BIOCHEMICAL BASIS OF RESISTANCE OF TOMATO (*Lycopersicon esculentum* MILL.) AGAINST BACTERIAL WILT (*Pseudomonas solanacearum* E.F. SMITH): CELL WALL MODIFICATION

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ABSTRACT

Three highly resistant accessions (Acc. #70, 75, and 508) and two highly susceptible cultivars (Bacolor and Yellow Plum) of tomato were selected to study cell wall modification as an induced structural defense response to the bacterial wilt pathogen.

Extensin, a hydroxyproline-rich glycoprotein (HRGP), was 25 to 45% higher in the resistant cell walls (Acc. # 70) than in the corresponding susceptible counterparts (Yellow Plum) in response to infection. Lignin increased in the root and stem cell walls of resistant (Acc. # 70) tomato by 14 to 24% and decreased by 10 to 24% in the susceptible Yellow Plum. Wall-bound proanthocyanidins were four to five times higher in the root cell walls and six to seven times higher in the stem cell walls of resistant Accs. # 70 and 75 relative to the susceptible cv. Yellow Plum. A time-course analysis showed that extensin in the root and stem cell walls of Acc. # 70 increased earlier (1st and 2nd day after infection) and to a higher extent (20 to 50 mg/g increase) compared to cv. Yellow Plum.

Root and stem cell walls of Acc. # 70, 75, and 508 were relatively stronger even before infection based on comparative structural resistance of extensin and lignin to chemical extraction and the effects of pre-treatments on their extractability. Modified cell walls (after infection) of Acc. # 70 became even more resistant to chemical extraction (twice decrease in extractability of extensin in root cell walls and 1.6 to 2.4x decrease in extractability of lignin in stem cell walls).

Average tensile strength of infected tomato stem tissues (Yellow Plum) decreased by 53% and by 14% in Acc. # 70. Infected stem tissues of the former were heavily macerated (absence of cell coherence) with great quantities of bacterial slime produced. Infected stem tissues of resistant Acc. # 70 still had cell coherence and bacterial slime was not evident.

In vitro digestion of cell walls of resistant and susceptible tomato plants by cell wall degrading polysaccharidases in bacterial culture filtrates showed that cells of resistant plants are less vulnerable to enzymatic digestion.

INTRODUCTION

Bacterial wilt is one of the most important plant diseases in the Philippines particularly of solanaceous vegetable crops. This disease was first reported in the Philippines by Reinking (1919) on tomato, eggplant, pepper, white potato, and tobacco. An excellent review of the biology and physiology of this vascular wilt disease was given by Buddenhagen and Kelman (1964) though a more recent review was made by Hayward (1991) which focused primarily on the fundamental aspects of the bacterial pathogen and its taxonomic relationships as determined by molecular biology, host range and geographical distribution, environmental interactions and epidemiology, and various strategies of disease control.

Bacterial wilt still remains to be one of the most destructive diseases of plants not only in the Philippines but also in other parts of the world. The disease is characterized by its widespread geographical distribution, extensive host range, genotypic complexity of the pathogen, rapid disease transmission by biotic and abiotic agents, lack of basic knowledge on disease ecology, and the lack of effective control against disease spread.

The causal microorganism is *Pseudomonas solanacearum* E.F. Smith, a Gram-negative, rod-shaped, soil-borne (as a saprophyte) and xylem-resident bacterium (as a pathogen). Based on ultrastructural studies, the natural entry point in the host plant is through the roots (Huang, 1986). The pathogen that infects tomato belongs to races 1 and 3 and biovars I, III, and IV (Valdez, 1986). Since 1984, the Institute of Plant Breeding (IPB) has screened more than a thousand accessions of tomato and has come up with accessions with high levels of resistance to the most virulent strains of the three biovars.

It is the aim of this study to investigate cell wall modification as an induced host defense-response of resistant tomato plants to bacterial wilt infection.

MATERIALS AND METHODS

Bacterial Culture. Isolate # 151 (Biovar III) is one of the most virulent strains of the bacterial pathogen. Biovar III is the predominant and most widely occurring biovar type in Luzon, Visayas, and Mindanao.

Plant Materials. Tomato seeds of resistant and susceptible accessions and cultivars were acquired from the Plant Pathology Laboratory and the Vegetables Breeding Division of the Institute of Plant Breeding. Resistant accessions and lines with 90 to 100% survival ability to the three most virulent isolates of the bacterial wilt pathogen representing three biovar types (biovar I, III, and IV) were used – Acc. # 70, Acc. # 75, and Acc. # 508. Yellow Plum and Bacolor are varieties which are highly susceptible to all biovar types of the pathogen.

Phytopathological Methods. The preparation of tomato test plants (6 week-old), the infection inoculum (isolate # 151/biovar III)s and the inoculation of test plants (root cut/injury technique) were all carried out according to standard procedures for screening tomato against bacterial wilt (Valdez, 1986; Laurena, 1993).

Sample Collection/Storage of Test Plants. Samples of all infected tomato test plants (resistant and susceptible) including controls healthy/uninfected were uprooted carefully from the trays. Extra care and attention were given especially during uprooting so that very little damage occurred in the root tissues. A major part of the experiments requires that samples be harvested when 95 to 100% of the suceptible types have wilted while the time-course analysis requires that a given number of tomato test plants be sampled each day right after inoculation up to the time that susceptible types have wilted. Right after sample collection, test plants were washed thoroughly with tap water, blotted dry and partitioned into three parts -- root, stem, and leaf tissues. These sampled parts were labelled properly in sealed plastic bags and stored indefinitely in the freezer. These various sampled plant materials were processed to isolate the cell wall fraction used as starting material for the various cell wall chemical analyses (Figs. 1 and 2).

Preparation of Cell Wall Material. Cell wall was prepared from tomato plant tissues according to the method of Cassab et al. (1985).

Pretreatments of Cell Wall. Cell walls were pretreated by the sequential depectination according to the method of Selvendran et al. (1975) and by partial digestion with fungal cellulase (Laurena, 1993).

Extensin Analysis by ELISA. Wall-bound extensin was extracted from isolated cell walls according to the method of Fry (1988). Extensin was quantitatively determined using an ELISA technique based on rabbit polyclonal antibodies generated from pure extensin (Laurena, 1993).

Lignin Analysis. Lignin was determined according to AOAC methods (1975) using the Klason procedure. Lignin was also extracted based on hot alkaline hydrolysis (Scalbert et al., 1985; Hammerschmidt and Kuc, 1982) and analyzed spectrophotometrically at 280 nm. Data obtained from the results were confirmed using the thioglycollic assay (Hammerschmidt et al., 1984) or the ionization difference spectral (Stafford, 1960) studies.

Analysis of Wall-bound Proanthocyanidins. Wall-bound proanthocyanidins were extracted and analyzed using the methods of Bate-Smith (1975, 1981).

Peroxidase Activity. Ionically-bound peroxidase was extracted using the method of Cadena-Gomez and Nicholson (1987) with some minor modifications and its activity determined according to the method of Kahn et al., (1981).

