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BIOLOGICAL CONTROL OF GOOSEWEED (*SPHENOCLEA ZEYLANICA* GAERTN.) WITH AN *ALTERNARIA* SP.

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ABSTRACT

Greenhouse and field experiments at the International Rice Research Institute (IIRI) evaluated the potential of a leaf blight pathogen (*Alternaria* sp.) for the control of gooseweed, *Sphenoclea zeylanica* Gaertn. In greenhouse experiments, *S. zeylanica* plants at all growth stages, from seedlings to flowering, were killed by the pathogen when applied as conidial suspensions of 6.3 x 10^3 to 1.4 x 10^6 conidia ml^{-1} over a range of dew period durations. Field trials confirmed the effectiveness of the *Alternaria* sp. to control *S. zeylanica* under varying environmental conditions. Concentrations of 10^5-10^6 conidia ml^{-1} applied at 50 ml 0.25 m^{-2} gave good control of gooseweed, reducing weed density by 80-99% and weed biomass by over 90% in all trials. The *Alternaria* sp. did not affect rice or other non-target species present in the field plots.

Keywords: *Alternaria;* biocontrol; mycoherbicide; rice; *Sphenoclea zeylanica;* weed control

INTRODUCTION

Sphenoclea zeylanica Gaertn. (Gooseweed) is a common, annual herbaceous weed of wetland rice (*Oryza sativa* L.) in Southeast Asia, the United States, the Caribbean area, India, Pakistan and West Africa (Holm *et al.*, 1977). Holm *et al.* (1977) found that *S. zeylanica* was never reported as a weed in any crop other than rice, but Sanders (1990) described it as one of the most common weeds in cotton (*Gossypium hirsutum* L.) in Louisiana. At densities of 20 plants m^{-2}, *S. zeylanica* causes significant yield reduction in transplanted rice (IRRI, 1989). *Sphenoclea zeylanica* competes with rice due to efficient nitrogen uptake (Biswas and Sattar, 1991) and it can also interfere with harvesting (Migo, Mercado and De Datta, 1986).

Several herbicides, such as 2,4-D (2,4-dichlorophenoxy acetic acid), provide good control of *S. zeylanica* (Migo *et al.*, 1986), but there are several problems associated with herbicide use. Application safety, environmental pollution, weed population shifts to more noxious weeds and the development of herbicide resistant forms are some of the concerns associated with the widespread use and misuse of herbicides (Watson, 1992b). The development of tolerant forms of *S. zeylanica,* due to the continuous postmergent application of 2,4-D, has been reported (Mercado *et al.*, 1990; Migo *et al.*, 1986; Sy and Mercado, 1983).

Biological control can offer viable, economic and effective alternatives to chemical herbicide for control of major weeds in rice and other crops in the tropics (Watson, 1992b). Biological control is the deliberate use of natural enemies to control a weed population and plant pathogens have been effectively deployed in the classical (inoculative) approach and in the mycoherbicide (inundative) approach in various parts of the world (Watson, 1992a). The mycoherbicide approach involves the augmentation of indigenous fungal weed pathogens to control or suppress the growth of a problem weed. It attempts to overcome disease constraints (such as low inoculum levels and poor inoculum dispersal) by supplying abundant, virulent inoculum at a time most conducive to disease development. The indigenous fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. f. sp. *aeschynomene* is being used effectively in the Mississipi Delta area of the Untied States on a commercial basis to control northern jointvetch [*Aeschynomene virginica* (L.) B.S.P.] in rice and soybeans [*Glycine max* (L.) Merr.] (Charudattan, 1991).

Few pathogens have been reported to occur on *Sphenoclea zeylanica*. *Cercosporidium helleri* Earle, a leaf mold, was recorded on *S. zeylanica* in India (Ponnappa, 1967) and this pathogen and a leaf blight are commonly observed on *S. zeylanica* in rice fields in the Philippines (Bayot, Watson and Moody, 1992). Bayot *et al.* (1992) suggested that the leaf blight pathogen (*Alternaria* sp.) had potential as biocontrol agent for *S. zeylanica.* Various *Alternaria* spp. have been examined as biocontrol agents of some weed species (Park *et al.*, 1992; Walker, 1981; Walker and Boyette, 1985; Walker and Riley, 1982; Yang, Johnson and Dowler, 1990). The objective of this study was to evaluate the potential of *Alternaria* sp. for control of *S. zeylanica* in rice.

