the nature of the viral proteins, have been noted. In the present study, the growth patterns and infectivities of these three virus strains were compared. The viruses were initially propagated in the mosquito cell line, C6/36 *Aedes albopictus*, maintained in Eagle's minimal essential medium containing 2% fetal calf serum (FCS) and incubated at 28°C. After 1 week, the infected culture fluids (ICF) were collected and pre-incubated with or without enhancing antibodies. These 2 types of ICFs were then inoculated at the same multiplicity of infection into K562 human myelomonocytic cells. After two hours of viral adsorption, cells were cultured in 24-well plates at a concentration of 2×10^5 cells/ml per well in 2% FCS-supplemented RPMI at 37°C in a CO₂ incubator. Cells were harvested everyday for 7 days. Virus growth was quantified by focus formation unit assay in BHK. Percent of infected K562 cells was detected through immunofluorescence test and correlated with severity of disease.

Key words: dengue virus, dengue fever, haemorrhagic fever, dengue shock syndrome . *Aedes albopictus*, enhancing antibodies, myelomonocytic cell, K562 cells, immunofluorescence assay test, dengue-2 viruses

110. THE ANALGESIC ACTIVITY OF THE ALKALOIDS OF (*Ipomea Muricata*) Jacq. FAM. CONVOLVACEAE: A CORRELATION OF IN VIVO AND IN VITRO STUDIES

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The analgesic activity of the indolizidine alkaloids from the seeds of *Ipomea muricata*, namely ipomine, ipalbidine, ipalbine, and ipalbinium were studied on mice using the hot-plate method. At a dose of 1 mg/kg BW, all alkaloids except ipalbine, elicited higher threshold to pain in mice than the 5 mg/kg-BW dose of indomethacin. The dose-activity relationship indicate that for all the alkaloids, 5 mg/kg/BW was observed as the dose that produced a time-course curve ideal of all analgesic agents. Based on the degree and nature of analgesic action, the alkaloids are arranged as follows: ipalbidine > ipalbinium > ipomine > ipalbine.

The alkaloids were assayed for their ability to inhibit prostaglandin synthesis in isolated rat leukocytes. Only ipalbidine showed significance inhibition of prostaglandin release at a concentration of 100µM. The results implicate the phenylindolizidine ring for the observed analgesic activity *in vivo*.

Key words: *Ipomea muricata*, alkaloids, analgesic, ipomine, ipalbidine, ipalbinium, ipalbine

111. CERVICAL ADENOCARCINOMA IN FILIPINOS ARSENIA A. CASAUAY, EDNA A. AMPARADO, SONIA D. JACINTO, ANNABELLE A. HERRERA, and RYAN C. FONTANILLA *Institute of Biology, College of Science University of the Philippines Diliman, 1101 Quezon City*

Paraffin blocks of cervical adenocarcinoma tissues taken from 23 patients were processed by light microscopy and morphometry. Three different grades of cervical adenocarcinoma, namely well-glandular lining, cell and nuclear pleomorphism, stromal invasions, and lymphatic involvement were observed. Mor-

phometry of transformed cells revealed that small cells predominate in the poorly differentiated stage. No notable differences in nuclear sizes were observed among the three histological anaplasia, that is, the nuclear-cytoplasmic ratio increases as the cell becomes more anaplastic.

Key words: cervix, adenocarcinoma, histology, morphometry

112. CYTOGENETIC ABNORMALITIES IN FILIPINO COLON AND COLORECTAL CARCINOMA PATIENTS

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Cancer of the colon and rectum is a common and often fatal disease. It is one of the three leading causes of cancer mortality worldwide. There is a dearth of cytogenetic data on solid tumors such as colon and rectal cancer, primarily because of the inherent technical problems associated with these studies. Identification of tumor-specific chromosomal abnormalities, important determining clinical remission or relapse, was conducted. Tumor tissues, all described as adenocarcinomas by histopathological examination, were surgically removed from one colorectal and 8 colon patients (7 males and 2 females). Primary cultures were prepared and chromosomes were stained using the Trypsin G-banding method. Structural aberrations included 3p and 5p deletions. Numerical aberrations such as hypodiploids, polyploids, absence of the Y chromosome, and presence of marker chromosomes were also observed. Cytogenetic findings were correlated with histopathological and flow cytometry data as well as cancer stage using the TNM and Duke's systems. Genetic abnormalities confirmed by fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH).

Key words: adenocarcinoma, cancer stage, colon cancer, colorectal cancer, cytogenetics, flow cytometry, histopathology, hypodiploid, malignancy, polyploid, trypsin G-banding

113. THE INDOLE ALKALOIDS FROM THE LEAVES OF *Alstonia scholaris* (L.) Don (APOCYNACEAE) – COMPARATIVE ANTIMYCOBACTERIAL ACTIVITY AND ANTICARCINOGENICITY AGAINST HUMAN ORAL EPIDERMAL CARCINOMA CELL-LINES

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The indole alkaloids comprise the second largest single group of plant bases reputed for their pharmacological and therapeutic properties. In this study, the indole alkaloids from the air-dried leaves of *Alstonia scholaris* (L.) Brown and *Catharanthus roseus* (L.) G. Don (both from family Apocynaceae), were studied for their potential antitubercular activity and comparative anticarcinogenicity against human-oral epidermal carcinoma cell lines (KB cells). Radiorespirometric assay of the crude alcohol leaf extracts and the crude alkaloids revealed a pronounced inhibition of *Mycobacterium tuberculosis* H37Rv and *Mycobacterium avium*. However, only the *A. scholaris* crude alcohol extract and the crude alkaloids were active against both mycobacterial species. Sulforhodamine-B colorimetric assay of the crude *C. roseus* alcohol extracts and alkaloids showed broad spectrum cytotoxicity against the KB cancer cells at LC₅₀ (0.3 and 0.2 mcg/mL). Crude *A. scholaris* alcohol extracts exhibited a very slight cytotoxicity (18.8 mcg/mL) whereas its crude alkaloids did not demonstrate a significant cytotoxicity at LC₅₀ (>20).

