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EVALUATION OF *FUSARIUM ROSEUM* 'CULMORUM' AS A BIOLOGICAL CONTROL AGENT FOR *HYDRILLA VERTICILLATA*

by

R. Charudattan, T. E. Freeman, R. E. Cullen
F. M. Hofmeister

University of Florida
Gainesville, Florida 32611

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An isolate of *Fusarium roseum* 'Culmorum' was isolated in 1974 from diseased *Stratiotes aloides* (Hydrocharitaceae) plants found near Wageningen, The Netherlands. In laboratory tests conducted in Gainesville, it was found to be pathogenic to a relative of *S. aloides*, *Hydrilla verticillata*, one of the most important aquatic plants in Florida. Hydrilla plants grown in different kinds of sterile water in containers ranging from 50-ml tubes to

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20. ABSTRACT (Continued).

20-ℓ aquarium tanks were killed following addition of macroconidial suspensions of this isolate and incubation for several weeks. A minimum of 25,000 conidia/ml of treated water were required to kill hydrilla. Following inoculation, shoots turned chlorotic and discolored in 2 weeks, and disintegrated completely in 3 weeks. Histological evidence of infection and the consistent reisolation of this fungus from dying shoots confirmed its pathogenicity. Five other fusaria, including three Florida isolates from hydrilla, did not cause any damage on this host, even at inoculum levels of 250,000 conidia/ml of treated water. However, an isolate of *F. roseum* 'Graminearum,' a weak foliar pathogen of *Eichhornia crassipes*, was lethal to hydrilla at a 60,000 conidia/ml level in the laboratory, but not in an outdoor test, and appeared to be less virulent than the Dutch Culmorum isolate.

The host range of the Dutch Culmorum isolate was tested on 80 plant species including crop cultivars and nontarget aquatic plants. Seed infestation and/or seedling inoculation methods were used. Results indicated that the fungus infected some nontarget hosts, but was not a significant primary pathogen of any of the terrestrial hosts tested. It was lethal to certain aquatic plants, which also may be controlled by this isolate.

The fungus survived in water or moist soil for at least 9 weeks, but the number of propagules in water declined to a low level during this period. The fungus was harmless to mosquitofish in an acute toxicity bioassay. A fungicide capable of controlling this fungus was identified.

Based on these results, it was considered desirable to test the fungus on hydrilla in a large-scale study outside the quarantine laboratory. Accordingly, the test was conducted under semioutdoor conditions in a series of large-scale pilot tests using 3.04-m-diam plastic pools containing hydrilla. Although the fungus-treated hydrilla was consistently more damaged than the nontreated controls, a practical level of biocontrol was not achieved. Therefore, the biocontrol efficacy of the Dutch isolate of *F. roseum* 'Culmorum' and the prospects for using it as a microbial herbicide are not considered good.

Further basic studies are needed to establish the mode of action that underlies the lethal effects of this fungus on hydrilla in small-scale tests. An understanding of the mode of action may explain the lack of efficacy of the fungus in the large-scale tests and may help overcome this problem.

Preface

This report presents the results of research aimed at evaluating an isolate of *Fusarium roseum* 'Culmorum' as a biological control agent for hydrilla. The research was conducted for the Aquatic Plant Control Research Program (APCRP) by the University of Florida, Plant Pathology Department, Gainesville, Florida, under Contract No. DACW39-76-C-0097.

The overall investigation was supported in part by the U. S. Army Corps of Engineers, the Florida Department of Natural Resources, and the Center for Aquatic Weeds, University of Florida. Funds for the Corps' part of this effort were provided by the Office, Chief of Engineers, under appropriation number 96X3122, Construction General, through the APCRP at the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss.

The investigators for the contract were Dr. R. Charudattan, Dr. T. E. Freeman, Mr. R. E. Cullen, and Mr. F. M. Hofmeister, University of Florida. Dr. Charudattan directed the work reported herein. The authors would like to extend their thanks to Mrs. J. Beskid and Mr. P. Hunter, who assisted in the research. Special thanks are extended to Dr. G. W. Erdos, Department of Microbiology and Cell Science, University of Florida, for help with scanning electron microscopy.

The fungus was isolated by Dr. Charudattan from diseased *Stratiotes aloides* plants collected in The Netherlands. It was tested in Gainesville under quarantine, under permission from the Florida Department of Agriculture and Consumer Services and the U. S. Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine.

The work was monitored at WES by Dr. D. R. Sanders, Sr., and Mr. E. A. Theriot of the Wetland and Terrestrial Habitat Group (WTHG), Environmental Resources Division (ERD), Environmental Laboratory (EL). The study was conducted under the direct supervision of Dr. H. K. Smith, WTHG, and the general supervision of Dr. C. J. Kirby, Jr., Chief, ERD. Dr. John Harrison was Chief, EL. Manager of the APCRP at WES was Mr. J. L. Decell.

Commanders and Directors of WES during the study and preparation of this report were COL John L. Cannon, CE; COL Nelson P. Conover, CE; and COL Tilford C. Creel, CE. Technical Director was Mr. F. R. Brown.

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Contents

EVALUATION OF *FUSARIUM ROSEUM* 'CULMORUM' AS A BIOLOGICAL CONTROL AGENT FOR *HYDRILLA VERTICILLATA*

Introduction

1. Among the few microbial agents known to be capable of killing hydrilla (*Hydrilla verticillata* (L. f.) Royle) is an isolate of the fungal pathogen *Fusarium roseum* 'Culmorum' (Lk. and Fr.) Snyd. and Hans. (Moniliales; Hyphomycetes) (Charudattan and McKinney 1977, 1978). This isolate (#621), hereafter called the Culmorum, was isolated from tissues of diseased *Stratiotes aloides* L. (Hydrocharitaceae) plants collected in The Netherlands and was shown to be lethal to hydrilla (Charudattan and McKinney 1978). The fungus produced numerous canoe-shaped, multicellular spores in liquid and solid cultures. These spores, called macroconidia, or conidia for short, when mixed with water containing hydrilla and incubated under conditions favorable for the plant's growth, were capable of killing hydrilla. Chlorosis of shoots and rotting of the entire plant, including tubers, roots, turions, and shoots, resulted. Details of pathogenicity tests on hydrilla have been previously reported (Charudattan and McKinney 1977, 1978); this report summarizes these tests and other results (Charudattan et al. 1980) of the evaluation of the Culmorum as a biological control agent for hydrilla.

Methods and Materials

Discovery and isolation

2. In 1974, a disease of *S. aloides* was discovered near Wageningen by Dr. J. C. J. van Zon, Center for Agrobiological Research, Wageningen, The Netherlands. Mature plants had symptoms of root and crown rot and severely diseased plants appeared to sink gradually as a consequence of tissue decay. A few infected plant parts were taken to Gainesville, Fla., where a group of fungi were cultured from them including, predominately, a *Fusarium roseum* 'Culmorum.' In view of the close taxonomic relationship between *S. aloides* and hydrilla, the pathogenic potential of these fungi to the latter host was determined. However, only the Culmorum was capable of killing hydrilla (Charudattan and McKinney 1977).

Effects on hydrilla

3. The effects of the Culmorum on hydrilla were determined initially in three test systems. The first one consisted of incubating 8- to 10-cm-long terminal portions of hydrilla shoots in 3- by 15-cm glass tubes with 40 ml of sterile tap water to which were added dense macroconidial suspensions. Control tubes were without conidia. There were three to six replicates and the test was repeated more than a dozen times. Fungal inoculum, consisting of filtered macroconicial suspension obtained from potato dextrose agar (PDA) cultures, was quantitated with a hemacytometer. Inoculum levels between 2.5×10^3 and 2.5×10^5 conidia per millilitre (10^5 and 10^7 conidia per tube containing 40 ml of water) were set up by mixing suitable concentrations of conidial suspensions. Treated and control hydrilla tubes were incubated under ca. 5000 lux at 22 $\pm$ 2° C for several weeks.

4. In the second system, 20-ℓ aquarium tanks were layered with river sand, filled with 14 ℓ of tap water, and planted with 100 terminal ends of hydrilla shoots, each with an active apical meristem. After 2 days, the tanks were treated with conidial suspensions of Culmorum at approximately 8×10^4 or 9×10^4 conidia per millilitre of water. The test was replicated three times. After incubation for 3 weeks at ca. 3500 lux, hydrilla shoots started to discolor and developed signs of rotting.

5. In the third system, the fungus was grown for 3 weeks on a sterilized mixture of 9 parts sand, 1 part oatmeal, and 3 parts water, and mixed with the bottom sand in hydrilla tubes at 1:1 and 1:10 proportions (w/w) of inoculum and sand. Controls had sand-oat-water mixture without the fungus, mixed with an equal weight of sand. A hydrilla plant with shoots, roots, and at least one tuber was planted per tube; each tube was filled with sterilized distilled water. The light intensity was ca. 3500 lux and there were three replicates.

Comparison of the Dutch Culmorum with other *Fusarium* isolates

6. In order to determine the specificity of the Culmorum isolate to hydrilla and to make certain that its effects were not due merely to the massive numbers of fungal spores in water, a comparative inoculation test was conducted. In this test, three *Fusarium* spp. isolated from hydrilla in Florida, a *F. roseum* from *Ficus elastica* Roxb., and a *F. roseum* 'Graminearum' from *Eichhornia crassipes* (Mart.) Solms were included. The tube assay

procedure (paragraph 3) was used with inoculum densities between 2.5×10^3 and 2.5×10^5 conidia per millilitre of treated water. The Graminearum isolate was a weak foliar pathogen of *E. crassipes*.

7. In another experiment, the Graminearum isolate was tested against rooted hydrilla shoots maintained in 4-ℓ glass jars in 2.5 ℓ of water at ca. 3500 lux and 23° ± 2° C. For inoculum, about 30 g of wet, filtered mycelium and conidia grown on potato dextrose broth were blended in 125 ml of sterile tap water. The resulting slurry was applied at 10-, 20-, and 40-ml portions consisting of 0.96, 1.92, and 3.84 g, respectively, of conidia and mycelium per litre. The inoculum was suspended over hydrilla in water or injected into the soil with a syringe. Control plants received equal amounts of sterile tap water.

Histopathology

8. To understand the Culmorum-induced death of hydrilla, histopathological events were followed with light and scanning electron microscopy. Hydrilla shoots in the tube assay system (paragraph 3) were treated with seven levels of inoculum, 0, 12.5×10^3, 25×10^3, 50×10^3, 75×10^3, 100×10^3, and 250×10^3 conidia per millilitre of treated water. There were six replicates. The conidia were washed before adding to the tubes to prevent possible carryover of toxins. On the first day following initiation of the test, every 6 hr one randomly selected leaf per replicate was removed and mounted on a microscope slide in aniline blue-lactophenol. The number of germinated versus nongerminated spores, infection structures, and evidence of host damage were recorded on 100 conidia per sample. The samplings and observations were then repeated once a day for 2 weeks. Photographic evidence was gathered.

9. For scanning electron microscopy, similar random sampling of leaves and stem pieces was conducted. The specimens were fixed in 4 percent osmium tetroxide, dried to critical point, sputter coated with gold, viewed under a Hitachi S-450 scanning electron microscope, and photographed on black and white plates.