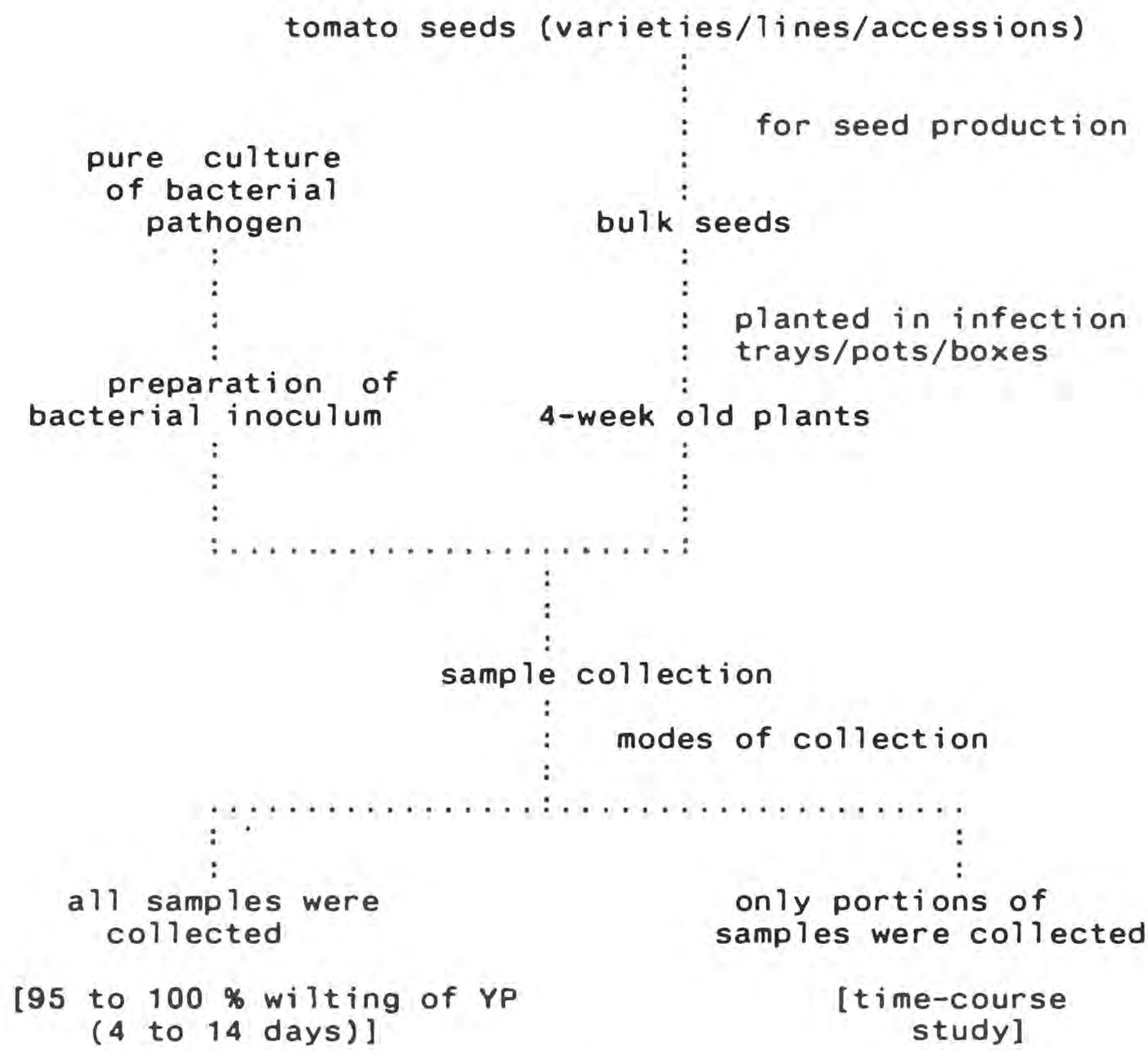

Figure 1. Schematic diagram of inoculation of test plants with the bacterial wilt pathogen and the modes of collection of plant samples.

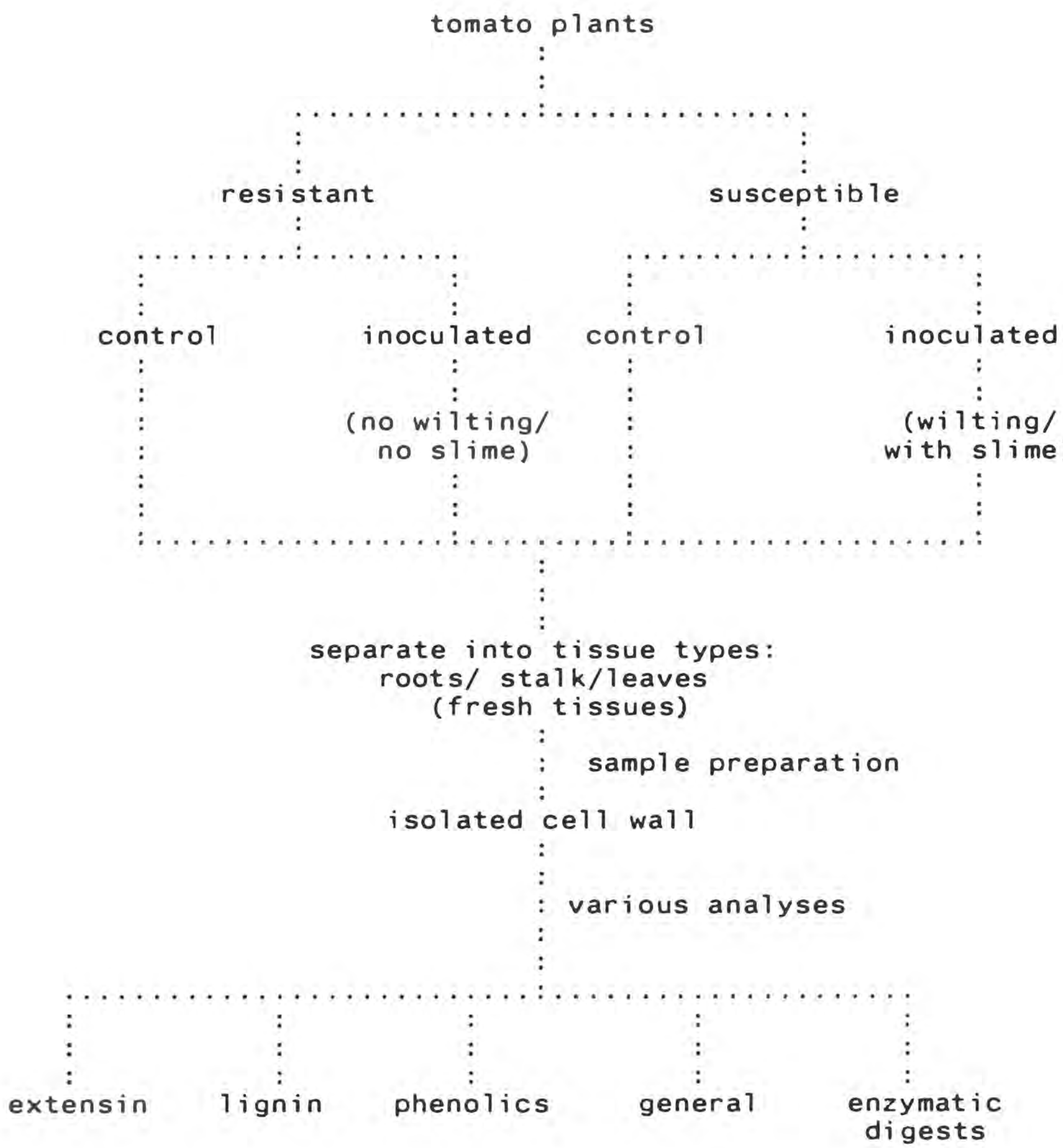

Figure 2. Schematic diagram of the different analyses on cell walls of resistant and susceptible tomato plants and the effect of pathogen inoculation/ infections.

Measurement of Tensile Strength. A Shimadzu (model SH-20) tensile strength testing machine with a maximum capacity of 20 kg force was used to measure the tensile strength of air-dried tomato stems.

Cell Coherence. Cell coherence of intact stem tissues of tomato test plants were estimated using the method of Ofuya (1986).

Enzymatic Digestion of Cell Walls. Cell wall material was digested enzymatically with fungal polygalacturonase, fungal cellulase, and culture filtrates of isolate 151 (biovar III) under optimum conditions (Laurena, 1993).

Analysis of Cell Wall Digestion Products. Galacturonic acid was determined colorimetrically by the thiobarbituric acid (TBA) assay as modified by Ayers et al. (1964). Glucose/reducing sugars were assayed by the Nelson-Somogyi procedure (Nelson, 1944).

RESULTS AND DISCUSSION

Soluble Monomeric and Wall-bound Extensin. Two molecular forms of hydroxyproline-rich glycoprotein (extensin) were determined in the cell walls of the various tissue parts of the resistant (Acc. # 70) and susceptible (Yellow Plum) tomato plant before and after infection: (1) the salt-soluble monomeric extensin and (2) the wall-bound insoluble extensin. The former represents newly-synthesized HRGPs attached loosely within the cell wall through ionic interactions and can be easily extracted from freshly prepared isolated cell walls (Cassab et al., 1985; Hood et al., 1988) by salt elution (Cassab and Lamport, 1988). ELISA values for soluble extensin ranged from 3.9 to 8.9 mg/g cell wall in the different plant parts (Table 1) and an increase of 40% in salt-soluble extensin was observed after infection. Except in the leaves, the salt-soluble extensin in the roots and stem cell walls of the resistant samples before and after infection were significantly higher (15 to 18% higher) than in the susceptible.

The latter represents the polymeric HRGP that is covalently linked via isodityrosine cross-linkages (Fry, 1982) mainly in the primary cell wall. Wall-bound extensin could not be extracted by chaotropic agents such as boiling SDS (Cassab and Lamport, 1988) but can be solubilized by hot acidified chlorite (Selvendran et al., 1975) through breaking of isodityrosine cross-linkages. ELISA values obtained for wall-bound extensin ranged from 18.9 to 97.5 mg/g in the cell wall of all samples and were 18 to 35% higher in all samples after infection than in healthy uninfected samples (Table 1). Wall-bound extensin was 25 to 45% higher in the resistant cell walls than in their corresponding susceptible counterparts.

The monomeric salt-soluble extensin does not contribute to the overall rigidity of the cell wall. It is only the polymeric extensin that can form cross-linkages with itself and other cell wall constituents such as pectins, cellulose, and lignin.

Table 1. Effect of bacterial wilt infection on salt-soluble monomeric and wall-bound polymeric extensin on a resistant accession (Acc. # 70) and a susceptible tomato cultivar (Yellow Plum).