Materials and Methods

Inoculum Production

Stock cultures of *Alternaria* were maintained on 10 ml half-strength potato dextrose agar (1/2 PDA) slants at 4°C. Cultures for inoculum production were started from stock cultures. The pathogen was grown either on 1/2 PDA or on sorghum [*Sorghum bicolor* (L.) Moench] seeds. For growth on 1/2 PDA, two agar slants with the fungus were macerated in 10 ml sterilized, distilled water and added to 250-300 ml acidified cooled 1/2 PDA, the mixture was poured in 90 mm diameter Petri dishes and the dishes sealed with parafilm. For growth on sorghum, 20 g boiled and twice autoclaved sorghum seeds in 250 ml Erlenmeyer flasks were seeded with three mycelia disks (5 mm diameter) from the leading edge of the pathogen growing on 1/2 PDA for one week. Plate cultures and seeded flasks were incubated on the laboratory bench with continuous fluorescent light or in a dark incubator at 30°C for 7-21 days. This *Alternaria* isolate will sporulate under both light and dark conditions. Conidia were collected by flooding plates with distilled water and scraping the surface of the colonies with a glass slide. Conidia from the flask cultures were harvested by adding 50 ml distilled water to each flask and stirring the contents with a spatula. The resulting suspensions were filtered through two layers of nylon cloth and conidial density was determined with a haemacytometer. One drop of Tween 20 per 50 ml was added to the final conidial suspension.

Greenhouse Tests

Six greenhouse trials conducted from October 1992 to February 1993 with conidia concentrations of 10^3-10^6 ml^{-1}, dew period durations of 0-24 h and initial plant heights of 5-25 cm. The experiments were arranged in a completely randomized design with four replications/treatment. *Sphenoclea zeylanica* plants were either grown from seeds or seedlings, collected from paddy fields, were transplanted into saturated soil (Maahas clay, Haplustic suborder) in 10 cm diameter pots and maintained in the greenhouse. Plants were inoculated with the pathogen using a hand sprayer with a volume of 20-25 ml per four pots. Control plants were sprayed with distilled water only. After inoculation, plants were placed in dark dew chambers for 0-24 hours at 25°C and then transferred to the mist room (Yeh and Bonman, 1986) for disease development. In the 0 hour dew period treatment, inoculated plants were placed directly in a corner of the mist room and one set was covered with cardboard for six hours to simulate a dark period. Symptom development on treated plants was observed over a two-week period.

Field Tests

Four field trials were conducted at the IRRI experimental farm from September to November 1992 under varying rainfall and temperature conditions. Rainfall and temperature data were provided by the Climate Unit of Agronomy, Plant Physiology and Agroecology Division, IRRI. Field research plots with naturally occurring high populations of *S. zeylanica* (173-380 plants m^{-2}) were selected as test sites. The experimental areas were puddled, levelled and weeds were allowed to grow. The experimental areas were occasionally flooded because of heavy rain. The treatments were arranged in a randomized complete block design with three or four replications. Plot size was 0.5 m x 0.5 m. The soil was saturated at inoculation. Plants were inoculated using a hand sprayer with a volume of 50 ml per plot except in experiments where spray volume was a treatment. Experiments were repeated in time; there were two experiments (1 and 2) on the effect of conidia concentration and two experiments (3 and 4) on the effect of spray volume. Treatments were applied between 4 and 6 pm; plants were 2-10, 9-20, 11-30 and 16-38 cm in height; relative humidities between 5 pm and 12 midnight were 70-90, 75-90, 80-88 and 72-88%; and the first rain occurred 6-7 hours, 2 days, 1 day and 4 days after inoculation for the first, second, third and fourth experiments, respectively. The number of living plants and the dry weight of above-ground biomass were determined two weeks after inoculation. Plants were cut at the soil level, dead tissues were discarded and living tissues dried in paper bags at 80°C for 3-4 days. Data were analyzed by the analysis of variance and treatment means were separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Greenhouse Tests