Effect on dissolved oxygen

10. The effect of treatment with the Culmorum isolate on the status of dissolved oxygen (DO) in the hydrilla containers was ascertained to determine whether or not a fungus-induced oxygen stress was the primary cause of hydrilla death. Five-litre, wide-mouth, clear glass jars were layered with 4 cm of

washed river sand, filled with a standard volume of tap water, and planted with 50 g of fresh, growing ends of hydrilla shoots (10-15 cm; approx. 35 sprigs). The jars were incubated under four different constant temperatures, 15°, 20°, 25°, and 30° C, in incubators programmed for 12 hr of light (5000 lux at the mouth of the jars) and 12 hr of darkness. Washed inoculum at the rate of 3.6×10^4 conidia per millilitre of water in the jars was added. Nontreated controls were maintained and the treatments were replicated three times. The oxygen levels were monitored polarographically with a battery-operated, YSI dissolved oxygen meter (Yellow Springs Instrument Co., Yellow Springs, Ohio).

11. In separate studies, the DO status of water in the experimental jars was determined following incubation of conidia alone (2.5×10^4 per millilitre) without hydrilla; hydrilla alone; or approximately 3 percent (v/v) of the fungal growth medium (carboxymethyl cellulose (CMC) or potato dextrose broth (PDB)) and hydrilla. The effects of continuous illumination as opposed to diurnal illumination and of continuous aeration were also determined.

Host range studies

12. Host range studies were conducted to determine host specificity and safety of the proposed biocontrol agent. Pathogenicity of the Culmorum isolate to nontarget terrestrial plants was determined by germinating seeds in fungus-infested soil (preemergence trial) or by spraying fungal inoculum on young seedlings (postemergence trial) (Table 1). Suspensions of conidia freed from mycelia by filtration served as inoculum for both trials. The trials were conducted in a greenhouse at 20° to 30° C.

13. In the preemergence trial, twice autoclaved potting soil mix (sandy loam and Perlite) in 10-cm pots was infested with a fungal inoculum of approximately 3.6×10^4 conidia per gram of soil, and sown immediately with host seeds. Twenty-four to sixty seeds per treatment per host were used except for peanuts, castor beans, and potatoes, which were tested at 6 seeds, 8 seeds, and 16 seed pieces per treatment, respectively. Control seeds were planted in similarly potted soil treated with uninoculated CMC broth medium (Booth 1971) used to grow the fungus. Percent seed germination in the controls, reduction in seed germination, and/or subsequent seedling mortality in the fungus treatments were the criteria used to determine the effect of the Culmorum on nontarget plants. Seedlings were maintained for 4 weeks following germination and observed for disease symptoms. Dead seeds, dead seedlings, or

symptomatic seedlings were plated on PDA in an attempt to recover the causal agent.

14. The postemergence trial was conducted on 10 to 20 seedlings per treatment per host; seedlings at the two-leaf stage were sprayed until runoff with a suspension of 10^6 conidia per millilitre by covering the leaves, stems, and the collar region. Control seedlings were sprayed with uninoculated broth medium. Seedlings maintained for 2 weeks were observed for symptoms of infection on the leaf, stem, or collar region, and sudden wilt or death. Roots were examined for damage before discarding plants; those with symptoms were plated on PDA to recover the causal agent.

15. The effects of the Culmorum on nontarget aquatic plants were also determined (Table 2). Actively growing shoots or entire plants were maintained in sterilized tap water in suitably sized glass containers, under ca. 3500 lux at 25° ± 4° C. The fungal inoculum consisting of filtered broth suspension of conidia was mixed with the water containing the test plants to yield conidial concentrations of 12.5×10^3 to 2.5×10^5 per millilitre of the total volume. Control plants were free of the fungus. Three to five replicates per host per inoculum level were used. Following inoculation, the plants were maintained for 3 to 4 weeks and rated for damage by assigning arbitrary values of 1 to 6 (1 = healthy, 6 = dead) to six equal zones of each replicate. The values of each replicate and of all replicates of a test species were averaged.

Effect of Culmorum on a fish

16. A bioassay on fish species was included as a further test of safety. The effect of the fungus on the mosquitofish *Gambusia affinis* (Baird and Girard) was tested by a static 96-hr acute toxicity bioassay procedure modified by the addition of aeration (American Public Health Association 1975, Conway and Cullen 1978, Doudoroff et al. 1951). Stocks of fish were collected from a freshwater reservoir and maintained and tested in aged tap water. To prevent any outbreak of fish diseases, the fish were held for 48 hr following collection in water treated with tetracycline at 15 mg/ℓ. They were then acclimatized for at least 20 days in a 76-ℓ aquarium tank provided with aeration and filtration, and fed daily with a commercial tropical fish food. Feeding was discontinued 2 days before exposure to the Culmorum and during the test.

17. Testing was conducted in 6-ℓ compartments of an equally divided

18-ℓ aquarium. Three such aquaria were maintained under a 12-hr day/night cycle at a constant temperature of 25° ± 0.5° C. In six of the nine compartments, the fish were exposed to the Culmorum, while the other three were Culmorum-free controls. The test was conducted twice; the first time, three of the Culmorum-treated compartments and two controls were nonaerated while the others were aerated. The second time, all nine compartments were aerated.

18. Five fish, each approximately 37 mm long with an average weight of 0.46 g, were placed in each compartment. A minimum of 10 fish per treatment was used. At the conclusion of the tests, a total of 45 fish had been exposed to the fungus, and 20 served as controls in the aerated treatments. In the nonaerated treatment, 15 and 10 fish were used in the fungus and control treatments, respectively. Dissolved oxygen, pH, total alkalinity, hardness, and nitrate and nitrite nitrogen levels of water were measured every 24 hr following initial readings that were taken just before the fungus was added. The same parameters were also measured in the stock tank at 5- to 7-day intervals.

19. A suspension of the Culmorum conidia in sterilized water was used as inoculum at the rate of 2.5×10^4 conidia per millilitre of treated water. The survival of the fungus at the completion of the bioassay was checked by plating water samples. Records of fish mortality, disease, and behavior during the test period were also maintained. Gills of dead fish were removed and examined microscopically for fungal conidia or mycelia. Fecal droppings were plated for recovery of the Culmorum.

Survival of Culmorum in water and soil

20. The fate of the Culmorum inoculum added to 20-ℓ glass tanks was monitored to learn what might happen to the inoculum over time. Two tanks containing hydrilla growing in sand and tap water were treated with the fungus at 8.4×10^4 conidia per millilitre. Hydrilla died between 14 and 21 days after treatment and a change in water quality ensued. Before and after hydrilla death, samples of water from each tank were dilution plated on PDA, generally at weekly intervals, and the numbers of the Culmorum and other microbial colonies were counted. Six water samples were drawn at different parts of each tank after uniformly mixing the contents. They were pooled and used for plating on PDA. Six replicates per dilution and four dilutions (total of 24 plates) were used to obtain average numbers of colonies. Each of

the Culmorum colonies was considered to have arisen from one conidium or propagule and, therefore, represented one unit of the fungus.

21. To determine the survival of the Culmorum isolate in soil, test tubes of sterilized sandy loam at 50 percent water saturation capacity were infested with 10^6 washed conidia per gram of soil and incubated at 10°, 18°, 20°, 25°, 30°, 35°, or 40° C. Six replicates per temperature were maintained. Attempts were made to recover the fungus at 2-, 3-, or 7-day intervals by plating approximately 30 mg of the soil from each tube on PDA. The survival of the fungus at these temperatures was indicated by its growth on plates.

Influence of some fungicides on Culmorum

22. To identify a fungicide capable of controlling the Culmorum in case of need, seven commonly used fungicides were screened. The fungus was mixed with wet, nonsterile sandy loam in test tubes and incubated for 3 days at 25° ± 2° C before adding aqueous preparations of fungicides. The fungicide concentrations were made up in predetermined volumes of water to yield 100 percent water saturation in each tube. Ten replicates per treatment were maintained and three concentrations of the following fungicides were tested: Cis N-(trichloromethyl) thio-4-cyclohexene-1, 2-dicarboximide [common name, captan; commercial preparation used, Captan 50 WP]; coordination product of zinc ion and ethylenebis (dithiocarbamate) manganese [mancozeb; Manzate 200, 50 WP]; cupric sulfate [copper sulfate, 53 WP]; manganese ethylenebisdithiocarbamate [maneb; Manzate 80 WP]; methyl-1-butylcarbamoyl-2-benzimidazolecarbamate [benomyl; Benlate 50 WP]; 2-(4-thiazolyl) benzimidazole [TBZ or thiabendazole; Mertect 340 F, 42 E]; and zinc ethylenebisdithiocarbamate [zineb; Zineb 57 WP]. Attempts were made to recover the fungus following its exposure to these fungicides for 6 and 22 days by plating a 1:10 (w/v) aqueous dilution of the fungus-infested soil. The *Fusarium*-selective pentachloronitrobenzene (PCNB) medium (Booth 1971) was used. The reduction in the number of the Culmorum colonies recovered from fungicide-treated soil was determined in comparison with a control without fungicides.

Conidial inoculum production for large-scale tests

23. Although the Dutch Culmorum isolate produced conidia easily and abundantly, methods for large-scale production were needed for evaluation. Therefore, various solid and liquid media were screened for maximum conidial

production. Solid media in 9- or 15-cm plastic petri plates and liquid media in shake flasks, Roux bottles, or a Virtis microbial fermentor were used for large-scale inoculum production. Conidia were separated by flooding plates with sterile deionized water and scraping, or by filtering the liquid cultures after mild agitation through four layers of cheesecloth. Conidial counts were made with a hemacytometer, and conidia were pelleted for wet weight determinations by centrifugation at 10,000 × g.

Chlamydospore production and survival

24. The Culmorum isolate produces thick-walled resting spores, the chlamydospores, which may persist in water and hydrosoil. It was necessary to determine their survival potential. Chlamydospores were induced by incubating either agar blocks containing mycelia in sterile water or conidia in a soil extract medium (Booth 1971).

25. Survival of chlamydospores in nonsterile soil was determined following the methods described under paragraph 21.

Large-scale pilot tests of biocontrol efficacy

26. The efficacy of the Culmorum isolate as a biocontrol agent was evaluated in a series of seven large-scale pilot tests (LSPT I-VII) to determine whether results from the small-scale studies can be duplicated on a larger scale under conditions closer to field conditions. Tests were conducted inside a fiberglass greenhouse in plastic pools measuring 3.04 m in diameter and 91 cm in height. Pools were prepared afresh for each test. The bottoms of the pools were layered with sand, filled with irrigation water, and planted for each test with approximately equal numbers of terminal segments of hydrilla shoots (15 to 20 cm long) collected from Manatee Springs, Florida. The shoots were allowed to acclimatize for 2 to 3 weeks before treating with the fungus. Dissolved oxygen levels, temperature, pH, hardness, and alkalinity of the pool water were monitored regularly during the tests.

27. Inoculum used in these tests consisted of an aqueous suspension of conidia without adjuvants or carriers, or conidia and mycelial homogenate in an agar sol or a broth. Conidia were produced either in a Virtis microbial fermentor as submerged liquid cultures or in solid agar cultures on 15-cm petri plates, or conidia from both types of cultures were pooled. Potato dextrose broth and potato dextrose agar were used, respectively, for liquid

and solid cultures. Typically, conidia were harvested from 3- to 4-week-old cultures. Conidia were washed by centrifugation at low speeds and resuspended in water at desired concentrations.