Vacuum liquid chromatography of the crude *A. scholaris* and *C. roseus* alkaloid fractions resulted in several alkaloid-positive fractions. Radiorespirometric assay of the alkaloid fractions (first two for *A. scholaris*) indicated a higher inhibition for fraction As and Cr B. Sequential gravity liquid column chromatography of the two bioactive fractions afforded a white amorphous solid (As-ISO) and a yellow solid (Cr-ISO). One-dimensional nuclear magnetic resonance analysis (¹H and ¹³C) of the isolates revealed the structure of As-ISO as akuammidine, a sampagine type (1) and Cr-ISO as vindoline, a plumeran type (2).

Key words: *Alstonia scholaris*, *Catharanthus roseus*, indole alkaloid, antimycobacterial, anticarcinogenicity, *Mycobacterium tuberculosis* H37Rv, *Mycobacterium avium*, human oral epidermoid carcinoma lines, sampagine plumen

114. MOLECULAR DETECTION OF ENTEROVIRUSES ASSOCIATED WITH DILATED CARDIOMYOPATHY

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Viral infections are the most common cause of inflammatory heart muscle disease aside from other known factors. Enteroviruses, particularly coxsackieviruses B (CVBs), have been implicated in the pathogenesis of dilated cardiomyopathy (DCM). However, viral cultures of myocardial tissue are almost always negative, even when the clinical history or serological studies indicate viral infections. In this study, a right ventricular biopsy (5 mg wet weight) was performed in patients with clinically suspected DCM. The myocardial biopsies were used in the detection of enteroviral RNA by the Polymerase Chain Reaction (PCR). PCR is ideally suited for this study since it can detect low copy numbers of viral genome in small tissue samples. Two sets of primers (A/B and C/D) are from two different consensus sites in the enteroviral genome which allowed the detection of either CVB or poliovirus (PV). Primers A/B are most homologous to CVB3, whereas primers C/D are 100% homologous with six virus types (CVB1, CVB3, CVB4, PV1, PV2, and PV3). Of the 28 patients enrolled in the study, 3 were positive for the first set of primers, while 11 were positive for the second set of primers. Two patients were found positive for both set of primers. Samples are also processed for electron microscopy but so far viral inclusion bodies or particles have not been detected. The results of this study indicate a link between viral infection and dilated cardiomyopathy in some patients.

Key words: enterovirus, coxsackie virus, poliovirus, endomyocardial, biopsies, dilated cardiomyopathy, RNA, cDNA synthesis, PCR, electron microscopy, inclusion bodies

115. DETECTION OF *Helicobacter pylori* FROM FORMALIN-FIXED, PARAFIN-EMBEDDED GASTRIC BIOPSY SPECIMENS; A STRATEGY FOR *vacA* GENOTYPING

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Helicobacter pylori is an important human pathogen, having been identified as the major causative agent of chronic gastritis. Although it is well-studied in the US, Europe, Latin America, Africa, and some Asian countries, i.e., Japan, Korea, and Thailand, there are no available data on its epidemiology in the Philippines. Due to the difficulty in culturing this bacterium, we established a method to detect *H. pylori* from formalin-fixed, paraffin-embedded gastric biopsy specimens. Forty-two samples from the Institute of Pathology of St. Luke's Medical Center were evaluated using polymerase chain reaction (PCR). The amplification targets are the genes for urease A (*ure A*), urease B (*ure B*) and urease C (*glmM*). Once a sample has been confirmed to be positive for *H. pylori*, the presence of the *vacA* gene was also evaluated. This gene encodes the vacuolating cytotoxin which induces the formation of intracellular vacuoles in epithelial cells. The signal region of the *vacA* gene occurs as either s1 or s2 allele, while the middle region is present as m1 or m2 allele. Different combinations of these alleles give rise to a specific *vacA* genotype and correlate with the severity of the disease. In this study, the PCR-based system developed by Atherton (1999 *J Clin. Microb. Vol. 37:9*) was used to determine the *vacA* genotype of 42 gastric samples obtained from paraffin blocks.

Key words: *Helicobacter pylori*, PCR, *ureA*, *UreB*, *glmM*, vacuolating cytotoxin, *vacA*, signal region, middle region, gastric biopsy.

**116. PRIMERS FOR CYSTEINE PROTEINASE GENE
COULD DISTINGUISH PATHOGENIC *Entamoeba histolytica*
FROM NON-PATHOGENIC *E. dispar***

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Cysteine proteinases and pore-forming peptides (amoebapores) have been found to be responsible for tissue lysis and cytopathic effects described in invasive amebiasis. A total of six distinct *Entamoeba histolytica* cysteine proteins genes (ehcp 1-ehcp6) have been identified and sequenced. Three of these genes, ehcp 1, 2, and 5 are expressed at high levels in the pathogenic *E. histolytica*. In this study, an Oligo 4.0 software was used to design primer pairs WRG 1&2 and WRG 3&4 from consensus sequences of this gene. Amoeba were isolated from stool samples, cultured, and maintained in Robinson's medium. Genomic DNA was extracted using phenol-chloroform method. Polymerase Chain Reaction (PCR) using both primer sets could distinguish pathogenic *E. histolytica* from the non-pathogenic *E. dispar*. A 570-bp PCR amplicon was observed in 6 out of the 8 samples using WRG 1&2, while a 755-bp product was obtained in 4 samples using WRG 3&4. These results were further compared with the PCR products generated by primers designed by Tachibana (1991) which also distinguish pathogenic from non-pathogenic *Entamoeba* species. Amplified products from the cysteine proteinase gene primers are currently being cloned for sequence analysis.