Tomato Accession/Cultivar *Tissue part/Treatment*	*Salt-soluble monoric extensin (mg/g)*	*Wall-bound polymeric extensin (mg/g)*
Yellow Plum (control)		
roots	4.6 h	47.0 de
stems	3.9 i	37.0 hi
leaves	6.1 h	22.4 k
Yellow Plum (infected)		
roots	7.3 c	73.0 bc
stems	4.7 g	45.4 efg
leaves	8.4 ab	18.9 kl
Acc. # 70 (control)		
roots	5.4 f	79.0 b
stems	4.6 h	45.7 ef
leaves	4.5 h	40.0 efgh
Acc. # 70 (infected)		
roots	8.9 a	97.5a
stems	8.1 b	63.9 d
leaves	6.8 d	34.6 hij

Values for ELISA (enzyme-linked immunosorbent assay) are means of two trials (3 replicates/trial). Treatment means followed by the same letter are not significantly different (5%, DMRT).

Klason Lignin. The root cell wall material has the highest lignin content (24 to 27%) compared to both the upper (15 to 18%) and lower (17 to 20%) stem cell walls (Table 2). Lignin content was even relatively higher in Yellow Plum (susceptible) than in Acc. # 70 (resistant) in the cell walls of the root, lower stem, and upper stem tissue parts during the pre-infectional stage.

Lignin increased in Acc. # 70 by 14% in the roots and in the upper stems and by 24% in the lower stems in response to pathogen inoculation/infection. These changes were significantly different. Lignin decreased in Yellow Plum by 14% in the roots and by 14% in the lower stems. No significant changes were observed in the upper stems of Yellow Plum in response to infection. The "delignifying effect" observed in response to infection in Yellow Plum root cell wall was also observed in an earlier study (Regaspi, 1992). This could be due to the pathogen producing an enzyme that is capable of hydrolyzing lignin. The infected tissues of the susceptible Yellow Plum could be heavily macerated by the cell wall degrading enzymes produced by the pathogen such that during the isolation of cell wall material, there is actual loss of lignin. Another point of consideration is that the "delignifying effect" was absent in the cell walls of resistant tissues suggesting that the ligninase enzyme is inhibited or that the cell wall is tightly cross-linked such there is no loss of lignin material during the cell wall isolation process.

To verify these results, lignin was also estimated by HPLC after alkaline hydrolysis of stem cell walls of Acc. # 70 and Yellow Plum and the "delignifying effect" was again observed in the susceptible samples as a consequence of infection (Laurena, 1993).

Fungal diseases are known to induce lignification of cell walls in infected tissues and such lignin formation has been suggested a mechanism to resist plant diseases (Ride,1978; Vance et al., 1980). Lignins have been proposed to be a resistance factor by one or more of the following mechanisms: (1) making existing cell walls or newly-synthesized lignin in existing cell walls resistant to enzymatic degradation or mechanical penetration by physically "sealing off"of an infection site or by shielding off substrate polysaccharides from degradative enzymes or by chemically altering such substrates making them less suitable for action by enzymes or by pathogens, (2) restricting the diffusion of enzymes and toxins from the pathogen to the host and conversely, nutrients and water from the host to the pathogen, and (3) the low molecular weight phenolic precursors and free radicals formed during polymerization of lignin may be toxic to the pathogen or capable of inactivating the enzymes.

Wall-bound Proanthocyanidins (Condensed Tannins). Condensed tannins covalently linked to cell walls can not be extracted even with hot aqueous methanol but can be estimated colorimetrically by heating the isolated cell wall material in butanol-HCl (19:1 v/v) at 96°C for 1 to 3 h in sealed hydrolysis tubes. Prior to infection, wall-bound proanthocyanidins were relatively higher (65.1 to 72.4 μg/g in the root cell walls and 94.7 to 101.9 μg/g in the stem cell walls) in the resistant

Table 2. Effect of bacterial wilt infection on lignin content in a resistant accession (Acc. # 70) and a susceptible tomato cultivar (Yellow Plum).

Tomato Accession/ Cultivar	*Plant response to infection*	*% Lignin*	
		Control	*Infected*
Yellow Plum	wilting		
roots		27.51 ab	24.09 de
upper stems		18.37 ghi	19.63 fgh
lower stems		17.21 ijk	20.35 fg
leaves		ND	ND
Acc. # 70	no wilting		
roots		24.25 d	28.34 a
upper stems		15.34 kl	18.02 hij
lower stems		20.81 f	27.27 abc
leaves		ND	ND

Lignin determined by Klason analysis (dry weight basis) and the values are means of three trials (2 replicates/trial). Treatment means followed by the same letter are not significantly different (5%, DMRT)

Cell walls were isolated from root, upper and lower stem tissues of 6-week old tomato plants sampled/collected at the time the susceptible tomato cultivar Yellow Plum had wilted.

ND - not determined. Aside from wilting as a response to pathogen inoculation of susceptible tomato plants, slime production was also observed. Neither of the two responses was observed in the inoculated resistant plant.

(Accs. # 70 and 75) tomato plants compared to the susceptible Yellow Plum (59.6 μg/g in the root cell walls and 76.8 μg/g in the stem cell walls (Table 3). In response to bacterial wilt infection, there were 4 to 5x more wall-bound proanthocyanidins in the root cell walls and 6 to 7x more in the stem cell walls of resistant plants relative to the susceptibles.

Condensed tannins are polyphenols which are highly reactive substances and when they become condensed, highly polymerized, and esterified with other cell wall components can make the cell wall resistant to enzymatic degradation by the pathogen. Condensed tannins covalently linked to the cell wall can also affect directly the pathogen since it can precipitate or interact with the extracellular polysaccharides (EPS) that envelop the Gram-negative bacterium.

Changes in Wall-Bound Extensin, Lignin, and Peroxidase Activity in the Root Tissue: A Time-course Analysis. The suceptible plants (Yellow Plum) wilted on the 5th day and in response to infection, wall-bound extensin in the roots decreased from the 1st day up to the 3rd day and increased only from the 3rd day up to the 5th day (when all the plants had wilted). The extent of increase was from 45.33 to 69.31 mg/g(Fig. 3).

In the resistant plant (Acc. # 70), the wall-bound extensin increased starting on the first day (75.48 mg/g) up to the 4th day (104.32 mg/g) and then levelled off up to the 7th day (95.37 to 97.12 mg/g). Based on these results, wall-bound extensin was induced as early as the first day when the pathogen was inoculated and accumulated constantly up to the 4th day. The amount of wall-bound extensin that accumulated was also relatively higher (104.32 mg/g in the resistant Acc. # 70 vs 69.31 mg/g in the susceptible Yellow Plum).

There were no observed changes in the lignin content of the root cell walls of the susceptible plant in response to bacterial wilt infection. Lignin increased in the root cell walls of the resistant plant only from the 3rd up to the 4th day and then leveled off up to the 7th day.

Ionically bound peroxidase activity in the root cell walls of the resistant plants was highest on the 2nd and 4th days. From the 4th day, enzyme activity gradually leveled off up to the 7th day. In the root cell walls of the susceptible plant, enzyme activity only started to increase during the 5th day (the day when the plant wilted).

Changes in Wall-Bound Extensin, Lignin, and Peroxidase Activity in Stem Tissues: A Time-course Analysis. In response to infection, wall-bound extensin of the resistant plant increased during the 2nd day (49.44 mg/g) up to the 4th day (69.12 mg/g) and gradually leveled off up to the 7th day (Fig. 4). No significant changes were evident in wall-bound extensin in the stem cell walls of the susceptible plant. Lignin in the stem cell walls of the resistant plant increased from the 2nd day up to the 5th day and then leveled off very gradually up to the 7th day. Lignin seemed to be degraded consistently during infection in the stem cell walls of the susceptible plant.

Table 3. Wall-bound proanthocyanidins in the isolated cell walls of root and stem tissues of resistant (Acc. # 70 and 75) and susceptible (Yellow Plum) tomato plants in response to bacterial wilt infection.

Tomato Plant/ Treatment	*Wall-bound proanthocyanidins (µg catechin equivalent/g)*	
	Control	*Inoculated*
Yellow Plum (control)		
roots	59.6 g	62.4 g
stems	76.8 f	55.3 h
Acc. # 70		
roots	65.1 g	229.5 d
stems	94.7 e	351.8 b
Acc. # 75		
roots	72.4 f	312.6 c
stems	101.9 c	372.2 a

Values for ELISA are means of two trials (3 replicates/trial). Wall-bound proanthocyanidins were estimated colorimetrically using the method of Bate-Smith (1981). Treatment means followed by the same letter are not significantly different (1%, DMRT).

Cell walls were isolated from old root and stem tissues of 6-week old tomato plants sampled/collected at the time the susceptible tomato cv. had wilted.