28. In each LSPT, one of two matched pools containing hydrilla or hydrilla and other submerged aquatic plants was treated with the fungus. The other pool served as the untreated control. The type of inoculum, water pH, water temperature, CO_2 concentration in water, light intensity, and plant density varied in each test. Important differences in the experimental design are discussed in the Results section. The average light intensity at the water surface was ca. 5500 lux. After varying incubation periods following fungus treatment, the damage to hydrilla was rated from the degree of chlorosis, discoloration, and death on 500 shoots collected along a randomly selected transect. Both treated and control hydrilla shoots were rated on a scale of 1 to 4: 1 = healthy, 2 and 3 = degrees of chlorosis and discoloration, and 4 = dead. The number of turions on sampled shoots and the number of tubers in a randomly selected square metre area were determined in some tests. The data were analyzed for significant differences due to the fungus.

Results

Effects on hydrilla

29. Damage to hydrilla from the Dutch Culmorum was usually evident as chlorosis and discoloration of treated shoots, usually 10 to 14 days after treatment (Figure 1). In 3 weeks, death and lysis or regrowth of partially damaged hydrilla can be observed. The threshold of inoculum needed to damage hydrilla was 1×10^6 conidia per tube or 2.5×10^4 per millilitre. A dose-and-effect relationship was seen on inoculated hydrilla; at lower inoculum levels the shoots were only partially damaged or killed while at higher inoculum levels the effects were drastic and lethal (Figure 1). In the second test system (paragraph 4), 3 weeks after treatment, hydrilla shoots started to discolor and rot. In about 5 weeks, the shoots broke down completely, and some that were still green were defoliated and uprooted, and floated to the water surface (Figure 2). In the third system (paragraph 5), the treated plants turned pale a week after treatment and were dead by the end of 14 days (Figure 3).

30. In all these systems the Culmorum isolate could be reisolated from treated hydrilla shoots, whether dead, dying, or green, after surface

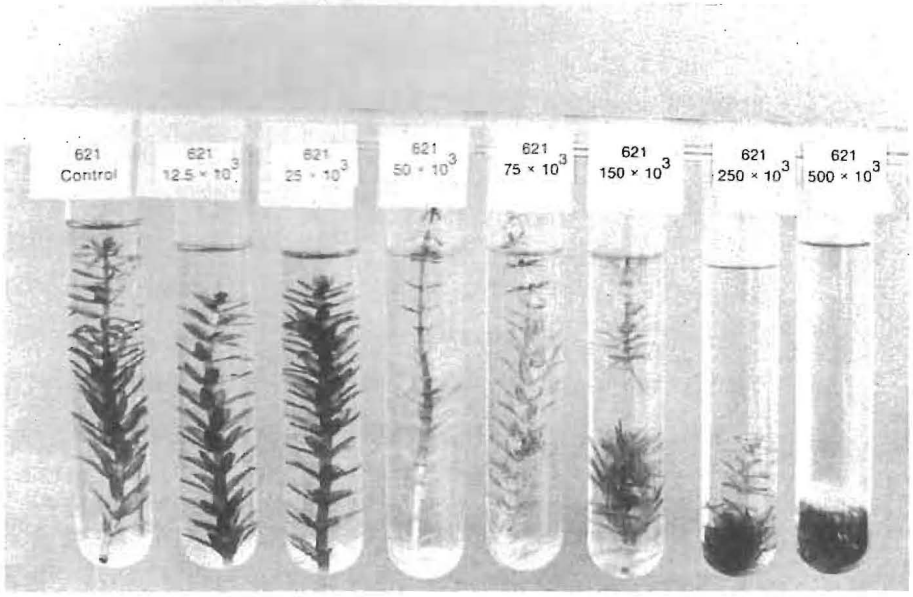


Figure 1. Effect of *Fusarium roseum* Culmorum on hydrilla; inoculum versus damage in tube assay system. Control (no inoculum) on the left; increasing inoculum levels from left to right

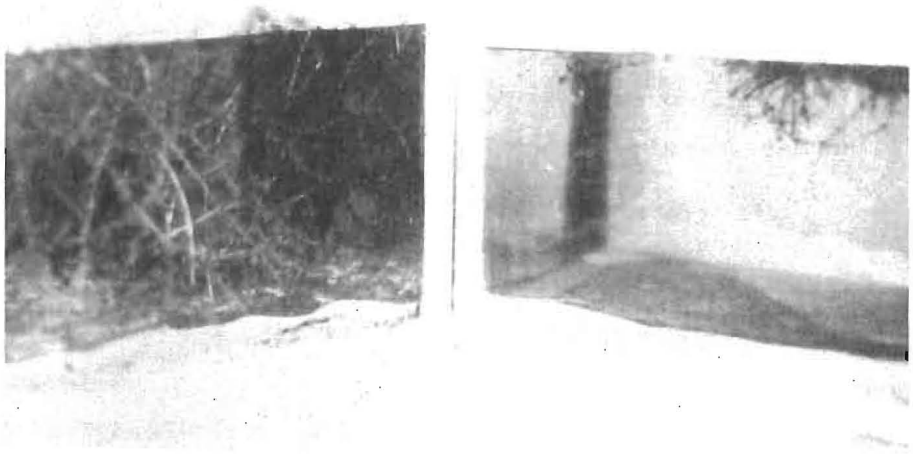


Figure 2. Effect of *Fusarium roseum* Culmorum on hydrilla in aquarium test. Control (left) and fungus-treated (right) aquaria

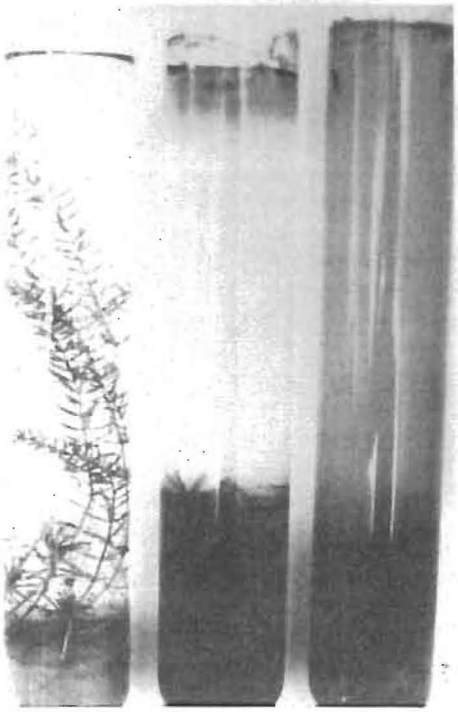


Figure 3. Effect of soil-inoculated *Fusarium roseum* Culmorum on hydrilla. Control (no inoculum) on the left; 1:1 inoculum in the middle; 1:10 inoculum on the right

sterilization and plating on PDA. Controls did not yield the fungus.

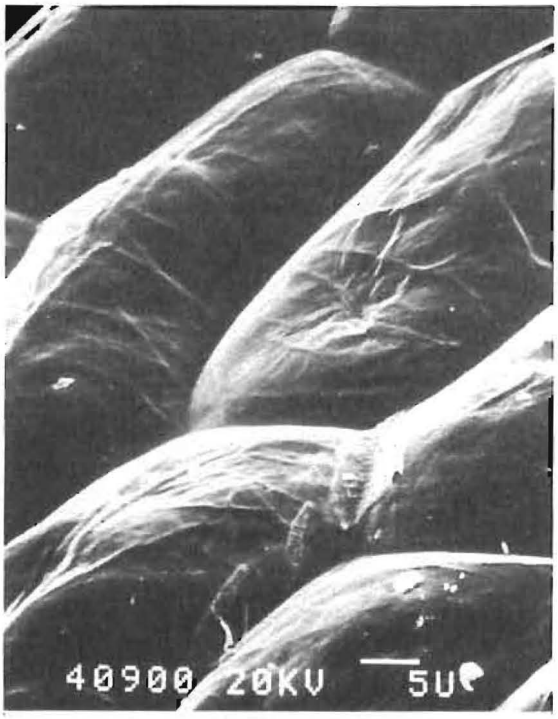
Comparison of the Dutch Culmorum with other *Fusarium* isolates

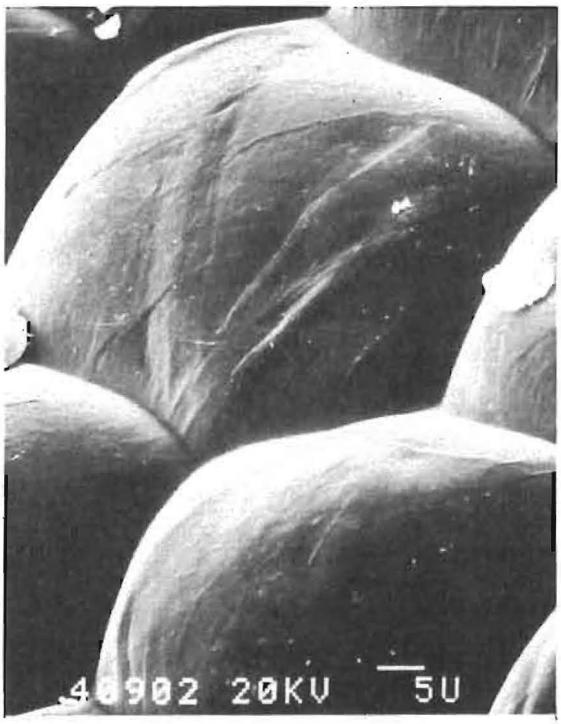
31. The results of this study (paragraphs 6 and 7) confirmed that the Dutch Culmorum was indeed unique in its effects on hydrilla. The three *Fusarium* spp. from hydrilla and the *Ficus* isolate of *F. roseum* did not damage hydrilla even at higher levels of inoculum. The Graminearum from *E. crassipes* was capable of damaging hydrilla, inciting similar symptoms as the Dutch Culmorum. When applied as a suspension, it caused considerable turbidity to water and was effective in killing most of the hydrilla by 3 weeks. In jars with soil-injected inoculum, some damage and death of shoots occurred, but the plants were mostly healthy, similar to the controls. However, the threshold of inoculum needed to cause damage by this isolate was approximately 6×10^4 conidia per millilitre, or 2.4 times higher than that for Culmorum. Therefore, the Dutch Culmorum isolate was considered to be more virulent towards hydrilla than any *Fusarium* tested.

Histopathology

32. Histopathological events were followed with light and scanning electron microscopy. Scanning electron microscope (SEM) pictures of control hydrilla cells are shown in Figure 4. The sequence of entry of the Culmorum isolate, depicted in Figures 5-14, was as follows. The conidia lodged on tissues and germinated from terminal and/or intercalary cells. The conidia were normal, canoe-shaped, or became thick-walled and moniliform before germination (Figures 5 and 6). Frequently, the germinating mycelium penetrated the tissue along the intercellular grooves (Figures 6-8). Appressorium-like structures and mycelial thickening were produced at the growing tips of the mycelium (Figures 5 and 9). The mycelium also entered directly through

a. Lower surface cells





b. Upper surface cells

Figure 4. Scanning electron microscope (SEM) pictures of control, non-treated hydrilla leaf cells. Bars in these and the following figures denote the relative measurements: 5 μm (5u) or 50 μm (50u).