Key words: *Entamoeba histolytica*, *E. dispar*, PCR, cysteine proteinases, *E. histolytica* gene (ehcp1-ehcp 6), amoebapores, Robinson's medium, genomic DNA extraction, cloning, gene sequencing.

117. POLYMERASE CHAIN REACTION-SEQUENCE SPECIFIC PRIMER (PCR-SSP) SYSTEM FOR BONE MARROW TYPING

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The Human Leukocyte Antigen (HLA) genes are highly polymorphic, co-dominantly expressed recognition genes that play an important role in determining tissue compatibility for transplantation. Because of this, HLA compatibility between donor and recipient must be determined prior to transplantation. Although HLA types have been defined using serology for many years, the resolution capacity of this method is limited. The advent of DNA-based techniques has allowed the identification of DNA sequence variations which cannot be distinguished by serologic typing. Sequence-specific primer (SSP) typing is DNA-based method that allows discrimination between the different alleles amplified by polymerase chain reaction (PCR). In this technique, PCR primers are designed to anneal only to a single or specific set of alleles. These primers contain sequences unique to the allele(s) and both primers must anneal to the DNA to get a positive amplification of the test sequence. The 96-well format for PCR allows simultaneous determination of 24 allelic groups 4 samples belonging to potential donors and the recipient. Amplified DNA fragments are separated by agarose gel electrophoresis and visualized by staining with ethidium bromide and exposure to UV light. Interpretation of the results is based on the presence or absence of amplified DNA fragment corresponding to the specific primer set. Determination of HLA type is by analysis of the pattern of positive wells either using an Analysis Program software or a reaction pattern worksheet.

Key words: HLA, transplantation, tissue compatibility, bone marrow, polymerase chain reaction, sequence-specific primer, serologic typing, donor, alleles, tissue typing

SOCIAL SCIENCES

118. AN ANALYSIS OF THE SEASONAL MOVEMENTS OF PALAY PRICES AND INTERTEMPORAL PRICE EFFICIENCY

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The study attempted to address the policy issue of how competitive or economically efficient the rice marketing systems are in terms of temporal price efficiency, that is, whether the incentive to carry inventory between two different time periods (from harvest to lean months) adequately covered costs of storage. Moreover, the seasonal price movements at the farm, wholesale, and retail levels are analyzed. Analyses were based on 1970-1995 price data series. Results showed that the seasonal price movements were largely due to the high seasonality of palay production. Between seasonal low and seasonal high price months, monthly seasonal price increase averaged at 3.4 percent, an incentive to induce traders to store excess stocks for the lean months. With the averaged costs of storage of 1.42 percent per month (in this case, it is the cost of working capital), the monthly seasonal price increase was adequate to cover the costs of storage. Although there were some years that the seasonal price increase were more than enough to cover costs of working capital, these were being balanced by other years of very low returns. Thus, there could be a year-in-year offsetting of profits and losses that the trader does assume.

Key words: seasonality, seasonal price increase, storage costs, price behavioral, intertemporal price, palay price, rice marketing

119. URBAN IMPACT OF THE QUALITY OF LIFE OF METRO MANILA RESIDENTS

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Sociologists agree that urbanization affects the quality of life of residents both positively and negatively. Using the survey method, this researcher investigated the impact of urbanization of Metropolitan Manila on the quality of life of its residents. Questionnaires consisting of 39 questions were distributed to 416 PLM students (43% male and 57 female) enrolled in courses like engineering, nursing, physical therapy, business administration, and arts and sciences at the start of the second semester of school year 1999-2000.

Results of the study indicated that 64% of the respondents live in their own houses and that their residential area is adequate (85%), spacious (56%), while 37% answered crowded. Sixty-seven percent (67%) think that their residential area is satisfactory because it is quiet (22%), nice and orderly (33%), and that neighbors are friendly and good (29%). Forty-seven percent (47%) indicated that transportation is adequate and accessible (74%) but traffic is heavy sometimes (58%), therefore, noise and air pollution is a problem (73%). Transportation system is satisfactory (45%). Drinking water is safe (79%) and sufficient (53%), and that they get their drinking water (68%) and bathing/washing water (71%) from the MWSS. They get their electricity from MERALCO (94%) and that it is very adequate (56%). Sewerage is clean and running well (40%) or defective (33%), and garbage is collected daily (45%). Jobs are adequate (52%) from the private sector (42%), which give sufficient income for basic needs and luxury (67%). Hospitals and puericulture centers are very adequate and accessible (59%), medicines are always available (64%); 54% indicated they have their own comfort and bathroom. Members of the family are mostly with education (85%). Leisure is always available and affordable (57%) in the form of megamalls (32%), movies (26%), parks (17%), and videos (17%). Malls and markets are available and accessible (93%), clean and nice (25%) where everything needed is available (35%) at affordable prices (25%). Peace and order is okay (74%), neighbors are friendly (35%), cooperative (22%), but sometimes become "isismo/a" (17%). However, 47% indicated that "bisyo" is minimal while 39% indicated that "bisyo" ay nagkalat. Summing up, 57% of the respondents think that life in the city is good and enjoyable; only 9% disagree.