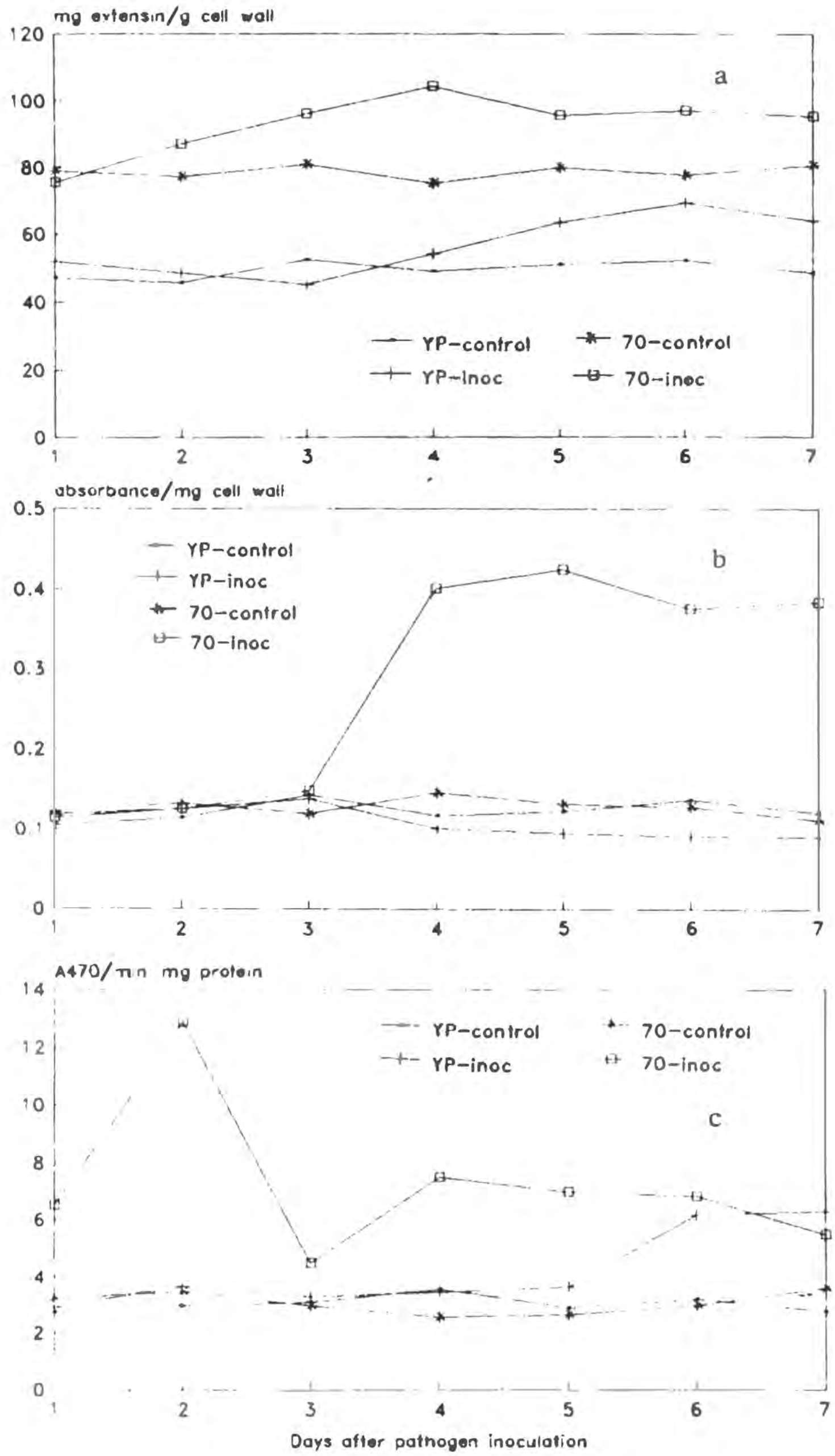

Figure 3. Time-course study of changes in (a) wall-bound extensin, (b) lignin, and (c) ionically bound peroxidase in the root cell walls of resistant (Acc. # 70) and susceptible (Yellow Plum) tomato plants response to pathogen inoculation or infection in root tissues.

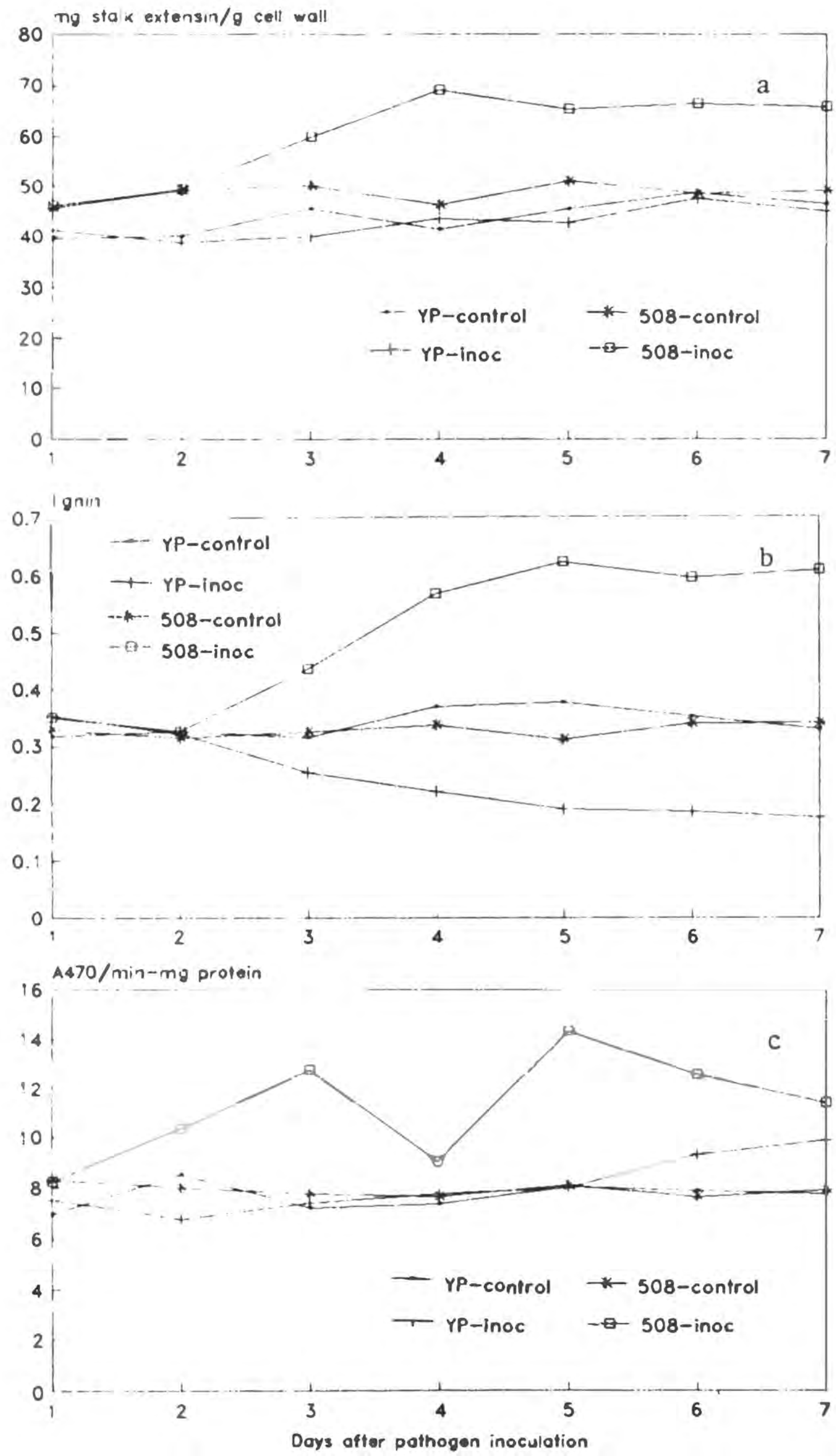

Figure 4. Time-course study of changes in (a) wall-bound extensin, (b) lignin, and (c) ionically bound peroxidase in stem tissues of resistant (Acc. # 508) and susceptible (Yellow Plum) tomato plants.

Peroxidase activity in the stem cell walls of resistant plants was highest on the 3rd day and the 5th day and then levelled off. No changes in peroxidase activity were observed in the stem cell wals of the susceptible plant except maybe on the 5th day where activity was gradually increasing up to the 7th day.

Time-course Analysis: Overall Discussion. The levels of wall-bound extensin, lignin, and peroxidase activity on a per day basis up to the 7th day after pathogen inoculation were determined. The results of the time-course analysis suggest the following: (1) wall-bound extensin can be a pre-infectional and a post-infectional resistance factor, (2) extensin serves as a matrix for deposition of lignin, and (3) different forms of peroxidases (isoenzymes) catalyze the polymerization of extensin and lignin.

That wall-bound extensin acts as a pre-infectional resistance factor is supported by data which showed that significantly higher levels of soluble monomeric and wall-bound extensin are present in the cell walls of resistant plants even before infection. Wall-bound extensin as a post-infectional resistance factor is mainly structural in function meaning extensin is predominantly found only in tissue types where collenchyma, sclerenchyma, and the vascular bundles are located and whose functions are primarily for structural support of the whole plant.