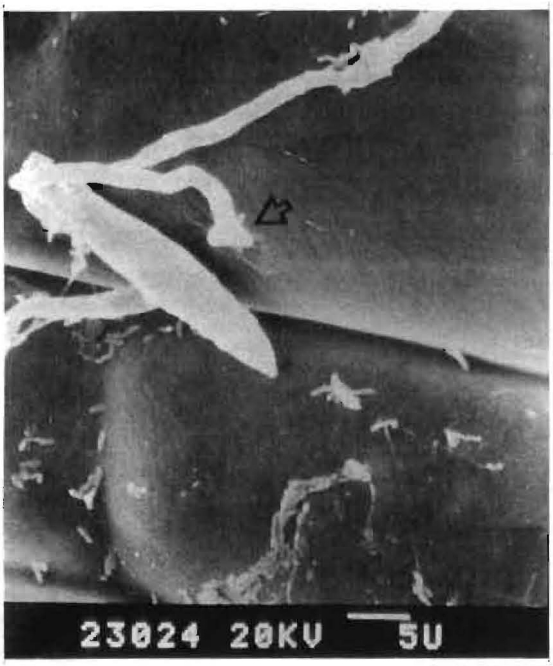


Figure 5. SEM picture of a normal, canoe-shaped conidium with germinating mycelia and an appressorium-like structure (arrow)

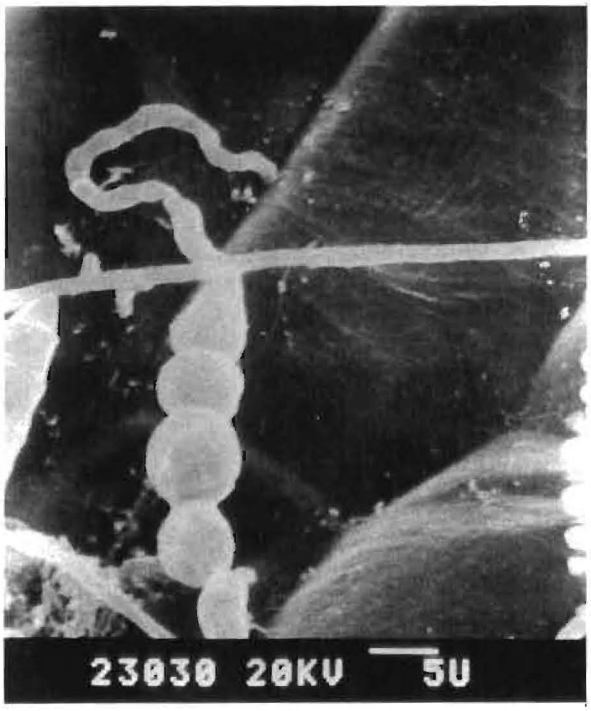
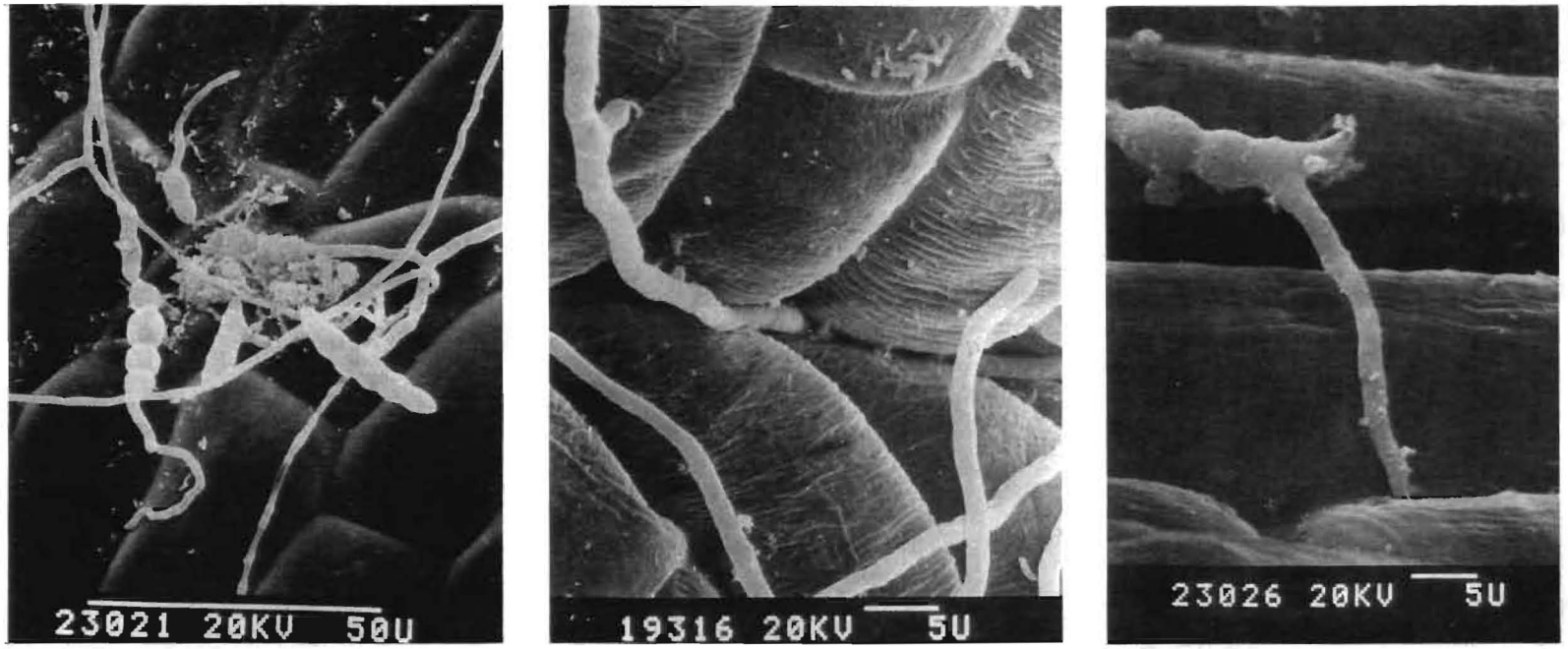
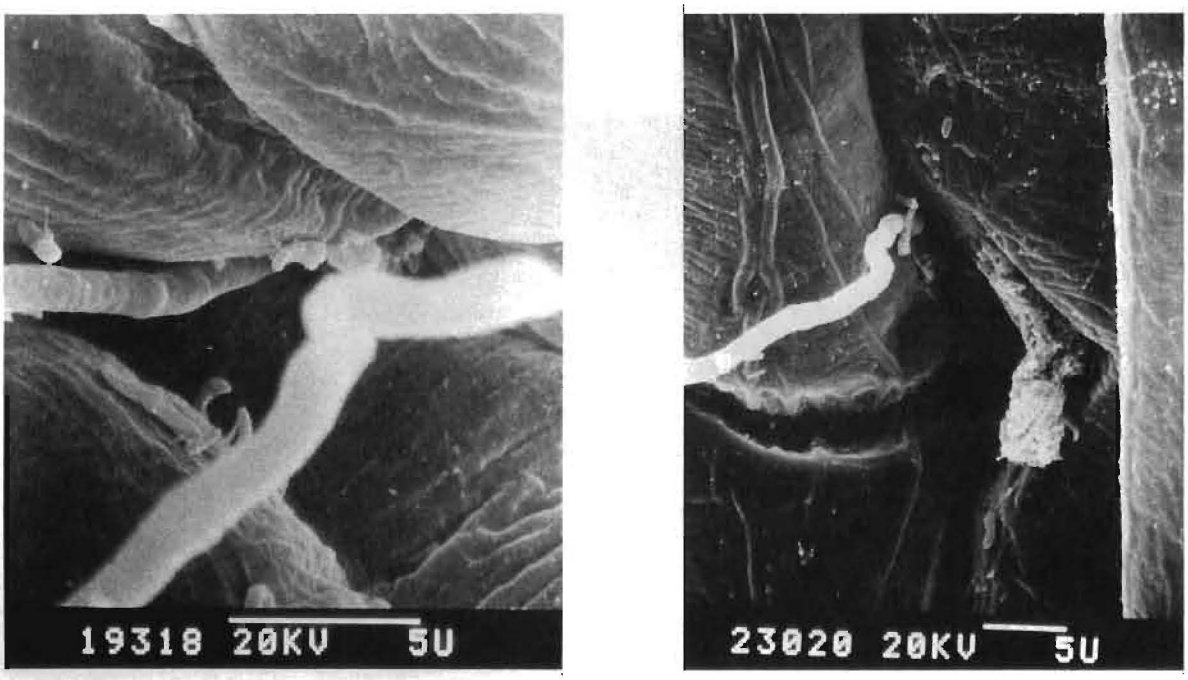


Figure 6. SEM picture of a moniliform conidium (a secondary structure) with germinating mycelium



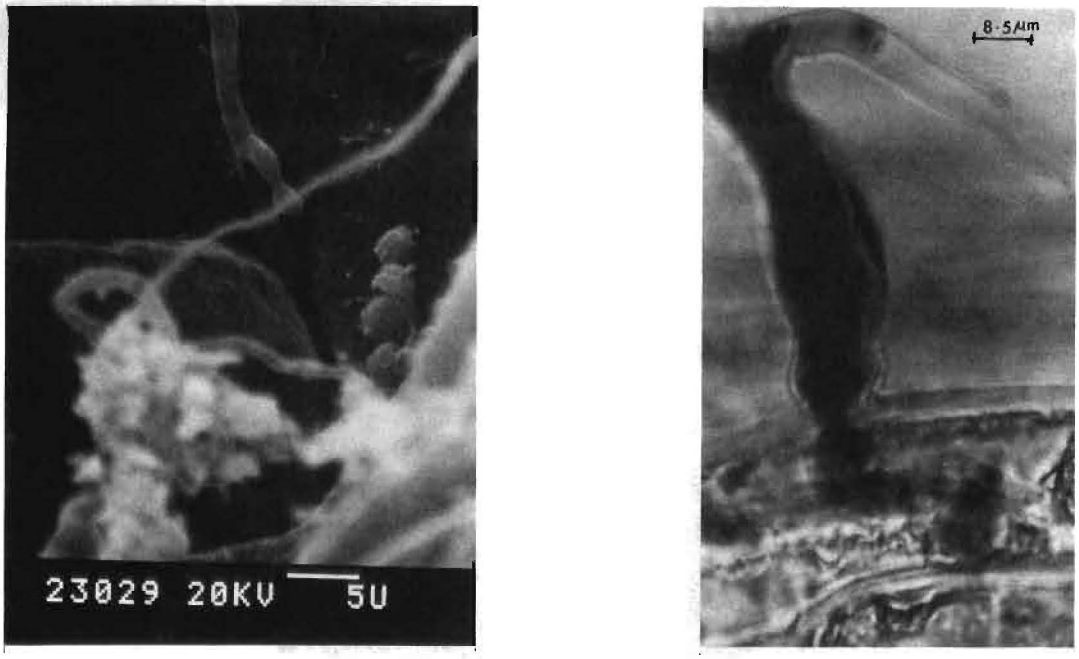
a. b. c.