Key words: urban impact, quality of life, Metro Manila residents

AUTHOR INDEX

A

Abalos MC, 271
Abrigo GNA, 266
Adunda JR, 291
Aganon CP, 298, 306
Aganon, TM, 269, 294
Agurnaldo AA, 246, 313
Aldemisa RR, 265
Alfoja EM, 290
Alfon JAR, 157
Alforja EM, 300
Amaparada EA, 311
Amarillo MLE, 137
Amisoso JO, 212
Angeles AT, 293
Anonico LP, 229
Antolin EG, 280
Aquino AG, 290, 291, 294
Aquino Rustivar L, 211
Aquino VM, 261, 262, 267
Aragones Jr, EG, 299
Asada AA, 224
Avellanosa ES, 265
Ayap JNB, 275, 280
Azanza PV, 281

B

Bacala AM, 91, 214, 217
Badayos CP, 303
Bagoic-Opulencia RD, 257
Balagot G, 284
Balatero CH, 264
Baldomaro-Columa C, 352
Banatlap PR, 187
Baonon ZG, 242
Barber BII, 255
Barcan JV, 300
Bartuan J, 290
Bayot RG, 290, 291, 294
Belizario Jr VY, 137
Bilgera BU, 286, 288
Binag CA, 226, 227

Bonrex AM, 275
Bonilla PS, 276
Botella JR, 268
Breno SR, 303
Briones AM, 266, 275, 318
Buerano CC, 310
Bugayong MPG, 137
Burleigh JR, 294

C

Caam-Lit M, 284, 287, 295
Cabling HIM, 275
Cabrera J, 244
Cacayuran ND, 286, 289
Cacho DR, 294
Caganda RFM, 229
Calapardo MR, 279
Caldaron LJI, 269
Canama AO, 264
Candelaria RB, 295
Candelario JR, 157, 317
Canoy Jr SR, 83
Cantanas MY, 309
Caparido UA, 296
Capricho MAA, 287
Carcillas RJL, 264
Carlos C, 157
Carunan PA, 240
Castaway AA, 311
Castaway AC, 240, 250
Cavayuran JR, 271
Castwon CB, 266
Caspilla AS, 294
Castro Bemas GD, 248
Cawagas RE, 220
Cayabyab BF, 290, 291, 294
Cayabyab FF, 294
Chan MA, 105
Chato Salvador R, 302
Chavez-Lapitan VP, 279
Chay BP, 228, 230
Chico MY, 274, 275, 278

Christou P, 279
 Comiso JC, 11
 Conroy JA, 105
 Cordell GA, 313
 Coronado MB, 305
 Corpuz AD, 3
 Corpuz ER, 273
 Cruz DJM, 137
 Cruz E, 250
 Cruz EM, 306, 307
 Cruz LB, 306
 Cruz R, 242
 Cu GWS, 227
 Cu JS, 199
 Cuadra DS, 225
 Cueto MF, 251, 258
 Cui XMR, 227
 Cureg ES, 211

D

Dahilo AS, 215
 Dalisay DS, 245
 Dalmacio IF, 252
 Danno MC, 266
 Danno MSMC, 289
 Dancel C, 228, 250
 Dante JA, 230
 Dapan EG, 249
 David RG, 206
 Dayrit FM, 228, 250
 De Guzman BB, 115
 De Guzman Jr AD, 137
 De Guzman R, 316
 De Leon T, 261
 De Leon WU, 137
 DE Ocampo NT, 221
 De Vera C, 311
 De Vera CM, 224
 Dela Villa AC, 263
 Delgado EB, 252
 Delos Reyes ABE, 137
 Demayo CG, 262
 Depositario PT, 167
 Desamero NV, 273, 274, 275, 278
 Diaz CL, 273
 Diaz MGQ, 258
 Dimasano EM, 157
 Dumanan YA, 271, 273
 Domingo EO, 280
 Domingo LG, 271, 274
 Domex NS, 276
 Duka IMA, 252, 258
 Duran EB, 224

E

Eduengo MD, 290, 291
 Elias ELL, 238
 Ella AB, 299
 Elhox FP, 187
 Enriquez MLD, 312
 Espantu EQ, 250
 Espantu G, 157
 Eusebio OL, 243
 Exuma RB, 279

F

Fashinger W, 217
 Faustino LP, 315
 Fegan M, 257
 Flores CEV, 272
 Follosco MP, 235
 Fontanilla RC, 311
 Francisco SR, 266
 Franco SS, 219
 Frarbleau SG, 246, 313
 Fujihara T, 306, 307
 Fujio K, 214

G

Gabius III, 229
 Galvez HE, 264
 Gamboa CP, 240
 Gasparin RM, 131
 Gaspar VP, 264
 Garcia MRC, 279
 Garcia TY, 224
 Garcia W, 212
 Gauran MJ, 246
 Gauran NL, 223
 Gergson FB, 287
 Gersacio SV, 212
 Germundo IM, 187
 Gonzales A, 157
 Gruezo KAG, 119
 Guzman PS, 305

H

Huete F, 157, 310
 Haurata DM, 264, 305
 Hayward AC, 257
 Herrera AA, 241, 244, 311
 Herrera EM, 280
 Herron AB, 272
 Hidalgo I C, 272
 Hidalgo MSP, 267
 Hipolito LB, 266
 Hoshi M, 244

Hufema AMM, 222
Hui M, 212

I

Igarashi A, 157, 310
Ilag LI, 285
Ilar GY, 265
Inoue S, 157, 310
Ishii K, 235
Iwawaki A, 255

J

Jabon JJ, 227
Jacinto SC, 311
Jacinto SD, 229
Javier EQ, 71
Josue ADL, 305
Jukalon MCN, 274
Julian FP, 286