At high inoculum concentration (10^5 conidia ml^{-1} and higher), symptom development on inoculated plants was evident within 24 hours as wilting and leaf cupping. The fungus causes necrosis of the host tissue, initially killing the leaves, then the stems and eventually the entire plant. With lower inoculum concentrations (10^4 conidia ml^{-1} and less) leaf lesions were initially pinhead in size which later expanded and coalesced forming irregular shaped necrotic lesions. Lesions also developed on the stems. Infected leaves were deformed, abscised and eventually the entire plant died. Three to five days after inoculation, most plants were dead and within two weeks all treated plants were dead (Table 1). With concentrations of 10^4 conidia ml^{-1} and higher, 100% kill was achieved within one week. At 10^3 conidia ml^{-1}, two weeks were required for 100% mortality.

Dew periods were not necessary to obtain 100% mortality when plants were subsequently maintained in a mist room in the greenhouse. In the treatment where

no additional free-moisture was provided (0-h-dew duration), all treated plants were killed. These results are quite different from those reported for other *Alternaria* spp. being examined as biocontrol agents of various weeds. Walker and Riley (1982) report at least eight hours of free moisture are required for *Alternaria cassiae* Jurair & Khan to cause severe disease on *Cassia obtusifolia* L., *Alternaria macrospora* Zimm. requires a 24-hour-dew period to incite over 80% mortality of *Anoda cristata* (L.) Schlecht. (Walker, 1981). Park *et al.* (1992) report a requirement of 24-hour-dew period for the *Alternaria* sp. on *Scripus planiculmis* Fr. Schm., and *Alternaria angustiovoidea* Simmons requires at least a 48-hour-dew period to control *Euphorbia esula* L. (Yang *et al.*, 1990).

Under controlled conditions, the *Alternaria* sp. was virulent on development stages of *S. zeylanica* from seedlings to flowering plants. With the other *Alternaria* spp. being evaluated as biological weed control agents, high levels of mortality are obtained only on seedlings to one-leaf stage plants (Walker, 1981; Walker and Riley, 1982). The rapid appearance of symptoms suggests that a phytotoxin(s) may be involved as reported for other *Alternaria* spp. (Stierle, Cardellina and Strobel, 1988; Walker and Riley, 1982; Yang *et al.*, 1990).

Field Tests

As in the greenhouse experiments, symptom development in the field occurred within 24 hours after inoculation. In experiment 1, aside from the usual leaf cupping and wilting observed the day after inoculation, lesion development and necrosis were also evident. The accelerated appearance of symptoms may have resulted from elevated moisture levels due to heavy rain that commenced 6-7 hours after inoculation (Figure 1). Conidia of this pathogen germinate within six hours and penetrate leaf tissues 12-16 hours after inoculation (Bayot *et al.*, 1992). It appears that conidia and germlings of this *Alternaria* sp. are not washed off by heavy rain (i.e., they are 'rainfast'). One week after treatment, most of the *S. zeylanica* plants were either dead or leafless. Two weeks after treatment, excellent control of *S. zeylanica* was achieved in plots treated with the *Alternaria* sp. based on significantly ($p < 0.05$) lower dry weight and number of living plants compared to plants in untreated plots (Table 2).

In experiment 2, 90% kill of plants occurred one week after inoculation in plots treated with 10^5 conidia ml^{-1}. There was no mortality after one week at lower concentrations, but lesions appeared on leaves causing deformation and stunting of *S. zeylanica*. The number of living plants was significantly ($p < 0.05$) reduced when 10^5 spores ml^{-1} were used, but not with lower concentrations (Table 2). Two weeks after inoculation, weed biomass was significantly ($p < 0.05$) reduced in plots receiving 10^4 and 10^5 conida ml^{-1}. Dry weight of *S. zeylanica* decreased with increasing concentration of the *Alternaria* sp. with the lowest dry weight obtained using 10^5 conidia ml^{-1}. Dry weight of plants treated with 10^3 conidia $ml^{-1}$was not significantly ($p < 0.05$) different from control plants. The effect of inoculum concen-

tration with this *Alternaria* sp. was similar to that reported for other *Alternaria* spp. (Walker, 1981; Walker and Boyette, 1985; Walker and Riley, 1982; Yang *et al.*, 1990), but not only seedlings were susceptible as all *S. zeylanica* growth stages including flowering plants were controlled in the field.