Figure 7. SEM pictures showing directional growth of mycelia towards grooves between cells



a. b.

Figure 8. SEM pictures of mycelial entry through grooves between cells



a. SEM view b. Light microscope view

Figure 9. Mycelial thickening at the points of contact between mycelial tips and hydrilla cells

Figure 10. SEM picture of mycelial entry through entry

Figure 11. Light microscope view of intracellular penetration and mycelial thickening

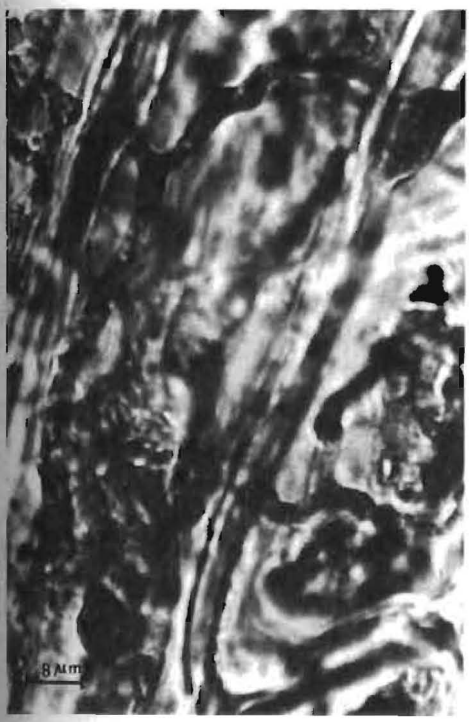


Figure 12. Light microscope view of mycelial ramification in the leaf tissue

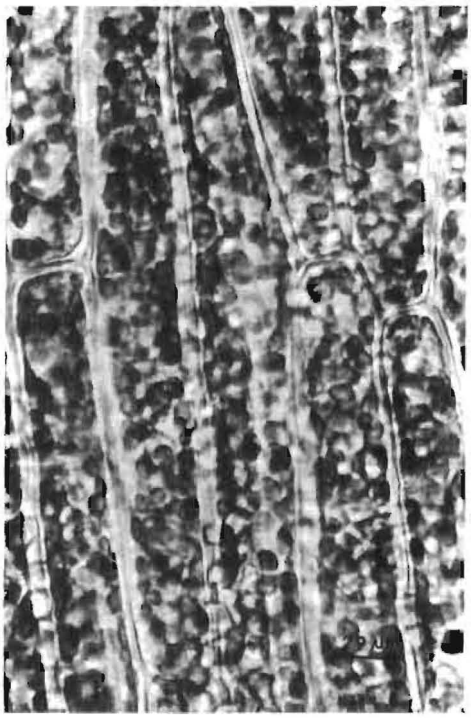
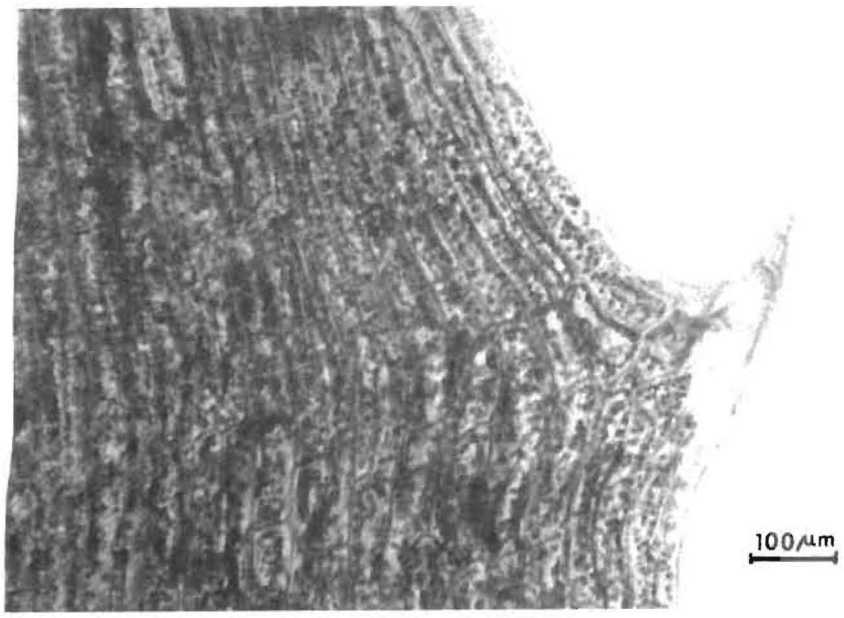
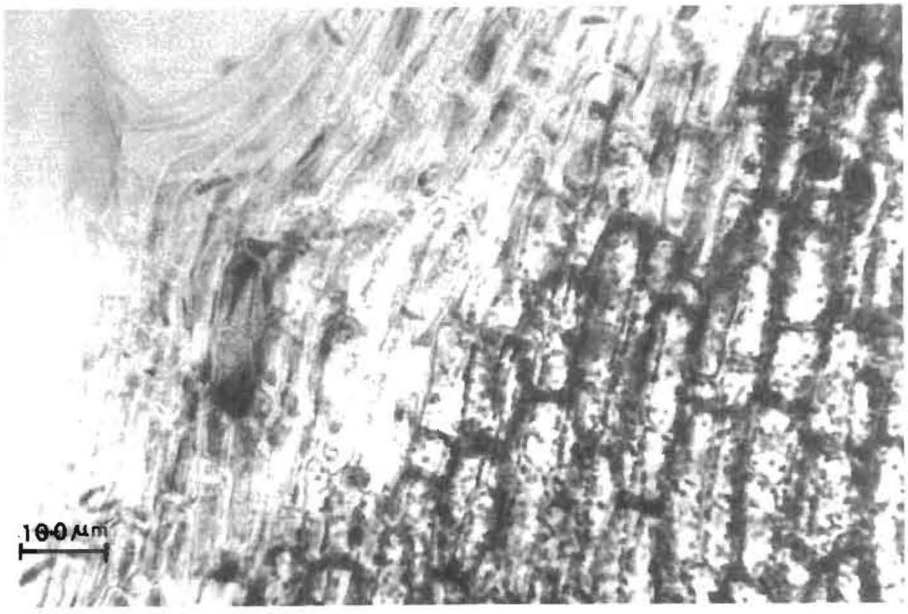


Figure 13. Light microscope view of healthy hydrilla leaf cells showing chloroplast organization. Compare with the chloroplast organization in Figures 11 and 12



a. Healthy



b. Fungus treated

Figure 14. Comparison of healthy and fungus-treated leaf tissues

wounds and fissures in cells (Figure 10). In the case of direct penetration, it is probable that formation of infection structures, mechanical pressure, cell dissociation at the point of entry, and mycelial invasion will occur.

33. Intracellular penetration and mycelial ramification occurred (Figures 11 and 12), the latter possibly via plasmodesmata. Mycelial thickening was seen at the point of entry between adjacent cells (Figure 11). All leaf cells including those of the midrib, vasculature, and leaf spines were observed to be invaded; extensive tissue colonization ensued (Figure 12).

34. Plasmolysis, loss of chloroplast structural integrity, and general disorganization of cellular contents followed mycelial penetration into the leaf tissue (Figures 11-13). These occurred only in the immediate vicinity of the invaded cells, suggesting the lack of toxic or osmotic damage in advance of the mycelium.

35. Disintegration of the leaf tissue started at the leaf apex and margins (older cells) and progressed toward the leaf center and base (younger cells) (Figure 14).

36. Macroscopically, the symptoms of chlorosis, discoloration, and lysis accompanied the above sequence of histopathological events.

Effect on DO

37. The Dutch Culmorum isolate had a drastic effect on DO content. During dark periods (night cycles), the DO reached critical levels of <2 mg/ℓ in the inoculated jars. These low levels were usually obtained about 48 to 60 hr after adding the inoculum to water (Figure 15). Such low DO levels were not observed in hydrilla jars without fungal inoculum (plain water with hydrilla), in fungus-treated jars under continuous 24-hr illumination, or in fungus-treated jars with continuous aeration.

38. The low levels of DO probably resulted from the respiratory demand on oxygen by both the plant and the fungus during dark periods as well as the disruption of the normal photosynthetic oxygen production by hydrilla (during light periods) due to infection. In other words, the infected plant did not produce enough photosynthetic oxygen to compensate for the oxygen lost by respiration at night.

Host range

39. Results of host range tests confirmed that the Culmorum isolate was not a virulent pathogen, but rather was an opportunistic parasite capable of depressing seed germination or inducing seedling mortality in 36 of 70

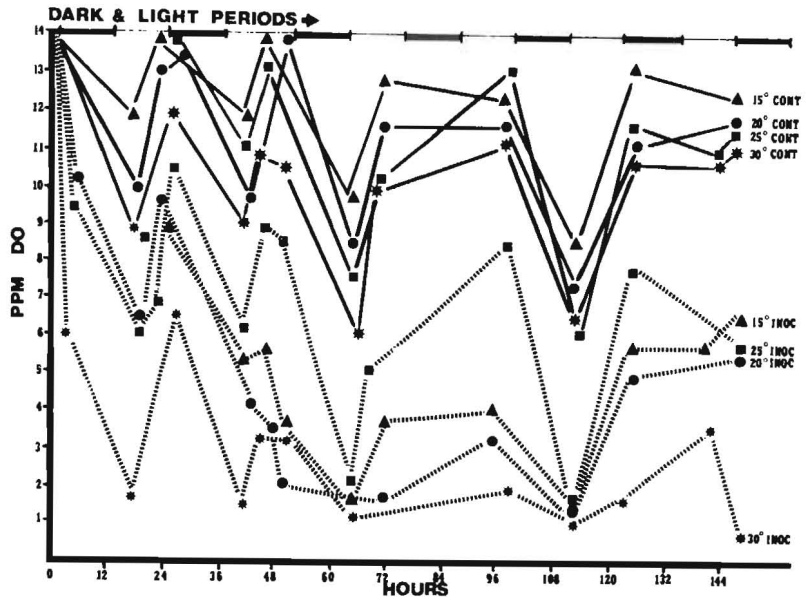


Figure 15. Changes in DO levels in hydrilla jars maintained at different temperatures. Dark and light periods are indicated, respectively, by thick bars and thin lines. INOC = inoculated jars; CONT = control jars

cultivars (preemergence trial, Table 1). Of these, germination of seventeen cultivars was reduced from 1 to 19 percent; eight, 20 to 39 percent; five, 40 to 59 percent; one, 60 to 79 percent; and five, 80 to 100 percent. However, the Culmorum isolate was recovered from diseased tissues of only 5 of these 36 cultivars. In 7 of the 36 cultivars, poor seed viability, as revealed by less than 50 percent seed germination, may have rendered the plants more susceptible. In the postemergence test the Culmorum was nonpathogenic. Seven plant species showed disease symptoms but did not yield the Culmorum on plating. Moreover, controls of four of these plant species were also diseased (Table 1), leading to the conclusion that these plants became diseased or wilted due to other causes.