K

Kobayashi K, 244
Korn M, 217

L

Lanigan FP, 223
Larona AR, 293
Laudé RP, 254, 258
Laurean CP, 303
Laurena AC, 258, 259, 267, 268
Laureta LV, 302
Lidasan HS, 37
Lit HL, 287, 293
Lo RW, 311, 317
Lopez NC, 240, 244
Lorena PDN, 311, 316

M

Mabesa IB, 205
Macabale SS, 274, 275, 278
Macabeo APC, 313
Macachor CP, 297, 301
Magallanes JM, 233
Magdalena PM, 268
Maghanoy MJ, 187
Maibanan FM, 303
Mangahas PMF, 247
Mapua C, 157
Masagca JY, 243
Matro LG, 294
Mateo ZF, 213
Matias RR, 157, 310, 314, 316
Mendiola MS, 119, 258

Miller SA, 131
Miyamoto A, 214
Morita K, 157, 310

N

Nagata AL, 285
Nalandaan JR, 219
Narciso EV, 315
Natividad DG, 280
Natividad FF, 157, 310, 312, 314, 315, 316, 317
Nato Jr AQ, 224, 253
Nifranco F, 91
Nidoes JM, 276
Nonato MG, 239

O

Obien SR, 287
Odiamar RV, 228
Orsh KI, 157
Opina O, 287
Orden EA, 306
Orden EM, 306, 307
Orden MEM, 307

P

Pahit E, 212
Padilla CL, 290, 291
Pagulayan IF, 242
Pagulayan RG, 242
Pahsac CP, 216
Palma MC, 119
Palmas Saloma C, 247
Pancho MA, 157
Panes VA, 277
Paredes MCB, 315
Pascual CB, 309
Patricio MG, 269
Peñalba F, 291, 294
Peralta MTB, 279
Perez BV, 268
Perez EA, 300
Perez EP, 291
Perez PA, 267
Pié C, 284
Pocido GN, 249
Porcuncula FL, 167
Portilla MCB, 309
Posas FEB, 314
Pulumbani Jr, FN, 250
Pyne NJ, 248

Q

Quistoriano MLV, 229
 Rayoite E, 284

R

Ramirez DA, 263
 Ramirez TQ, 280
 Ramos MCR, 248
 Ramos MDR, 299
 Ramos O, 298
 Ramirez CV, 131
 Ranzon IR, 275
 Rcaño CE, 264
 Rodondo GO, 266
 Reserva RL, 217
 Reyes JB, 187
 Reyes JD, 314
 Reyes R, 224
 Rillon GS, 293
 Rivera R, 264
 Robinson HL, 255
 Roderos RS, 238
 Romano LP, 274
 Romano SR, 301
 Romero GO, 277
 Romero MV, 278, 292

S

Sajise JC, 279
 Saldia LT, 317
 Saludes JP, 246
 Sana EA, 197
 Sanchez ALC, 214
 Santiago B, 284
 Santiago KS, 226
 Santos DS, 318
 Santos GA, 264
 Santos Ocampo PS, 1
 Sando ML, 187
 Sano RB, 297
 Sebastian LS, 266, 270, 271, 277, 279
 Segovia SJE, 264
 Serra AB, 306
 Sevilla III FS, 225
 Simbillo CF, 311
 Smith DR, 254
 Solidum RS, 217
 Solisloy AD, 280
 Solisloy TS, 286
 Sonico MGI, 229
 Sonazo Jr JS, 269
 Sta. Maria LB, 274

T

Talven RE, 270, 271, 273
 Takahashi K, 292
 Talekar N, 284
 Tan I, 311
 Tanaka Y, 213
 Tandang RN, 119
 Tanduyan SN, 237
 Tecson Mendoza, 258, 259, 267, 268
 Terio RM, 91
 Tiong-Palacios S, 217
 Tolentino LT, 187
 Tolentino V, 277
 Tolentino VS, 236
 Tomolete L, 317
 Torres-Villanueva CT, 255, 256
 Tolones FI, 249
 Tumimbang EB, 262

U

Ulat VJM, 251, 258
 Unico JRS, 228
 Ureta S, 245
 Uriza RP, 295
 Ursua MG, 235

V

Valdez RE, 275
 Valdez RE, 280
 Valencia MF, 311
 Valente BM, 303
 Vanquez GM, 245
 Villafructe LS, 254
 Villamael LN, 119
 Villanar C, 245
 Villagas VN, 268, 279
 Visvanathan C, 222
 Vivar G, 256

W

Wu R, 279

Y

Yebon MGN, 262
 Ysrael MC, 311
 Yulo Nazarea T, 224, 253

Z

Zamora PM, 236
 Zara AL, 187
 Zulueta NV, 280, 281

SUBJECT INDEX

A

abaca, 262
 academic waste, 221
 ACC synthase, 267
 acclimation, 340
 ACP, 258
 Acyl carrier protein, 258
 adenocarcinoma, 312
 adjacency matrix, 211
 adsorption, 252
 adverse environment, 275
 AFLP, 265, 276
 agricultural extension, 187
 agriculture, 71
 Agrobacterium, 235
 Agrobacterium tumefaciens, 265
 agronomic potentials, 303
 agrotechnology transfer, 303
 air gamma dose rate, 224
 albina, PCR-SSCP, 247
 alkaloids, 311, 313
 antimycobacterial, 313, anticarcinogenicity, 312
 human oral epidermal carcinoma lines, 312
 alkaloids, 239
 Alstonia scholaris, 313
 ambient, 297
 ammonia excretion, 301
 amorphopores, 315
 Amorphantha lous, 286
 amplified fragment length polymorphism, 264
 Amaranthus bigramis, 284, 289
 analgesic, 311
 analysis, 297
 animal feed, 230, 292
 annealing temperature, 259
 anodal protein, 251
 antagonistic plants, 131
 anther culture, 273, 274, 275, 278
 anitropogenic, 224
 antibiotics, 245
 anti-fertility drug, 249
 antigen sandwich ELISA, 157
 antigen, 255

anti-Listeria, 252
 antimicrobial spectrum, 252
 Aphis gossypii, 289
 aquatic, 300
 arc, 213
 arden pea, 286
 Argemone mexicana, 290
 Artemisia vulgaris L., 119
 arthropods, 287
 artificial seawater, 240
 Ascaris, Trichuris, 137
 aseptic processing, 296
 ASM cells, 248
 Aspergillus niger, 230
 assessment, 224, 299
 autotrophy, 103
 axillary shooting, 235