In experiment 3, application of 50 ml of the conidial suspension 0.25 m^{-2} gave superior control of *S. zeylanica* (Table 3). However, this was not significantly different from using 25 ml 0.25 m^{-1}. Good control of *S. zeylanica* was also achieved when 12.5 ml was applied and when 50 ml of a three-week-old suspension was used. Thus, conidia stored in water might still be usable for application depending on length and condition of storage.

In experiment 4, *S. zeylanica* plants inoculated with 4.8 x 10^5 conidia ml^{-1} had significantly ($p < 0.05$) lower dry weight and significantly ($p < 0.05$) lower dry weight and significantly ($p < 0.05$) fewer living plants compared to plants in the control plots after two weeks (Table 3). Dry weight, however, was not significantly ($p < 0.05$) affected by spray volumes used but a significant ($p < 0.05$) reduction in numbers of living plants occurred when 50 ml was used compared to 12.5 ml 0.25 m^{-2}. Higher volumes of spray solution may contribute to an extended free moisture period on the plant surface and subsequent increased disease expression.

Echinochloa spp., *Fimbristylis miliacea* (L.) Vahl, *Cyperus difformis* L., *Cyperus iria* L., *Eclipta prostrata* L., *Leptochloa chinensis* (L.) Nees *Ludwigia octovalvis* (Jacq.) Raven, *Monochoria vaginalis* (Burm. f.) Presl, *Ammannia* sp., *Sesbania* sp. and volunteer rice were not affected by the *Alternaria* sp. in these field trials.

These results demonstrate the potential of this pathogen as a biological control agent for *S. zeylanica*. The pathogen gave excellent control of the weed during field trials under varying environmental conditions. Further studies are underway to identify the pathogen to species, determine its host range, determine the possible involvement of phytotoxins, evaluate aspects of virulence and efficacy and optimize inoculum production and formulation. Additional testing of the pathogen in field trials is in progress.

This *Alternaria* sp. has major advantages over other *Alternaria* spp. being evaluated as potential bioherbicides. It does not require extended periods of leaf wetness and it is effective on all stages of plant growth, from seedlings to flowering plants. *Alternaria* spp. do not sporulate in liquid fermentation and must be mass produced using solid fermentation techniques. The inability to economically adapt solid fermentation on an industrial scale is a major reason why the commercialization of *Alternaria* cassiae (CASST ^{R}) has not proceeded. The relatively small market niche for *S. zeylanica* will not likely warrant development of this *Alternaria* sp. as a "commercial product." Therefore, studies are underway to develop methodologies for "onfarm" or "cottage industry" production of inoculum to satisfy local needs and to be cost effective on a small scale.

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Table 1. Effects of dew period duration, conidia concentration and plant height at inoculation on the efficacy of an *Alternaria* sp. to control *Sphenoclea zeylanica* 2 weeks after inoculation

Dew Period duration[a] (h)	Inoculum concentration range (conidia ml^{-1})	Height[b] (cm)	Mortality[c] (%)
24	6.3×10^3-1.4×10^6	5-25	100
7	6.3×10^5	5-25	100
6	2.0×10^4-1.4×10^6	5-25	100
3	4.8×10^4	20-25	100
0*	4.8×10^4	20-25	100
0**	4.8×10^4	20-25	100

[a]The 0-h-dew period duration consisted of two treatments; two sets of inoculated plants (0* and 0**) were placed directly in the mist room wherein one set (0*) was covered with a cardboard carton for 6 h.

[b]The 20-25 cm height represents the pre-flowering to flowering stage of growth.

[c]Determined 2 weeks after inoculation. At concentrations greater than 1×10^4 plants died within 3-7 days afer inoculation.