40. The inoculum levels used in the host range trials, i.e., 3.6×10^4 conidia per gram of soil for the preemergence trial and 10^6 per millilitre for the postemergence trial are from several to twelve times higher than the population levels recorded for other Culmorums in soil (Baker and Cook 1974). Such abnormally high inoculum levels may have caused unduly adverse effects on the host. Furthermore, *F. roseum* Culmorum isolates are generally pathogens of seeds, seedlings, and weakened mature plants. Healthy vigorous plants

are rarely susceptible to attack (Baker and Cook 1974). Seeds that are slow to germinate and seedlings that emerge slowly are especially susceptible to this fungus (Holmes 1979). Therefore, the results of this host range test (Table 1) support the opinion that the Dutch Culmorum is a parasite of terrestrial plants only under conditions of host immaturity and adversity.

41. The Culmorum was also lethal to six of seven nontarget aquatic plants screened (Table 2). At 2.5×10^5 conidia per millilitre, it completely killed coontail, elodea, southern naiad, and spatterdock; severely discolored eelgrass; and caused alligatorweed to turn completely chlorotic. However, the susceptibility of these plants differed at lower inoculum levels; coontail and southern naiad, the most susceptible, were significantly damaged even at the 12.5×10^3 level. Alligatorweed, elodea, eelgrass, and spatterdock were more resistant to the fungus. These results supported an earlier qualitative evaluation of the Culmorum damage on these hosts (Charudattan and McKinney 1978), and confirmed that the fungus was less damaging to some of these aquatic plants than to hydrilla (see also paragraph 55).

Effects on fish

42. The fungus did not cause any ill effects on the test fish. In fact, the fish fed voraciously on the conidia as they were being added to water, subsequently resulting in the littering of the bottoms of the compartments with red (color of the Culmorum conidia) fecal droppings. The fungus-free control compartments remained relatively clean, and the feces were of a different color. In the nonaerated fungus treatments, four fish deaths (27 percent) were recorded. Two fish died in the aerated compartments due to malfunctioning of the aerator which resulted in low oxygen levels (<4 mg/ℓ). The fish in the properly aerated treatments showed no ill effects from the fungus (Table 3).

43. Water temperature throughout the test remained at 25° ± 0.5° C in all compartments. Oxygen levels were close to saturation in the aerated compartments, whereas in the nonaerated the levels dropped as low as 2.8 mg/ℓ. The other water quality parameters, pH, hardness, total alkalinity, nitrate, and nitrite levels, were not appreciably different between the treatments (Table 3).

44. No Culmorum or other fungus was found in the dissected gills of dead fish and no lesions or other external fungal growths were observed on any of the fish. Fecal particles from the fungus-treated and control

compartments gave 100 percent recovery of the Culmorum, whereas isolations from the stock tank yielded *F. solani*, *Mucor*, members of the Zoopagales, and bacteria.

45. It is important that distinction be made between the direct and indirect effects of the Culmorum on the fish. In order to detect and evaluate lethality of the fungus, adequate DO must be maintained (Doudoroff et al. 1951). When the oxygen level was above 4.0 mg/ℓ, the fish showed no adverse effects. Therefore, the apparent high biological oxygen demand (BOD) due to the fungus in the nonaerated compartments was indirectly implicated in the fish kill, and it is clear that the test fish was not directly harmed by the Culmorum.

46. The recovery of the Culmorum isolate from fish feces suggested the possibility of its survival after passage through the fish gut, although surface contamination of the feces by the ambient inoculum is a more likely explanation for the recovery. The recovery of the fungus from the control compartments that were intended to be free of the fungus indicated cross-contamination, which probably occurred via conidia-bearing aerosol spray produced by the vigorous bubbling of air through water.

Survival of Culmorum

47. In nonsterile water, the number of colony-forming units (propagules) of the Culmorum decreased drastically from 6426 per millilitre on the seventh day to 41 per millilitre on the 70th day after the addition of conidial inoculum to water (Table 4). At the same time, the proportion of other colony-forming microorganisms increased, possibly contributing to competition or antagonism towards the Culmorum. Therefore, in field performance the Culmorum inoculum will probably decline in numbers following hydrilla death. This would lessen the danger of Culmorum damage to the more tolerant species of aquatic plants that are likely to succeed hydrilla.

48. In sterilized sandy loam at 50 percent water saturation, the Culmorum survived for at least 9 weeks at 18° to 30° C. The recovery of the fungus was reduced after 3 to 4 weeks, respectively, at 10° and 35° C. The fungus did not survive at 40° beyond 2 days, suggesting that the survival of the Culmorum in terrestrial soils will be limited by temperatures of 30° C and above. Such temperatures are common in the summer in the topsoil of Florida. Moreover, propagules of Culmorum are known to reside only in the top 10 cm of soil (Baker and Cook 1974, p 136). Culmorums are cool temperature

pathogens, and the Dutch Culmorum has a temperature optimum for in vitro growth between 19° and 21° C (see paragraph 51). Therefore, this isolate is unlikely to establish in the terrestrial soils of Florida.

Fungicides

49. The relative efficacy of the fungicides screened was determined from the percent reduction in the number of the Culmorum colonies relative to the fungicide-free control. Captan and mancozeb gave significant reductions (70 to 100 percent) at both plating dates (Table 5). Benomyl, TBZ, and zineb were not effective, while copper sulfate and maneb were moderately effective at the first plating. Maneb and zineb were more effective at the second plating, while copper sulfate was not. Since the samples were drawn from the same tubes for both platings, the recovery of the fungus in the subsequent plating and not in the earlier one suggested a fungistatic rather than a fungicidal activity for copper sulfate. The lack of a linear dose-response relationship with some of the fungicides was probably due to sampling error.

50. In view of the cost and efficacy, captan should be the preferred fungicide for control of this Culmorum. However, it must be emphasized that none of these fungicides is registered for use in water, and captan is toxic to fish. Therefore, the use of captan or any of the fungicides in or around water must be specifically approved.

Conidial inoculum production

51. The effects of temperature, media, and culture age on sporulation by the Culmorum isolate were determined for the purpose of inoculum production. The optimum temperature for growth on PDA was between 19° and 21° C. Out of ten solid media tested, PDA was the best for abundant spore production. Among three liquid media screened, PDB and a medium with carboxymethyl cellulose were suitable for submerged liquid-culturing of the fungus. In submerged liquid culture using PDB and a Virtis microbial fermentor, the fungus produced conidia as early as 72 hr after seeding. By 96 hr, as many as 6.8×10^4 conidia per millilitre of the medium were produced. By 12 days, 1.204×10^6 conidia per millilitre were obtained. The PDA, however, yielded ca. 3×10^8 conidia per plate (15 cm diam) per 20 ml of medium, and therefore was most suitable for mass production of Culmorum inoculum in the laboratory.

52. Although the laboratory capabilities for inoculum production were limited, enough inoculum was produced for large-scale experiments. To treat

a pool 152 cm in radius and 30 cm in water depth with an inoculum at the rate of 5×10^4 conidia per millilitre, 1.11×10^{11} conidia were needed. This many conidia were obtained from about 8 ℓ of solid medium, and weighed as a thick paste ca. 96.5 g, or equaled 6.4 tablespoonsful.

Chlamydospore production

53. The Culmorum isolate produces two types of chlamydospores: conidial and mycelial. The conidial chlamydospore is formed as a secondary spore from the thickening of one or more cells of the macroconidium. The mycelial chlamydospore is produced by the thickening of one or more cells at the tips or in the middle of a hypha. Both types of chlamydospores could be produced readily by incubating conidia or mycelia in aqueous soil-extract or sterile water, respectively. The Culmorum isolate, therefore, is expected to produce chlamydospores when used as a biocontrol agent, irrespective of the type of inoculum used.

54. The conidial chlamydospores did not survive incubation in non-sterile soil at 50 percent water saturation and temperatures of 35° and 40° C, but survived up to 80 days at 10° to 30° C. The chlamydospores of the Culmorum isolate, therefore, are not expected to survive beyond 35° C, but may survive for prolonged periods at <35° C. Such long chlamydospore survival may be advantageous to the biocontrol attempt; any residual chlamydospore inoculum may help sustain the biocontrol pressure on hydrilla.

Large-scale pilot tests

55. In LSPT I (7 Jan 81-23 Feb 83), the fungus was applied as an aqueous suspension at the rate of 5×10^4 conidia per millilitre of treated water. Plant species tested included common arrowhead (*Sagittaria latifolia* Willd.), coontail, eelgrass, southern naiad, spatterdock, and hydrilla. Although no germination, penetration, or colonization could be confirmed, damage to hydrilla and southern naiad was apparent 1 week after treatment with the fungus. After 1 month, about 90 percent of hydrilla and 100 percent of southern naiad plants in the treated tank were moderately to severely chlorotic, whereas eelgrass, common arrowhead, coontail, and spatterdock were unaffected by the fungus. All six plant species in the control tank were healthy.

56. Less than 5 percent of the conidia in the tank had germinated. This was partly due to the high pH (9.08 to 9.87) of the water. Conidia in deionized water in a companion tube test germinated at about 90 percent.

57. Nine days after treatment with the fungus, the hydrilla in the tubes

turned chlorotic at inoculum levels of 1×10^5 and 2×10^5 conidia per millilitre. By day 22, hydrilla at these inoculum levels was completely dead and decayed. Similar effects were not seen at the 5×10^4 inoculum level.

58. Unlike the tube tests, the large-scale tests may be a true indicator of how the Culmorum isolate would affect nontarget plants in the field (see paragraph 41).

59. Damage ratings of the second through fifth LSPT are summarized in Tables 6-9, along with important experimental conditions used. Statistical significance was not tested for number of turions per square metre in Tables 6 and 7. The water quality data for LSPT I through V are listed in Table 10.

60. In LSPT II, at 4 weeks after adding the fungus, the fungus-treated plants had a symptom rating of 2.46 which was significantly higher than the control at 2.32. There were more turions (0.11 per plant) and more tubers ($154/m^2$) in the fungus-treated plants than in the nontreated control (0.05 turion and 125 tubers).

61. In LSPT III, at 6 weeks after adding the fungus to the pool, the fungus-treated plants had, again, significantly more damage than the control plants (rating of 2.08 and 1.68, respectively).

62. In all these tests the DO levels of the fungus-treated and control pools remained comparable. The oxygen-limiting condition observed in the small container tests (see paragraph 37) was not observed in these large pools. The fungus when used as a biocontrol agent may not cause any problems with DO in the field.

63. The pH of water in the pools was alkaline (8.0 to 9.5). At these levels, the conidia did not germinate, whereas germination was unaffected at pH 5.0 to 7.0. By bubbling carbon dioxide gas through water, the pH of the pools was lowered in LSPT II and III to around 6.5 in order to obtain conidial germination and damage to hydrilla. Other aspects of water quality (hardness, carbon, and nitrogen statuses; alkalinity; etc.) must be critically studied to determine their influence on spore germination and efficacy of the Culmorum isolate.

64. Tests VI and VII were rated only qualitatively. In all these tests (I-VII) there was consistently more damage on the fungus-treated hydrilla when compared to the untreated control. However, although statistically significant, the differences were barely noticeable in terms of

reduction in the biomass of the fungus-treated hydrilla. Therefore, based on the LSPT results, the Dutch Culmorum cannot be considered efficacious for practical use as a biocontrol agent for hydrilla. However, to date only nonformulated, fresh inoculum has been tested. The efficacy of other types of inocula must be evaluated before dismissing this isolate as a biocontrol agent.