B

BAC, 276, RGA, 276, TAIL-PCR, 276
 bacilli, 245
 bacterial blight, 271
 bacitracin, 252
 bamboo insects, 287
 bamboo pests, 287
 bamboo, 295
 banana bunchy top virus, 261
 banana, 230, 261
 BASTA, 279
 BBTV, 261
 Beawulf system, 215
 3-beta-hydroxysteroid dehydrogenase, 249
 beverage industry, 222
 bioconversion, 230
 biodiversity, 266
 biopsy, 315
 biotechnology, 71, 230
 blood, 306, 307
 body weight, 301
 Bol loops, 220
 bulls, 286
 bollworm, 287
 bone marrow, 317

- Bongabon, 290
 bruchid, 265
 BSA Dupont, 226
 Bt rice, 282
 bumpy top virus, 262
Burkholderia andropogonis, 258
 butterflies, 293
- C
Caligus, 245
 calli, 265
 callus induction, 273
Caltharantus roseus, 313
 CAMAC, 217
 Camarines Norte, 272
 Camotes Sea, 237
 cancer, 312
Carica papaya, 267, 268
 carnivores, 240
 carp, 240
 Catanduanes, 243
 catfish, 303
Catharantus roseus L., 119
 cathodal proteins, 251
 caustic soda, 222
 cDNA, 262, 267
 Cebu, 301
 cell growth, 248
 Central Luzon, 298
 ceramics, 219
Cercopithecus, 265
 cereal grains, 292
 cervix, 312
 CFAS, 229, 250
 Chaimat, 245
 chemotaxonomy, 239
 chemotherapeutic, 246
 Chikungunya (CHIK) virus, 157
Chilo suppressalis, 293
 chromosome walking, 276
 circuit, 213
 circular matrix, 211
 circulant graphs, 211
Charias garipinus, 301
 climate, 7, 11
 cloning, 258, 262, 267, 315
 clustering, 238
 coat protein, 267
 coconut, 264
 codon usage, 256
 collection, 299
 coloa, 312
 common intestinal helminths, 137
 common variance, 214
 communication, 9
 community development, 167
 community structure, 301
 comprehensive strength, 221
 computer simulation, 214
 Copuriet, 215
 computing, 215
 conchology, 242
 concrete, 221
 conducting polymer, 226, 227
 conductivity, 276
 consumer preference, 274
 contaminants, 221
 coning, 276
 continuous lysate production, 228
 control cost, 287
 cooling qualities, 281
 corn diet, 293
Corynebacterium glutamicum, 228
 cosmic ray, 91
 cotton, 286, 287
 eutyledonary nodules, 235
 cotyledons, 236
 crops, 71
Crotalaria uncana, 131
Crotalaria macronota, 131
 crustacea, 245
 culture, 237
 cycle graph, 211
 cyclic voltammetry,
 cyclosporin, 309
 cysteine protease gene, 316
 cytogenetic effects, 119
 cytogenetics, 312
 cytotoxicity, 229
 cytotoxin, 315
- D
 2,4-D, 273
 damage assessment, 291
 damping-off organisms, 289
 Dasao del Norte, 240
 dengue fever (DF), 157
 dengue hemorrhagic fever (DHF), 157
 dengue virus, 157
 dengue-2, 310
 dense planting, 286
 density, 243
 deposition, 252 well-diffusion assay, 252
 detection, 11
 development, 241
 devolution, 187

- desulfurizing, 137
- daggraph, 213
- diapers, 219
- diet, 301
- differential expression, 251
- dihydroturpene, 236
- Dipletanum*, 245
- direct plasmid regeneration, 273
- disease resistance, 271
- diversity, 243, 301, 305
- DNA immunization, 255
- DNA marker, 266
- DNA sequence, 258
- DNA vaccines, 255, 256
- doctors, 197
- Dofichos lablab*, 229
- doubled haploid line, 273
- dragon kites, 219
- dry season, 272
- drylands, 290
- dye, 299
- E
 - ecological stress, 233
 - ecology, 233
 - ecosystem, 300, 301
 - eggmasses, 293
 - eggplant, 262, 264
 - eigenvalue, 214
 - El Niño, 272
 - electrophoresis, 238
 - electropolymerization, 227
 - electroweak interactions, 215
 - elementary particle, 215
 - embryo, 236
 - embryogenic calli, 279
 - empowerment, 187
 - emryogenesis, 242
 - energy, 219
 - Entamoeba histolytica*, 315
 - entropy, 259
 - environment, 7, 11, 233
 - ESI-MS, 229
 - ethylene, 262, 267, 268
 - Euborella annulata*, 286
 - exotic weed, 290
 - exposure, 233
- F
 - fallow, 131
 - farmer choice, 275
 - fingerlings, 250
 - flow cytometry, 312
 - histopathology, 312
 - food additive, 252
 - forage, 306
 - free cells, 228
 - freeze drying, 296
 - freeze-drying rate, 296
 - freshwater, 251
 - fry, 240
 - fuelwood, 219
 - fungicide, 289
 - fungicide, 303
 - Fusarium wilt*, 286
 - gain-of-function mutation, 247, 249
 - garden soil, 219
 - gastropods, 243
 - gauge field theory, 215
 - gauge invariance, 215
 - gelling agent, immobilized cells, 228
 - gene tree, 255
 - genetic engineering, 265
 - genetically modified, 71
 - genome polymorphism, 255
 - genotypic methods, 258
 - genotyping, 315
 - gill parasites, 245
 - GlmM, 315
 - glycoproteins, 229
 - GNA, 279
 - Gnathia*, 245
 - gne sequencing, 315
 - goats, 306, 307
 - graceful labeling, 213
 - grain quality, 275, 280
 - grain-negative, 245
 - grain-positive, 245
 - greatest common divisor, 211
 - ground meristem, 236
 - growth patterns, 310
 - infectivity, 310
 - growth performance, 307
 - growth stage, 287
 - guppy, 250
- H
 - haemorrhagic fever, 310
 - Hunga, 236
 - haplotypes, 255
 - hatching, 240
 - health, 7
 - Helicobacter pylori*, 315
 - Helicoverpa armigera*, 289
 - Hemstock integral, 83
 - hepatotoxicity, 250
 - herbal drug, 249
 - herbivores, 240