Table 2. Effect of conidia concentration of an *Alternaria* sp. on dry weight and number of *Sphenoclea zeylanica* 2 weeks after application[a]

Concentration[b] (conidia ml^{-1})	Dry weight (g 0.25 m^{-2})	Living plants (No. 0.25 m^{-2})
September 1992 (Exp. 1)		
Control	11.2b	201.8b
7.0×10^5	0.2a	16.3a
1.8×10^6	0.04a	5.3a
CV(%)	53.2	33.2
October 1992 (Exp. 2)		
Control	17.1c	81.5b
8.0×10^3	13.7c	93.0b
3.0×10^4	8.0b	79.5b
2.6×10^5	0.2a	4.3a
CV(%)	29.3	25.4

[a]Values are means of four replications. In a column, means followed by a common letter within a date are not significantly different at 5% level according to Duncan's multiple range test.

[b]Volume of spray = 50 ml 0.25 m^{-2}

Table 3. Effect of spray volume on dry weight and number of *Sphenoclea zeylanica* 2 weeks after application of a conidial suspension of an Alternaria sp[a]

Volume of spray (ml 0.25 m^{-2})	Dry weight (g 0.25 m^{-2})	Living plants (No. 0.25 m^2)
September 1992 (Exp. 3)		
Control	17.6d	145.0c
12.5	3.0b	40.7ab
25	0.8ab	9.3a
50	0.2a	1.3a
50 (3-week-old)[b]	5.5c	74.7b
CV(%)	23.6	45.0
November 1992 (Exp. 4)		
Control	28.2b	169.3c
12.5	10.5a	89.7b
50	2.1a	33.3a
CV(%)	35.2	17.7

[a]Conidia concentration of September experiment was 1.6×10^6 and 4.8×10^5 in November. Values are means of three replications. In a column, means followed by a common letter within a date are not significantly different at 5% level according to Duncan's Multiple Range Test.

[b]Conidia harvested previously and stored in water at 4°C for 3 weeks prior to use in this experiment.

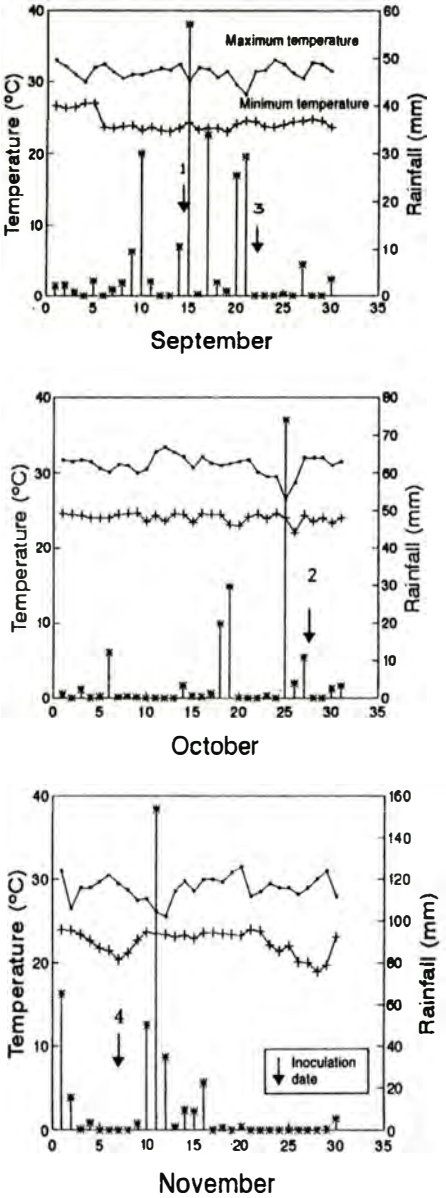

Figure 1. Daily rainfall (mm) and maximum and minimum temperatures (°C) at the International Rice Research Institute, Los Baños, Laguna, Philippines, during September-November, 1992. Arrows marked 1, 2, 3 and 4 indicate the date of inoculation of *Sphenoclea zeylanica* with conidia of an *Alternaria* sp. in the four field trials.

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