Discussion

65. The results from laboratory tests have confirmed that isolate 621 of *F. roseum* 'Culmorum' is lethal to hydrilla and, in this respect, is unlike any other fungus or bacterium that we have screened against hydrilla (Charudattan and McKinney 1978). The mode of action of this isolate appears to be typically pathogenic; histopathological data support this assumption. However, it is not clear whether any preinfection and postinfection changes take place in hydrilla that predispose it to the fungus. Preliminary data (not included here) and histopathological evidence appear to rule out the involvement of toxin(s) as predisposing factors. However, in the small-scale assay systems, the fungus may create a physiologically critical stress point at which hydrilla is rendered photosynthetically inactive and susceptible to the fungus. At this point, increasing demand on oxygen may set in, leading to a drastic reduction in DO levels and the eventual death of hydrilla. More basic work, however, needs to be done to confirm the mode of action of the Culmorum isolate against hydrilla.

66. The overall host range data, including those from the preemergence host range trial, suggest that the Culmorum isolate is not a threat to the terrestrial plants screened. Among the nontarget aquatic plants screened, it is certain that only southern naiad is susceptible to the fungus (paragraph 55 and Table 2). Coupled with the observation that the conidia and chlamydospores persist in water and soil for only a few weeks (paragraphs 47, 48, and 54), the Culmorum isolate is not likely to pose any threat to nontarget host plants in Florida. It is also safe against one species of fish. Therefore, the safety aspects of biocontrol with the Culmorum isolate are acceptable.

67. The adverse effects on the DO levels that followed Culmorum-induced hydrilla decline in small-scale systems did not happen in the large-scale

pilot tests. Either such reductions in DO levels are limited to small test systems or the lack of reductions in DO levels in the large-scale tests was related to the lack of fungus efficacy.

68. The results of the small-scale tests, in which complete hydrilla kill was achieved, were not duplicated in the large-scale tests. Although in the first LSPT 90 percent of the hydrilla shoots turned chlorotic and discolored, total kill and disintegration of hydrilla were not seen. Therefore, the results of the pilot tests of biocontrol efficacy were less satisfactory than anticipated.

69. There is a need to reevaluate the type of inoculum used in the large-scale tests. Several methods of inoculum production, especially those that maintain the efficacy of inoculum, must be evaluated.

Conclusions

70. An isolate of *Fusarium roseum* 'Culmorum' that is consistently lethal to hydrilla in small test systems has been found. On the basis of host range tests to nontarget plants, nontoxicity to a species of fish, and survival characteristics, this isolate is considered to be a safe biocontrol agent. However, in large-scale tests of biocontrol efficacy using a nonformulated, fresh inoculum, it was less satisfactory than anticipated. Therefore, it cannot be considered a suitable biocontrol agent for hydrilla unless further studies prove it otherwise.

Recommendations

71. Further basic studies are recommended to understand the mode of lethal action of the Culmorum isolate in small test systems and ways to improve its inoculum efficacy. These studies will be conducted as part of the ongoing research at the University of Florida.

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Table 1

Effect of Isolate 621 of *Fusarium roseum* Culmorum on Nontarget Terrestrial Plants

Family Genus, Species Common Name, Cultivar	Preemergence			Postemergence†							
				Percent of Control Plants Exhibiting Symptoms				Percent of Culmorum Plants Exhibiting Symptoms			
	Percent Reduction in Germination*	Culmorum Reisolated from Tissue**	Percent Seed Viability	LS	CR	W	H	LS	CR	W	H
Aceraceae											
Rufacer rubrum (L.) Small											
Red maple	NT††	NT	NA††				100				100
Alliaceae											
Allium cepa L.											
Onion, Texas grand	0	NT	58				100				100
Onion, Spanish white	NT	NT	NA				100				100
Altingiaceae											
Liquidambar styraciflua L.											
Sweet gum	NT	NT	NA				100				100
Apocynaceae											
Vinca rosea L.											
Periwinkle	50	-	8				100				100
Chenopodiaceae											
Spinacia oleracea L.											
Spinach, Bloomsdale	0	NT	58				100				100
Beta vulgaris L.											
Beet, Detroit dark red	12	-	71			6	94			11	89
Beet, Early wonder	5	-	79				NT				NT
Compositae											
Cichorium endivia L.											
Endive, Broad leaf Batavian	0	NT	13				100				100

(Continued)

* Percent reduction in germination of fungus-infested seeds in relation to control seeds treated with uninoculated medium.

** -/+ = Culmorum not isolated/isolated.

† LS = leaf spots; CR = collar rot; W = wilt; H = healthy, no symptoms.

†† NT = not tested; NA = not applicable.

Table 1 (Continued)

Family Genus, Species Common Name, Cultivar	Preemergence Percent Reduction in Germination	Preemergence Culmorum Reisolated from Tissue	Preemergence Percent Seed Viability	Postemergence Percent of Control Plants Exhibiting Symptoms LS	CR	W	H	Postemergence Percent of Culmorum Plants Exhibiting Symptoms LS	CR	W	H
Compositae											
Helianthus annuus L.											
Sunflower	0	NT	83				100				100
Lactuca sativa L.											
Lettuce, Bibb	0	NT	92			29	71				100
Lettuce, Great lakes	NT	NT	NA				100				100
Cruciferae											
Brassica juncea (L.) Czern. and Coss.											
Mustard, Florida broad leaf	0	NT	88				100				100
B. oleracea L. (Capitata group)											
Broccoli, Italian green sprout	0	NT	71				100				100
Cabbage, Charleston Wakefield	15	-	86				100				100
Cabbage, Greenback	0	NT	4				NT				NT
B. oleracea L. (Botrytis group)											
Cauliflower, Snowball	10	-	83				100				100
B. oleracea L. (Acephala group)											
Collards, Georgia	8	-	50				100				100
B. oleracea L. (Gongylodes group)											
Kohlrabi, Early white Vienna	0	NT	67				100				100
B. rapa L.											
Turnip, Purple top	0	NT	29			70	30		5	11	84
Raphanus sativus L.											
Radish, Scarlet globe short top	0	NT	71			3	97		8	13	79
Cucurbitaceae											
Citrullus vulgaris Schrad.											
Watermelon, Charleston grey	0	NT	21				NT				NT
Watermelon, Congo	11	-	79				100				100

(Continued)

Table 1 (Continued)

Family Genus, Species Common Name, Cultivar	Preemergence Percent Reduction in Germination	Preemergence Culmorum Reisolated from Tissue	Preemergence Percent Seed Viability	Postemergence Percent of Control Plants Exhibiting Symptoms LS	CR	W	H	Postemergence Percent of Culmorum Plants Exhibiting Symptoms LS	CR	W	H
Cucumis melo L.											
Cantaloupe, Hales' jumbo	38	-	46				NT				NT
Canteloupe, Smith's perfect	16	-	74				100				100
C. sativus L.											
Cucumber, Poinsett	0	-	92				100				100
C. sativus L.											
Cucumber, Marketer	NT	NT	NA				100				100
Curcurbita pepo L.											
Squash, Early prolific straightneck	41	-	79				100				100
Euphorbiaceae											
Ricinus communis L.											
Castor bean	100	-	13				100				100
Gramineae											
Lolium multiflorum Lam											
Ryegrass, Gulf annual	29‡	-	93				100				100
Paspalum notatum Fluegge											
Argentine Bahia grass	25	-	17				100				100
Secale cereale L.											
Rye, Weser	25	-	33				100				100
Setaria italica (L.) Beauv.											
Millet	0	NT	67				100				100
Sorghum vulgare Pers.											
Sorghum	0	NT	38	50			50				100

(Continued)

‡ Some seedlings of these varieties showed symptoms of collar rot, hypocotyl lesions and/or rots, or mummified cotyledons (cotyledons that did not emerge out of seed coats following germination).

Table 1 (Continued)

Family Genus, Species Common Name, Cultivar	Preemergence Percent Reduction in Germination	Preemergence Culmorum Reisolated from Tissue	Preemergence Percent Seed Viability	Postemergence Percent of Control Plants Exhibiting Symptoms LS	CR	W	H	Postemergence Percent of Culmorum Plants Exhibiting Symptoms LS	CR	W	H
Triticum aestivum L.											
Wheat, Coker	46	-	85				100				100
Wheat, Hadden	100	-	63				NT				NT
Wheat, McNair	0	NT	83				100				100
Wheat, Nebeoka	5 ‡	-	88				NT				NT
Zea mays L.											
Corn, Funks 5945	5 ‡,§	+	92				NT				NT
Corn, Bantam golden cross	18	-	92				100				100
Corn, Silver queen	0	NT	92				100				100
Leguminosae											
Arachis hypogaea L.											
Peanut, Florunner	0	NT	75				100				100
Glycine max (L.) Merr.											
Soybean, Hood	42	-	79				100		10		90
Soybean, Forrest	0	NT	92				100				100
Soybean, Pickett	29	-	88				100	19			81
Pisum sativum L.											
Pea, English acre cream	83‡	-	54				100				100
Pea, English Alaska	0	NT	88				100				100
Pea, English little marvel	10	-	88				NT				NT
Phaseolus limensis Macf.											
Butter bean, Henderson	0	NT	100			16	84				100
Lima bean, Fordhook	34‡	-	79				100				100
Lima bean, Thorogreen	100§	+	33				100				100

(Continued)

‡ Some seedlings of these varieties showed symptoms of collar rot, hypocotyl lesions and/or rots, or mummified cotyledons (cotyledons that did not emerge out of seed coats following germination).

§ Ungerminated seeds or symptomatic hypocotyls yielded the Culmorum isolate on reisolation from inoculated plants of these hosts.

Table 1 (Continued)

Family Genus, Species Common Name, Cultivar	Preemergence Percent Reduction in Germination	Preemergence Culmorum Reisolated from Tissue	Preemergence Percent Seed Viability	Postemergence Percent of Control Plants Exhibiting Symptoms LS	CR	W	H	Postemergence Percent of Culmorum Plants Exhibiting Symptoms LS	CR	W	H
P. vulgaris L.											
Pole bean, Blue lake	0	-	96			43	57			18	82
Pole bean, Dade	0	NT	62				100				100
Bush bean, Harvester	33	-	100				100				100
Bush bean, Tendergreen	4	-	96				100				100
Trifolium hybridum L.											
Clover, LA S-1	0	NT	58				100				100
Vigna unguiculata L.											
Cowpea, Knuckle purple hull	62 ‡,§	+	84				100			6	94
V. sinensis (L.) Endl.											
Blackeye pea, California #5	86	-	64				100				100
Liliaceae											
Asparagus officinalis L.											
Asparagus	NT	NT	NA				100				100
Malvaceae											
Gossypium barbadense L.											
Sea island cotton	7	-	58				100				100
Hibiscus esculentus L.											
Okra, Clemson spineless	13	-	63				100				100
Nyssaceae											
Nyssa aquatica L.											
Tupelo gum	NT	NT	NA				100				100

(Continued)

‡ Some seedlings of these varieties showed symptoms of collar rot, hypocotyl lesions and/or rots, or mummified cotyledons (cotyledons that did not emerge out of seed coats following germination).

§ Ungerminated seeds or symptomatic hypocotyls yielded the Culmorum isolate on reisolation from inoculated plants of these hosts.