Higgs boson self-energy, 215
 Higgs boson, 214
 high energy physics, 214
 high protein,
 high yield, 271
 Hilbertian, 83
 histogenesis, 241
 histology, 242, 312
 HLA, 317
 homologous recombination, 256
 honeybees, 255
 host plant resistance, 284
 HPLC, 309
 human reason, 3
 hybrid, 303
 hygromycin, 279
 hyperactive, 249
 hyperactivity test, 249

I

IgG indirect ELISA, 157
 IgM capture ELISA, 157
 IgM-IFA, 157
 immobilization, 221
 immune response, 255
 immunoblot, 248
 immunomodulatory, 229
 immunofluorescence assay, 310
 in situ observations, 11
 inbred lines, 305
 indica rice, 273, 278
 indigenous, 299
 inflorescence, 265, 275
 influence function, 214
 influential observations, 214
 information, 9
 insect pests, 292
 insect resistance, 279
 insecticide, 289
 interspecific variation, 234
 introgression, 262
 invertible, 220
 IP, 220
 ipalbidine, 311
 ipalbine 311
 ipalbinium, 311
 IPM, 269, eggplant, 269,
 ipomoea, 311
Ipomoea muricata, 311
 iron, 281
 irrigated lowland, 269
 isozymes, 238

J

JLC, 214

K

K562 cells, 310
Kappaphysa alvarezii, 237
 Kennon, 303
 ketosteroids, 246

L

Laguna Lake, 251
 lahar, 219, 306
 larvae, 293,
 LAS, 250
 laser ablation, 212
 laser-produced plasma, 212
 lattice-matched, relaxation, 217
 leaching, 221
 leaf chewers, 291
 leaf hopper, 284
 least common multiple, 211
 Jertin, 229
 Lepidopteran pests, 291 cutworms, 291
 leukocytes, 119
 LGUs, 187
 limestone, 303
Limnocharta flora, 300
Limnocharta, 300
 Lingayen Gulf, 245
 local government units
 loops, 220
 loss-of-function mutation, 247

M

maize, 305
 management, 286
 management, 287
 mango powder, 296
 mango puree, 296
 mangroves, 243, 301
 MAPK, 248
 mass screening, 276
 maize treatment, 137
 maturation, 262
 MBE, 217
 meiosis, 247
Metolodyni graminicola, 131, 287
 membrane technology, 222
 Message passing interface, 215
 Metro Manila, 318
 mice, 249
 microbial, 297
 microfiltration, 222

microsatellite markers, 305
 microsatellite, 264
 microsatellite, 266
 milk, 307
 milkfish, 250
 minerals, 306
 Mist-polishing, 260
 mitochondria, 255
 mitotic index, 119
 mitotic inhibitor, 119
 molecular fingerprinting, 251
 molluscs, 240
 mollusks, 243
Momordica charantia L., 119
 monitoring, 137
 monogenic, 245
Morinda citrifolia, 246
 morphoanatomy, 242
 morphometry, 312
 Moufang, 220
 Mount Banabau de Lucban, 293
 mt DNA, 255
 Mt Pinatubo, 219
 multiplet shooting, 235
 mungbean, 265
 Muon lifetime, 217
 mutagenic, 119
Mycobacterium avium, 313

 N
 NAFL, 220
 nanofiltration recovery, 222
 narra, 235
 natural radioactivity, 224
 Nd-YAG laser, 212
Neobranchella, 245
 Nerostian, 226
 n-heptane, 236
 NIM modules, 91
 NIM, 217
 nitrogen, 212, 286
 Non abelian, 215
 non-associative, 220
 non-isomorphic, 220
 nonsingular graphs, 211
 northern blot, 267
 nuclearity, 83
 Nueva Ecija, 272, 290, 291

 O
 omnivores, 240
 onion, 131, 290, 291

optical emission, 312
Oreochromis niloticus, 241
 organogenesis, 242
 ovarian follicles, 249