The induced accumulation of wall-bound extension occurs at an earlier period relative to the accumulation of lignin in both the root and stem cell walls of resistant plants in response to infection. This sequence of events suggests that extensin can serve as an initial matrix for lignin deposition at a later period. In the susceptible plants, not enough initial sites (extensin) are available for lignin to be cross-lined so that fewer lignin molecules can be deposited.

Peroxidase activity increases in the resistant plants in response to bacterial wilt infection which coincides with the dual events of induced extensin and lignin accumulation. Peroxidase in the cell walls catalyzes the incorporation of soluble monomeric extensin into the wall by isodityrosine crosslinkages between extensin monomers. The enzyme also catalyzes the free radical polymerization of monolignols and phenolics (such as condensed tannins) into lignin in the cell wall. Data from the time-course analysis suggest that different molecular forms of the peroxidase enzyme catalyze the polymerization of both extensin and lignin in the cell wall.

Cell Wall Modification as an Effective Inducible Host Defense Response of Resistant Tomato Against Bacterial Wilt. The experimental approach that was undertaken in order to prove that changes in cell wall composition (extensin, lignin, and wall-bound proanthocyanidin) can contribute to the overall resistance mechanism of tomato against the bacterial wilt pathogen was to characterize the modified cell walls of root and stem tissues of resistant plants and compare them to the "unmodified" cell walls of roots and stems of susceptible plants. Characterization of the modified cell walls was done by: (1) studying the extractability of wall-bound extensin and lignin before and after infection, (2) measuring some physical param-

eters of cell wall strength such as tensile strength of tomato stems and estimating the degree of tissue maceration, and (3) determining the resistance of the modified cell walls to enzymatic hydrolysis by enzymes produced by the pathogen during disease development.

Comparative Resistance of Wall-Bound Extensin in Root Cell Wall to Chemical Extraction. The extent or ease of extraction of wall-bound extensin could be a direct measure of the degree of isodityrosine cross linkages between extensin molecules in the cell wall. Without any cell wall pretreatment (control) of the susceptible tomato cultivars (Yellow Plum and Bacolor) and the resistant accessions (Acc. # 70, 75, and 508), there were no significant changes in the levels of wall-bound extensin as extracted chemically using acidified chlorite. These results negate the assumption that it is easier to extract wall-bound extensin because of its lower degree of isodityrosine crosslinkages.

Pretreating the isolated cell wall by the partial removal of pectins (depectination) and partial hydrolysis of the cellulose microfibrils increased the extractability of wall-bound extensin from both the susceptible and the resistant tomato cultivars and accessions (Fig. 5). However, the extent of extraction is significantly greater in the resistant tomato plant than in the susceptible.

Depectination increased extractability of wall-bound extensin in the root cell walls of the susceptible plants by 18% in Yellow Plum and by 28% in Bacolor. In the root cell walls of the resistant plants, extractability increased by 55% in Acc. # 508, by 47% in Acc. # 70, and by 52% in Acc. # 75. On the other hand, partial digestion of cellulose microfibrils further increased the extent of extractability even more than the depectination treatments. Cellulase digestion increased wall-bound extensin extractability by 70% in Acc. # 508, # 70, and # 75. The extent of extractability in the susceptible Yellow Plum and Bacolor was only 40%.

Thus, the results obtained from pretreatment experiments suggest that wall-bound extensin can form multi-crosslinkages with other cell wall components such as pectins and cellulose which can affect its extractability.

Comparative Resistance of Lignin in Stem Cell Walls to Chemical Extraction. The effects of pretreatment on the extractability of lignin were determined by hot alkaline hydrolysis of plant cell walls and analyses of the hydrolyzates spectrophotometrically. In the susceptible Yellow Plum, depectination increased lignin extractability only by 2% while cellulase digestion increased digestibility by 11% (Fig. 6). In the resistant accessions (# 70 and # 508), depectination increased lignin extratability by 6 to 8%. The effect of cellulase digestion was more prominent at 35 to 38% increase in lignin extractability.

The extent of lignin extractability was relatively higher when the cell walls were treated with the cellulase enzyme. These same trends were observed on the extractability of wall-bound extensin. These results suggest that extensin and lignin are tightly interlinked with the cellulose microfibrils more than with pectins such

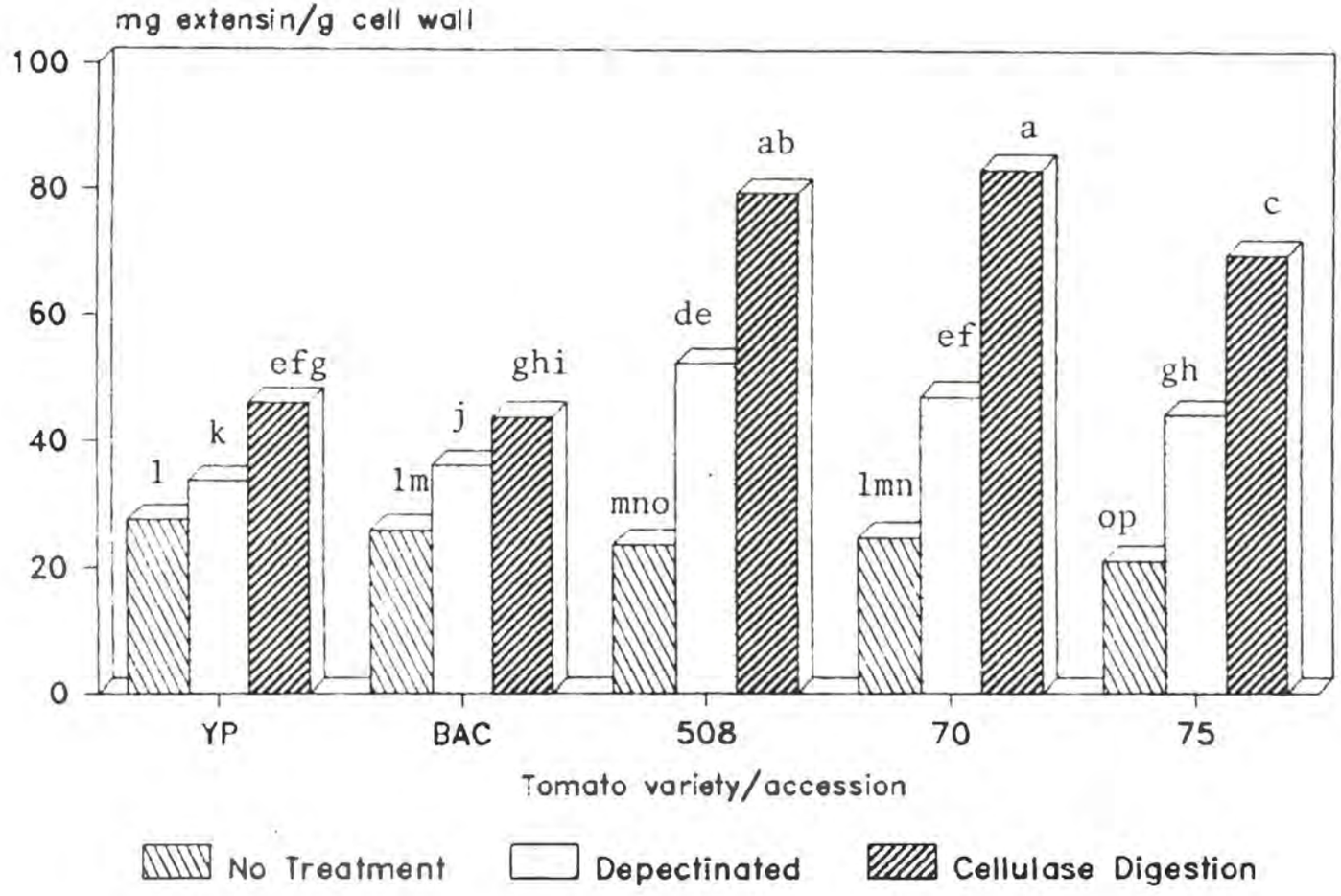

Figure 5. Effects of pre-treatments on the chemical extractability of wall-bound extensin in the root cell walls of five tomato cultivars and accessions. YP-Yellow Plum; BAC-cv. Bacolor; 70, 75, and 508 are resistant accessions. Vertical bars followed by the same letter are not significantly different (1%, DMRT).