Table 1 (Continued)

Family Genus, Species Common Name, Cultivar	Preemergence Percent Reduction in Germination	Preemergence Culmorum Reisolated from Tissue	Preemergence Percent Seed Viability	Postemergence Percent of Control Plants Exhibiting Symptoms LS	CR	W	H	Postemergence Percent of Culmorum Plants Exhibiting Symptoms LS	CR	W	H
Oleaceae											
Fraxinus pennsylvanica Marsh.											
Green ash	NT	NT	NA				100				100
Pinaceae											
Cedrus sp.											
Red cedar	NT	NT	NA				100				100
Picea australis Small											
Southern spruce	NT	NT	NA				100				100
Pinus caribaea Morelet											
Slash pine	NT	NT	NA				100				100
P. taeda L.											
Loblolly pine	NT	NT	NA				100				100
Platanaceae											
Platanus occidentalis L.											
Sycamore	NT	NT	NA				100				100
Polygonaceae											
Rheum rhaponticum L.											
Rhubarb	0	NT	21				100				100
Solanaceae											
Capsicum annuum L.											
Pepper, Early California wonder	13 ‡	-	67				100				100
Pepper, Yolo	0	NT	79				100				100
Lycopersicon exculentum Mill.											
Tomato, Homestead	0	NT	67				100				100
Tomato, Walter	0	NT	83				100				100

(Continued)

‡ Some seedlings of these varieties showed symptoms of collar rot, hypocotyl lesions and/or rots, or mummified cotyledons (cotyledons that did not emerge out of seed coats following germination).

Table 1 (Continued)

Family Genus, Species Common Name, Cultivar	Preemergence Percent Reduction in Germination	Preemergence Culmorum Reisolated from Tissue	Percent Seed Viability	Postemergence Percent of Control Plants Exhibiting Symptoms LS	CR	W	H	Postemergence Percent of Culmorum Plants Exhibiting Symptoms LS	CR	W	H
Nicotiana tabacum L.											
Tobacco, NN	0	NT	92				100				100
Tobacco, CG-1	0	NT	79				NT				NT
Solanum melongena L. var. *esculentum* Nees											
Eggplant, Florida strain M	0	NT	71				100				100
S. tuberosum L.											
Potato, White Kennebec.	31 ‡,§	+	100				100				100
Potato, Red LaSoda	16 ‡,§	+	100				100				100
Taxodiaceae											
Taxodium distichum (L.) L. C. Rich.											
Bald cypress	NT	NT	NA				100				100
Tetragoniaceae											
Tetragonia expansa Murr.											
New Zealand spinach	5	NT	66				100				100

(Continued)

‡ Some seedlings of these varieties showed symptoms of collar rot, hypocotyl lesions and/or rots, or mummified cotyledons (cotyledons that did not emerge out of seed coats following germination).

§ Ungerminated seeds or symptomatic hypocotyls yielded the Culmorum isolate on reisolation from inoculated plants of these hosts.

Table 1 (Concluded)

Family Genus, Species Common Name, Cultivar	Preemergence Percent Reduction in Germination	Preemergence Culmorum Reisolated from Tissue	Preemergence Percent Seed Viability	Postemergence Percent of Control Plants Exhibiting Symptoms LS	CR	W	H	Postemergence Percent of Culmorum Plants Exhibiting Symptoms LS	CR	W	H
Umbelliferae											
Apium graveolens L. var. *dulce* (Mill.) Pers. Celery, Pascal	0	NT	83				100				100
Coriander sativum L. Coriander	0	NT	33				100				100
Daucus carota L. subsp. *sativus* (Hoffm.) Arcaug. Carrot, Imperator	0	NT	79				100				100
Heracleum sp. Parsnip, Hollow crown	50	-	8				100				100

Table 2

Effect of Isolate 621 of *Fusarium roseum* Culmorum on Nontarget Aquatic Plants*

Family Genus, Species Common Name	Plant Part Tested**	Rating of Damage† at Inoculum Level per ml, × 1000 0	12.5	25	50	100	250
Amaranthaceae							
Alternanthera philoxeroides (Mart.) Griseb.							
Alligatorweed	S	1.0	NT	1.1-	NT	NT	4.3+
Ceratophyllaceae							
Ceratophyllum demersum L.							
Coontail	S	2.5	5.3+	6.0+	6.0+	6.0+	6.0+
Hydrocharitaceae							
Egeria densa Planchon							
Brazilian elodea	S	3.4	4.8-	4.4-	4.4-	5.6+	6.0+
Valisneria americana Michx.							
Eelgrass	E	1.0	NT	2.0-	1.4-	3.1-	5.2+
Najadaceae							
Najas quadalupensis (Spreng.) Magnus							
Southern naiad	S	1.0	6.0+	6.0+	6.0+	6.0+	6.0+
Nymphaeaceae							
Nuphar luteum (L.) Sibthorp & Smith							
Spatterdock	E	1.4	1.1-	1.1-	1.6-	6.0+	6.0+
Ruppiaceae							
Ruppia maritima L.							
Widgeongrass	S	0.0-	0.0-	0.0-	0.0-	0.0-	NT
Hydrilla verticillata used as control	S	1.1	5.8+	5.8+	5.8+	5.8+	5.8+

Note: The isolate caused root rot on waterhyacinth (*Eichhornia crassipes* [Mart.] Solms) at 125,000 conidia/ml level.

* The ability of the Culmorum isolate to cause chlorosis, discoloration, and/or death was rated.

** S = 15- to 20-cm terminal portion of shoot; E = entire plant, rooted in sand.

† Rating scale: 1 = healthy, 6 = dead. NT = not tested. -/+ = rating not significantly/significantly different from uninoculated control (0 inoculum level) at 0.05 level using the t-test.

Table 3

Effects of Isolate 621 of *Fusarium roseum* Culmorum on the Fish *Gambusia affinis* and on Water Quality*

Test	Oxygen Concentration, mg/ℓ, at Time, hr					pH at Time, hr		Total Alkalinity, mg/ℓ $CaCO_3$, at Time, hr		Hardness, mg/ℓ $CaCO_3$, at Time, hr		Nitrate mg/ℓ N		Nitrite mg/ℓ N		Fish Kill at 96 hr
	0	24	48	72	96	0	96	0	96	0	96	0	96	0	96	
Test 1 (Aerated)																
Control	7.4	5.8	7.8	7.8	7.4	6.9	7.2	55	56	111	111	24	25	0	0.02	0
Culmorum	6.5	7.2	7.8	7.6	7.9	7.0	7.2	56	67	118	138	24	24	0	0	2
Test 1 (Nonaearated)																
Control	7.4	4.2	4.2	5.0	6.0	6.9	7.1	55	56	111	111	24	25	0	0	0
Culmorum	6.5	3.1	2.8	5.1	4.0	7.0	7.2	58	63	118	122	24	29	0	0	4
Test 2 (Aerated)																
Control	8.2	8.0	7.8	7.8	7.8	6.8	7.0	57	57	124	128	24	26	0	0	0
Culmorum	8.1	8.0	7.9	7.8	7.8	7.0	7.2	58	60	120	122	24	26	0	0	0

* The water quality data were measured according to the methods in American Public Health Association (1975).
** There were no fish deaths prior to the 96-hr data collection.

Table 4

Decline of Isolate 621 of *Fusarium roseum* Culmorum in Hydrilla Tanks

Days After Mixing Conidial Suspension	Number of Culmorum Colonies per Millilitre of Tank Water	Percentage of Culmorum Relative to Total Microbial Colonies Recovered per Millilitre of Tank Water
0	84,000 (Inoculum level)	Not tested
7	6,426	75
14	3,488	33
21	1,360	32
28	2,432	25
35	436	17
42	555	27
49	674	31
58	593	24
63	543	18
70	41	6

Table 5

Effects of Fungicides on the Recovery of Isolate 621 of *Fusarium roseum* Culmorum from Water-Saturated Soil*

Fungicide Concentration**	Percent Reduction in Culmorum Colonies†						
	Benomyl	Captan	$CuSO_4$	Maneb	Mancozeb	TBZ	Zineb
	After Exposure for 6 Days						
1	0-	99+	19-	75+	100+	0-	0-
2	11-	72+	50+	22-	95+	0-	0-
3	12-	86+	52+	49+	91+	0-	0-
	After Exposure for 22 Days						
1	NT	99+	0-	99+	100+	51+	100+
2	24-	82+	0-	86-	100+	8-	85+
3	38+	87+	0-	46+	99+	51+	75+

* Percent reduction in the number of Culmorum colonies from fungicide treated versus nontreated control soil was the measure of fungicidal efficacy.

** Concentrations used, respectively, at levels 1, 2 and 3 were (g/ℓ): Benomyl, 3.43, 1.71, 0.86; captan and mancozeb, 6.86, 3.43, 1.71; $CuSO_4$, 10.29, 5.14, 2.57; maneb and zineb, 5.14, 2.57, 1.29; TBZ 3.34, 1.67, 0.84. Concentration level 2 is the generally recommended rate.

† Average of 10 replicates. -/+ = difference between fungicide treatment and control was not significant or significant, respectively, at 0.05 level using the t-test.

Table 6

LSPT II* Damage Rating and Number of Turions and Tubers

	Treated	Control
Mean damage rating**	2.46	2.32
Mean no. turions/shoot	0.11	0.05
No. tubers/m^2	154	125

* The fungus was applied twice, each time at the rate of 5×10^4 conidia per millilitre of treated water. The first application was on 30 Mar 81 and the second on 13 May 81. Test was completed on 3 Jun 81. The inoculum used was an aqueous suspension of conidia.

** The difference between the treated and the control was significant at the 0.05 level using the t-test.

Table 7

LSPT III* Damage Rating and Number of Turions and Tubers

	Treated	Control
Mean damage rating**	2.08	1.68
Mean no. turions/shoot	Too few to count	
No. tubers/m^2	17	41

* The fungus was applied on 1 Jul 81 and the test was completed on 11 Sep 81. The inoculum consisted of an aqueous suspension of 5×10^3 pregerminated conidia per millilitre and 8.7×10^4 ungerminated conidia per millilitre of treated water.

** The difference between the treated and the control was significant at the 0.001 level, using the t-test.

Table 8

LSPT IV* Damage Rating and Number of Turions and Tubers

	Treated	Control
Mean damage rating**	1.97	1.74
Mean no. turions/shoot	Too few to count	
No. tubers/m^2	Too few to count	

* The fungus was applied twice; the first time at the rate of 6×10^4 conidia per millilitre of treated water and the second with a 2-ℓ homogenized culture of the fungus. The test began 1 Sep 81 and ended 27 Oct 81. Inoculum consisted of an aqueous suspension of conidia (first) and a suspension of homogenized mycelia and conidia in PDB (second).

** The difference between the treated and the control was significant at the 0.02 level, using the t-test.

Table 9

LSPT V* Damage Rating

	Treated	Control
Mean damage rating**	2.02	1.99

* The test began 20 Oct 81 and ended 25 Jan 82. The inoculum was not quantitated. It consisted of 11.36 ℓ of blended agar culture of the fungus. Inoculum concentration in the tank was 0.26% (v/v).

** The difference between the treated and the control was significant at the 0.05 level, using the t-test.