 P
 PAA, 273
 PAGE, 255
 PAK peptide assay, 248
 palay price, 318
 palay, 318
Pandanus, 239
 papaya, 267, 268
 Parallel virtual machine, 215
 parasites, 292
 parasitologic assessment, 137
 particle bombardment, 279
 partnership, 187
 path, 213
 pathogen, 245
 PCR, 259, 261, 262, 267
Pectinophora gossypiella, 289
 peclings, 230
 perennate, 119
 perturbation theory, 214, 215
 piezoelectric crystal, 225
 pest management, 282
 pH potentiometry, 227
 pH, 226, 286
 Philippines, 157
 philosophy, 3
 phosphorylase kinases, 248
 photon flux density, 195
 physical mapping, 276
 physicians, 197
 official curriculum, 197
 operational curriculum, 197
 hidden curriculum, 97
 medical education, 197
 clinical internship, 197
 professional socialization, 197
 physicochemical, 280, 281, 297
 pigmented mace, 249
 Pin2, 279
Pinus radiata, 105
 pipefish, pufferfish, 240
Piper betle, 287
Pitiorporum resiniferum, 236
 planktivores, 240
 plant regeneration, 265
 plastic scintillator detector, 91
 polyaniline, 226

Polythiophene, poly(3-methylthiophene), 227

poppy, 290

population, 282

PorsnairR, 250

postharvest, 292

potentiometry, 226

prawn, 251

predators, 292

preservative, 252

primer design, 258, 269

primers, 315

principal factor analysis, 214

probiotics, 245

procambium, 236

protoderm, 236

provenance, 235

PRSV, 267

Pterocarpus indicus, 235

pulsed laser deposition, 212

purification, 252

Q

quality of life, 318

quartz crystal, 225

quasigroups, 220

rabbitfish, 240

radiative corrections, 215

radionuclides, 224

rainfall, 272

rainshelter, 294

ranked C_N-space, 83

RAPD, 268

RAPD, 276, 278

red pinny, 291

refrigerated, 297

remilling, 280

reproductive

resettlement, 167

residually graceful labeling, 213

resistance gene analog, 265

resolutions, 7

RFLP, 276,

Rhizospora, 301

ribotyping, 258

rice breeding, 273

rice flour, 281

rice hull burning, 287, 298

rice hulls, 298

rice marketing, 318

rice noodle, 281

rice, 131, 265, 266, 269, 271, 273, 275, 276,

278, 279, 280

rice, 303

rice-based cropping, 269

rice-onion system, 287

ringspot virus, 267

risk assessment, 233

risk factor, 233

Rhopalocera, 293,

root knot, 287

root rot, 286

root stock, 294

root-knot nematode, 131

r-separated, 83

r-th power graph, 211eigenvalues, 211

RT-PCR, 157

RT-PCR, 258

RT-PCR, 267

S

Saccharum, 262

saline, 275

salt tolerance, 276

Salvinia molesta, 300

Salvinia, 300

Samal Island, 240

sanitary napkins, 219

satellite, 11

school-based control, 137

science, 3

seion, 294

SCUs, 187

SDS PAGE, 248, 251

sea water, 276

seagrass fishes, 240

seasonality, 318

secretion, 255

seed culture, 275

seed quality, 303

selenium, 307

SEM, 212, 226, 227

senescent, 105

sensor, 226, 227

sensory evaluation, 280

sequence analysis, 256

sequence specific primer, 317

shelf life, 297

shoot culture, 105

shoots, 295

Short-period superlattice, 217

simple sequence repeats, 264

singular graphs, 211

S-matrix pinch technique, 215

Social reproduction, 197

sodium nitrate, 249
 soil taxonomy 303
 soil, 298,
 soils, 303
Solanum aethiopicum, 262
Solanum melongena, 262
 solidification, 221
 solidified waste, 221
 Southern blot, 258, 267
 SrCl_2 , 228
 SSR, 278
 stabilization, 221
 stakeholders, 187
 state colleges and
 stemborer, 282, 293
 steroid biosynthesis, 249
 stigmata, 246
 storage costs, 318
 stored products, 292
 stringbeans, 269
 strong Lusin, 83
 Substrate-temperature, 217
 sucrose, 105
 sugarcane, 262
 supermale, 241
 Surfactant analysis, 229
 surfactant toxicity, 250
 suspensor, 236
 sustainable, 71
Syzygium cumini L., 119

T
Tagetes, 131
 technology development, 269
 temperature, 286
 tempura sauce, 297
 tempura, 297
Terapon jarbua, 245
 thidiazuron, 235
 thymidine incorporation assay, 248
 tilapia, 240, 241, 250
 tissue compatibility, 317
 tissue typing, 317
 titanium nitride, 212
 titanium, 212
 tomato, 294
 toston, 242
 tournament, 213
 toxic heavy metals, 221
 toxicity tests, 250
 traffic, 7, 37
 transformation, 235, 262, 268, 279, 317,
 transplanted rice, 269

transportation, 7, 37
 trap crops, 286
Tribolium castaneum, 292
Stegobium paniceum, 292
 TRIS, 226
 trochophore, 242
Troides rhadamantus, 293
 tuberculosis, 246
 tyrosinase, 247

U
 uniform elite, 197
 unique variance matrix, 214
 universities, 187
 urban impact, 318
 Urea, 315
 US military bases, 224

V
 variability, 282
 variant, 275
 Vector NTI, 259
 vegetable, 295, 269, 298
 veliger, 242
 vertex, 213
 Vigan jars, 219
 viscosity sensor, 225
 vitrification, 105, 219
Vivipara costata, 242
 viviparous, 242
 volatile oil, 289
 Western blotting, 248
 wet season, 272

X
 Xa-21 gene, 271
 XPS, 226
 XPS, 227
 XRD, 212
 XRD, 217

Y
 yield loss, 267
 yield performance, 273

Z
 zeatin, 235
 zinc deficiency, 275
 Zingiberaceae, 238
 ZnSe buffer, 217
 ZnTe well, 217
 ZnTe:Zn (S, Te), 217