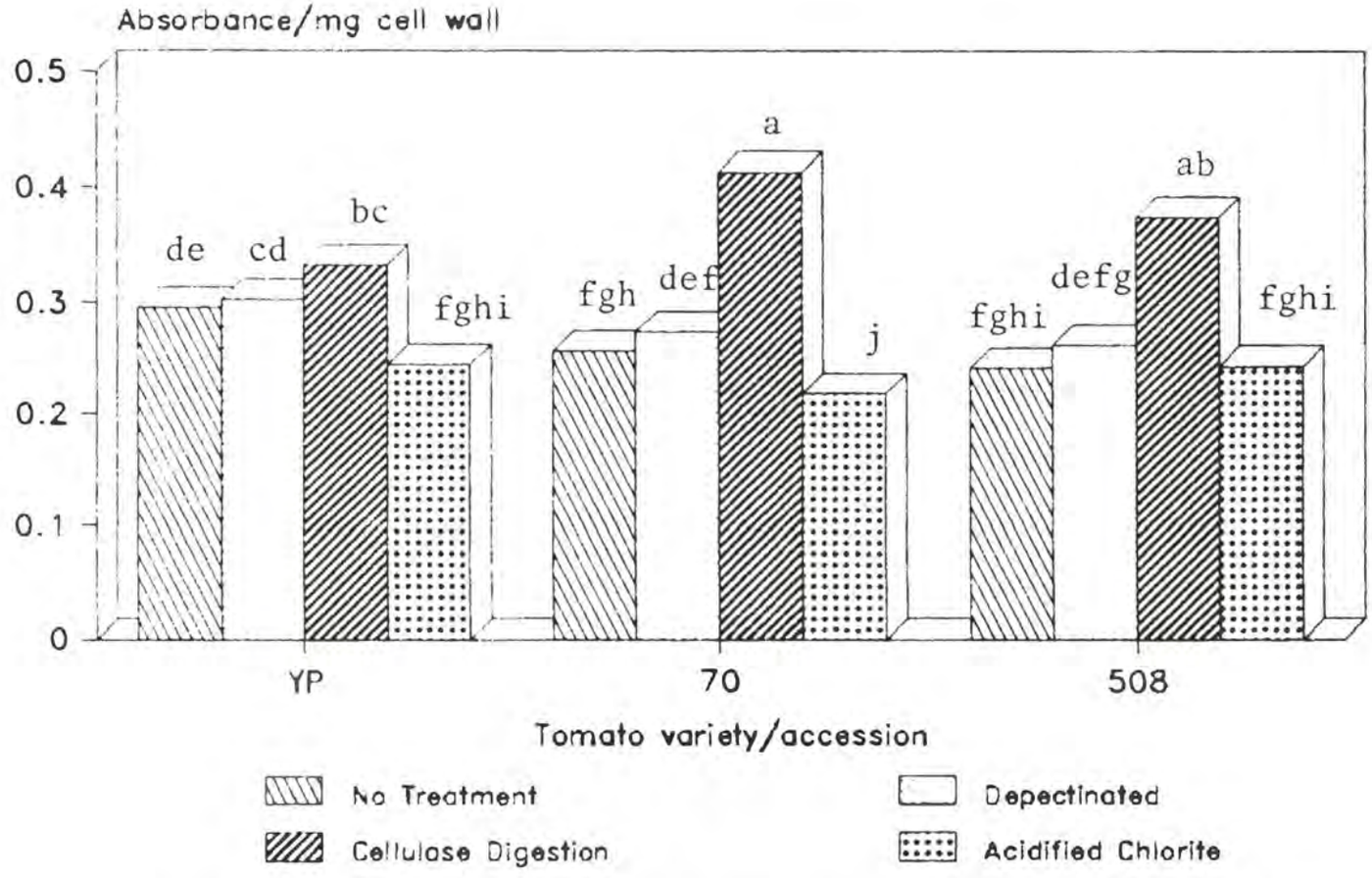

Figure 6. Effect of pre-treatments on the chemical extractability of lignin in stem cell walls of three tomato cultivar and accessions. YP-Yellow Plum; 70 and 508 are resistant accessions. Vertical bars followed by the same letter are not significantly different (1%, DMRT).

that partial digestion of the cellulose microfibrils led to the increased extractability of both macromolecules from the plant cell wall.

Comparative Resistance of Wall-Bound Extensin in Modified Root Cell Walls to Chemical Extraction. Without cell wall pretreatments, extractability of wallbound extensin in the root cell walls of Acc. # 70 before and after infection decreased by 2x from 5 to 15 min extraction time (Fig. 7a). This is strong indication that extensin became more tightly bound with the other cell wall macromolecules such as cellulose and pectins during cell wall modification in response to infection.

Comparative Resistance of Lignin in Modified Stem Cell Walls to Chemical Extraction. Without cell wall pretreatments, extractability of lignin in stem cell walls of the resistant Acc. # 70 before and after infection was lower by 2x (Fig. 7b) which strongly suggests that in the altered or modified cell walls, lignin became more tightly bound with other cell wall macromolecules such as pectins and cellulose.

Tensile Strength of Stem Tissues. In response to bacterial wilt infection, the tensile strength of susceptible tomato stems decreased from 7.6 to 10.0 kg-force to 1.0 to 2.5 kg-force (Table 4). Initially, both the resistant and susceptible tomato stems had similar tensile strength from 7.6 to 10.0 kg-force. The tensile strength of the resistant tomato stems only decreased to a range from 5.1 to 7.5 kg-force. The tensile strength of two other tomato plants, one resistant (Acc. # 508) and one susceptible (Bacolor), was also determined and similar results were obtained.

Test for Cell Coherence. One physiological sign of the bacterial wilt syndrome is maceration of cells in infection tissues. This is caused by the enzymatic digestion (by pectic enzymes) of the middle lamella of plant cell walls.

The extent of tissue degradation in the susceptible Yellow Plum increased from the first day up to the 6th day (Table 5). There was no tissue degradation in the controls of both the resistant and the susceptible. There was also little or no tissue degradation in the inoculated resistant plants.

Comparative Resistance to Enzymatic Digestion of Cell Walls of Resistant and Susceptible Tomato. Cell walls isolated from the roots, lower and upper stems, and the leaves were subjected to in vitro polygalacturonase digestion for 8 h and 16 h and the free galacturonic acid released was analyzed colorimetrically. No significant difference in the levels of free galacturonic acid released during the first 8 h of digestion in the roots, upper and lower stems of both the resistant and the susceptible plants (Fig. 8). was observed. The extent of pectin degradation was highest in the leaf cell walls of both the resistant and the susceptible. However, after 16 h of digestion, pectin degradation in all the cell wall types of the susceptible was considerably greater compared to the resistant.

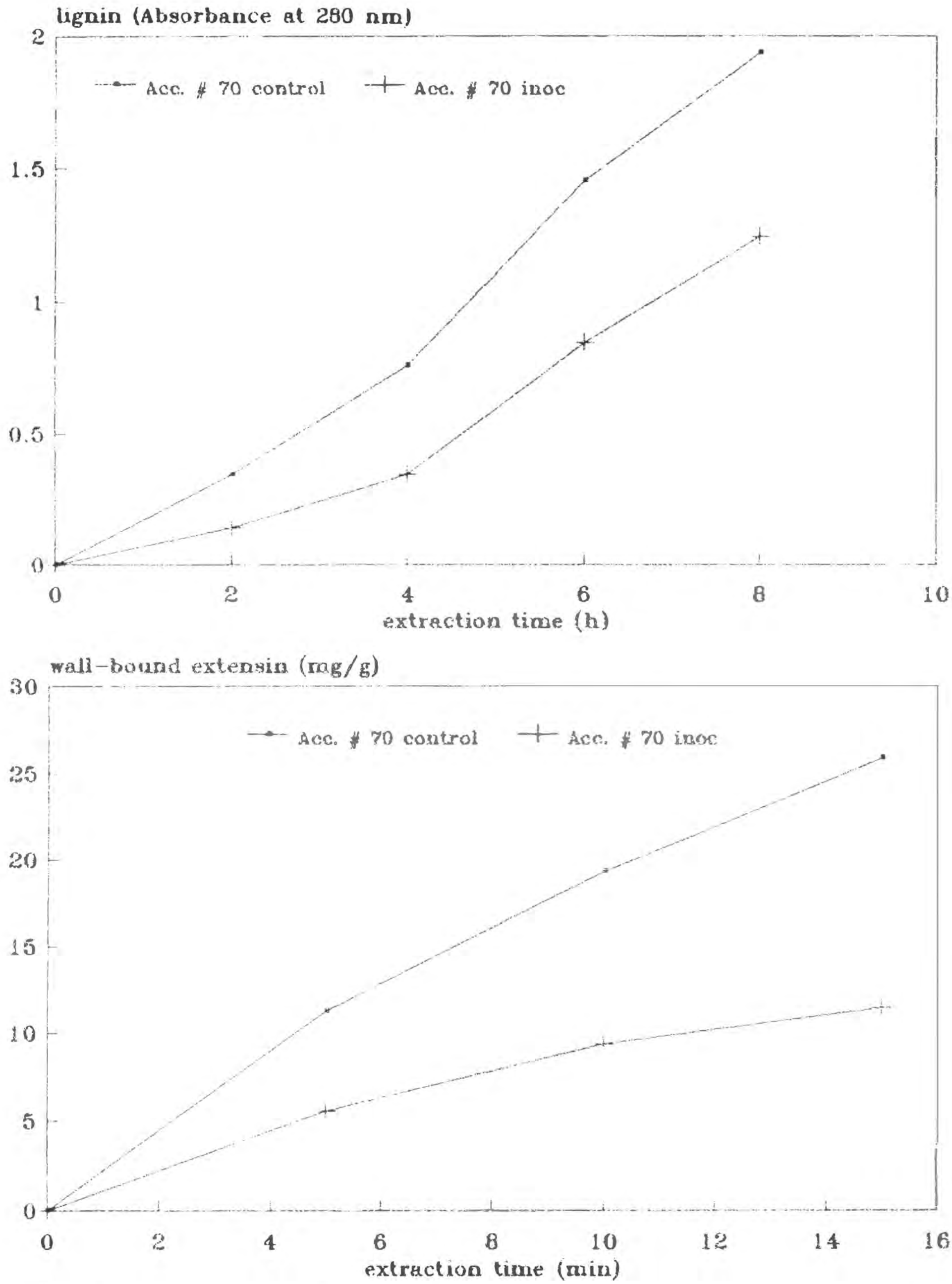

Figure 7. Effect of induced cell wall modification on the chemical extractability of (a) lignin and (b) wall-bound extensin in the stem and root cell walls of Acc. # 70, respectively.

Table 4. Effect of pathogen inoculation on tensile strength of stems of resistant (Acc. # 70) and susceptible (Yellow Plum) tomato plants.

Tensile strength (kg-force)	*Number of tomato plant stems*			
	Yellow Plum		*Acc. # 70*	
	Control	*Inoculated*	*Control*	*Inoculated*
1.0 - 2.5	–	19	–	–
2.6 - 5.0	3	10	3	4
5.1 - 7.5	12	8	10	21
7.6 - 10.0	15	3	17	7
10.1 - 12.5	6	–	10	6
12.6 - 15.0	–	–	5	4
15.6 - 20.0	–	–	–	–
Total no. of tomato stems	36	40	44	42
Average tensile strength (kg-force)	7.9	3.7	9.1	7.8

Table 5. Time-course study on cell coherence in infected stem tissues of resistant (Acc. # 508) and susceptible (Yellow Plum) tomato plants.

Days after inoculation	Maceration index			
	Yellow Plum		Acc. # 508	
	Control	Inoculated	Control	Inoculated
0	0	0	0	0
1	0	0	0	0
2	0	1	0	0
3	0	2	0	1
4	0	2	0	0
5	0	3	0	0
6	0	4	0	1
7	0	4	0	0

Values are means of 15 stem tissue discs. Stem tissue discs were tested by the ease with which the discs were pulled apart with two dissecting needles after incubation for 48 h in culture filtrates of the virulent bacteria (isolate # 151/ biovar III).

A maceration index from 0 to 5 was used for estimating cell coherence: a rating of 0 indicated strong cohesion similar to controls or freshly cut tissues and a rating of 5 meant complete tissue dissociation.

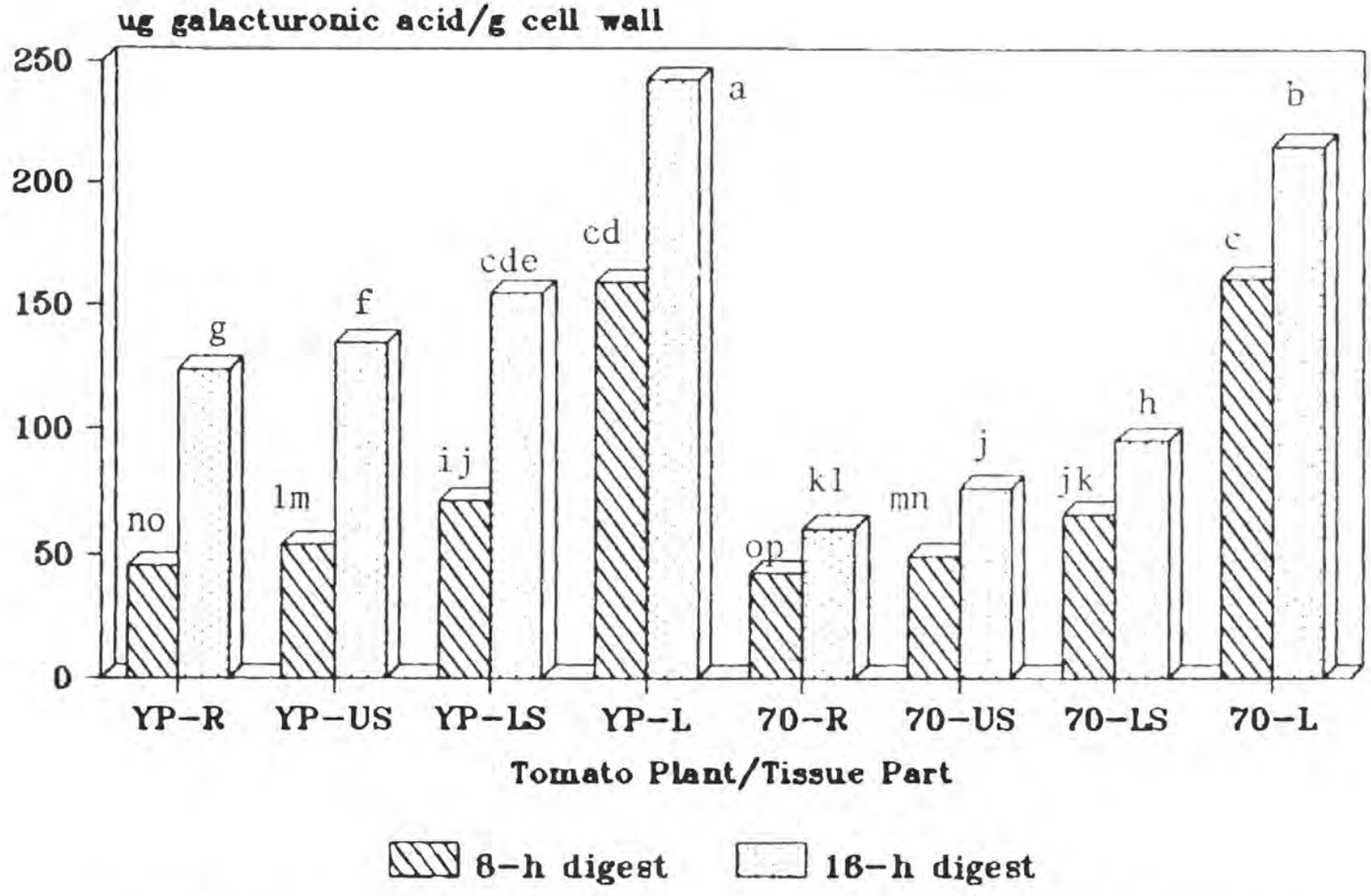

Figure 8. Polygalacturonase digestion of cell walls of root, stem, and leaf tissues of resistant (Acc. # 70) and susceptible (Yellow Plum) tomato plants. Vertical bars followed by the same letter are not significantly different (1%, DMRT).

There was no significant difference in the levels of glucose released upon cellulose digestion for 4 h in all tbe cell wall types (roots, lower and upper stems, and the leaves) of both the resistant (Acc. # 70) and the susceptible (Yellow Plum) tomato plants (Fig. 9). However, the extent of cellulose digestion was relatively higher in the susceptible than in the resistant after 8 h of enzymatic digestion.

The root and stem cell walls of the susceptible (Yellow Plum) and the resistant (Acc. # 70) were also subjected to enzymatic digestion with filtrates prepared from bacterial cultures. The filtrates were analyzed and found positive for some cell-wall degrading enzymes (Laurena, 1993) such as endo-polygalacturonase, pectate lyase, and cellulase. Protease activity was also observed in the culture filtrates. In the unmodified cell walls of uninfected root and stem tissues (controls), the extent of enzymatic degradation was greater in the susceptible (1.4x higher in the roots and 1.2x higher in the stems) compared to the resistant (Fig. 10). In the infected root and stem tissues of susceptible plants, the extent of cell wall degradation was even higher (33% increase in the root and 24% increased in the stems: compared to controls. These data suggest that root and stem cell walls have already undergone cell wall degradation during the process of natural infection such that additional exposure of these defective cell walls to the culture filtrates containing the hydrolytic enzymes further increases wall degradation.

The results obtained on enzymatic digestion indicate that the root and stem cell walls of the resistant tomato plants are more difficult to hydrolyze even before infection. In the altered/modified cell walls (infection-induced) of the root and stem tissues of the resistant plants, the extent of enzyme degradation was even lower. Therefore, cell wall modification in resistant plants as induced by bacterial wilt infection increases the resistance of plant cell walls to degradation by enzymes produced by the pathogen.

SUMMARY AND CONCLUSIONS

The biochemical nature, number, and timing of changes in cell wall components of root and stem tissues of tomato plants such as extensin, lignin, and wall-bound proanthcyanidins in response to pathogen inoculation were established in the absence of disease expression – absence of wilting and survival of inoculated resistant plants. In infected susceptible plants, there is disease expression (wilting and death of infected plants) and there is no induced accumulation of these cell wall components. Stem tissues of infected susceptible plants are macerated by cell wall degrading enzymes produced by the pathogen and whole plants wilt and finally die.

Studies on the effect of cell wall pretreatments, chemical e
enzymatic digestion experiments provided strong evidences that
walls are indeed resistant to the hydrolytic enzymes of the pat
wall modification plays a major important role as an induced host

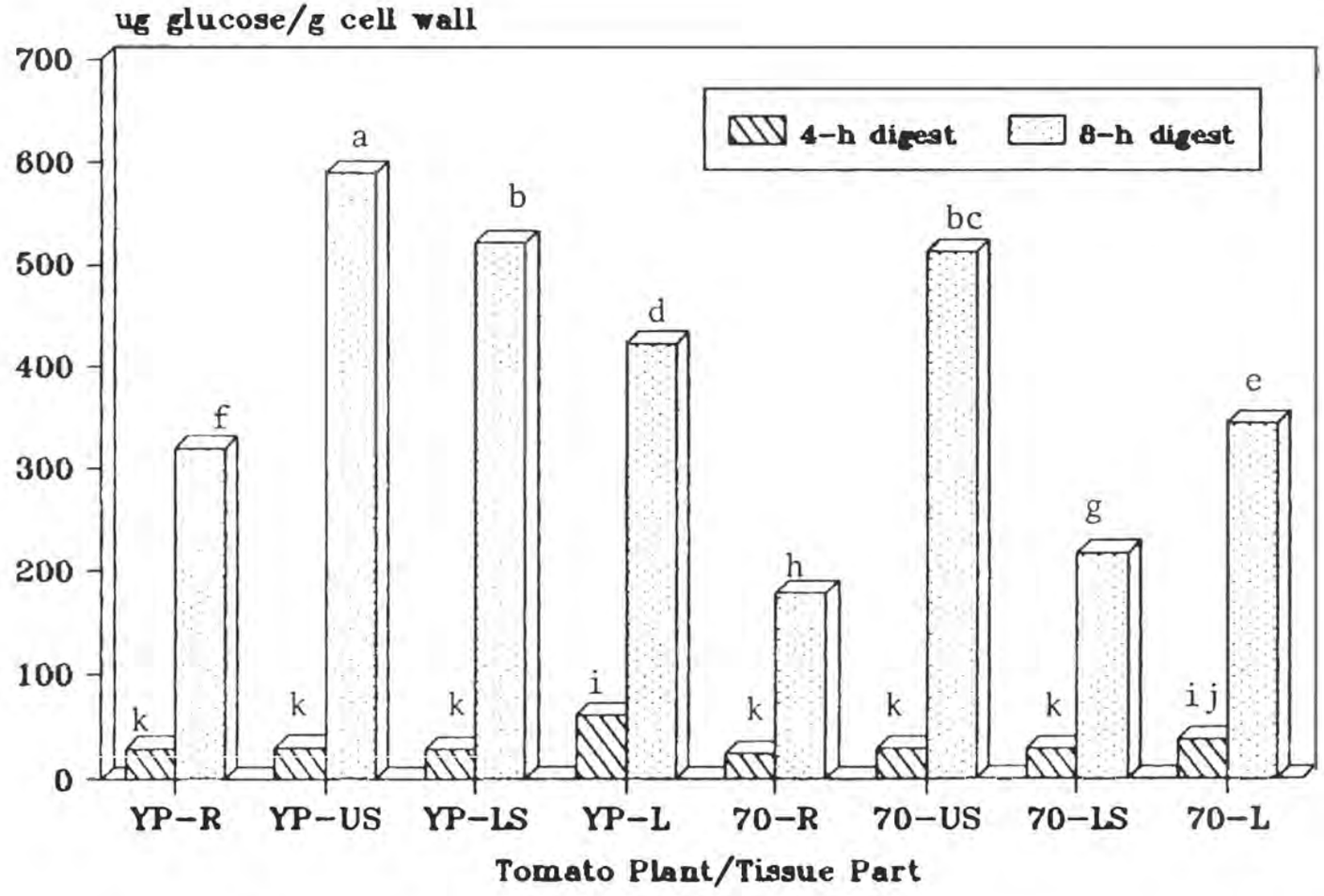

Figure 9. Cellulase digestion of cell walls of root, stem, and leaf tissues of resistant (Acc. # 70) and susceptible (Yellow Plum) tomato plants. Vertical bars followed by the same letter are not significantly different (1%, DMRT).

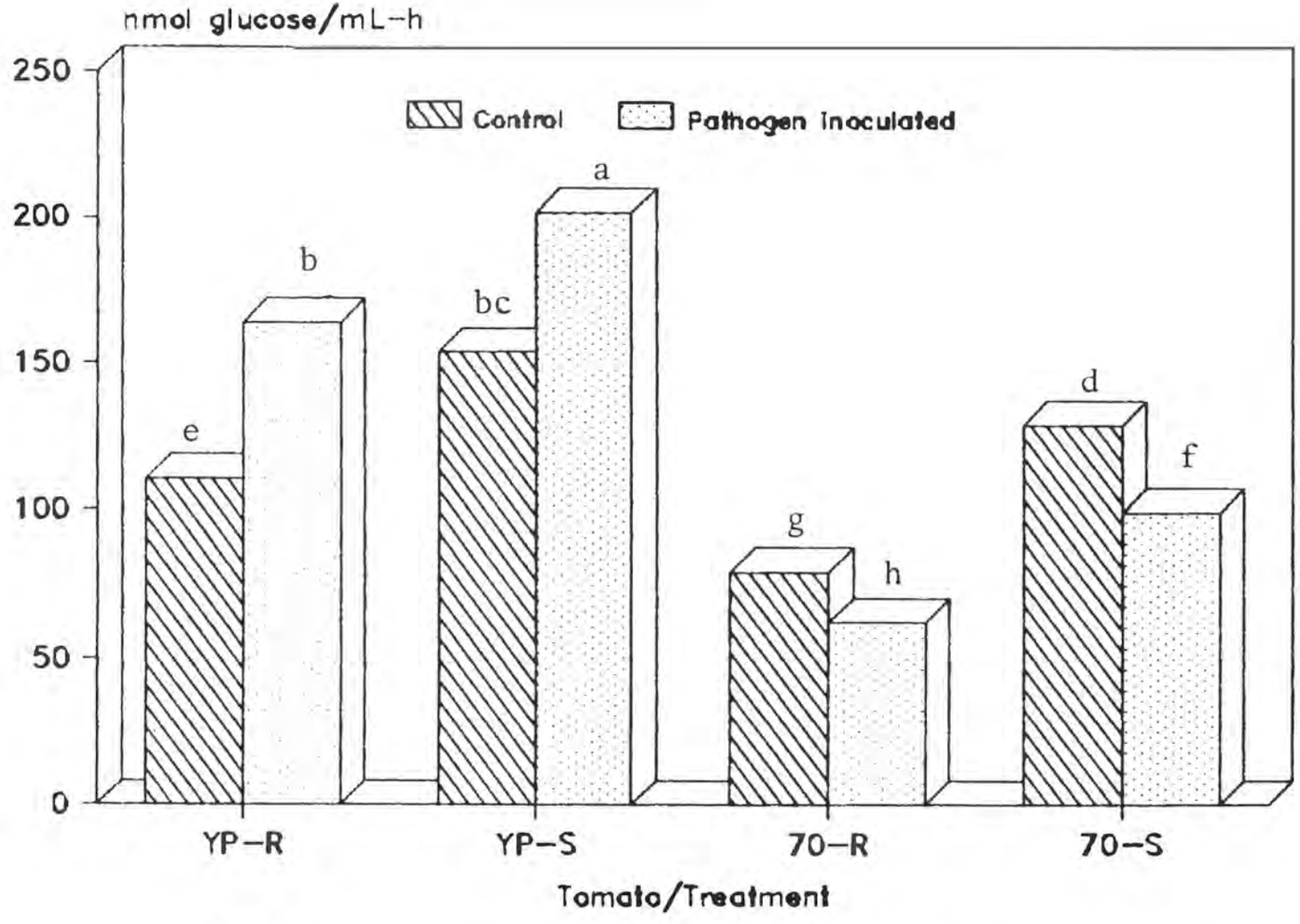

Figure 10. Digestion by cell wall-degrading enzymes in culture filtrates of cell walls of root and stem tissues of resistant (Acc. # 70) and susceptible (Yellow Plum) tomato plants. Vertical bars followed by the same letter are not significantly different (1%, DMRT)

of resistant tomato against the pathogen by neutralizing the destructive effects of the cell wall degrading enzymes produced by the pathogen. However, other mechanisms can not be discounted and might be functioning in coordination with cell wall modification. The pathogen is a very complicated microorganism and the expression of high/stable disease resistance will probably require an array of defense mechanisms.

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