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Antagonism of Trichoderma spp. to sclerotia of Typhula incarnata.

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ANTAGONISM OF *TRICHODERMA* SPP. TO SCLEROTIA OF *TYPHULA INCARNATA*

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INTRODUCTION

The ultimate accomplishment for a researcher working on plant disease control is to be able to utilize the living organisms within the environment to inhibit a pathogenic organism. The development of a successful biological control process is truly a productive advancement. The renewed awareness of humanity toward all living organisms on our planet emphasizes the need for greater efforts in controlling the use of synthetic formulations that may cause unintended damage to our environment.

This study was initiated to determine whether a practical means of biologically controlling Gray Snow Mold (Typhula incarnata Lasch ex. Fries) could be developed. In order for biocontrol to be successful, the mechanism of control must be economical and have a practical means of application on such high-value turf areas as golf greens. The overriding concern in this study is the practicality of the biological control system which utilizes the Trichoderma-Typhula interaction to achieve predictable productive results.

LITERATURE REVIEW

The Disease

Gray Snow Mold (Typhula incarnata Lasch ex. Fries) is a serious and economically important pathogen causing widespread damage on specialized turf areas and home lawns during winter months. Various other species of Typhula cause "blights" of fine turf and grains throughout the temperate regions of the United States (5, 20, 32, 34, 40, 46). Most of the serious infections occur when snow falls early in the season on unfrozen ground (4, 7, 29). Although snow cover is not essential for infection, the presence of snow favors the pathogens by maintaining relatively high humidity to permit mycelial growth (4). Under these favorable conditions wheat plants covered by snow on unfrozen ground are subject to depletion of carbohydrate reserves (7). The depletion of carbohydrates combined with an accountable respiration rate near 0°C results in a weakened plant subject to infection by low temperature organisms such as Typhula spp. Lack of light under the snow cover prevents the maintenance of adequate carbohydrate reserves.

Sclerotia are the most important sources of infection on winter barley (29). They germinate and mycelia infect the leaves and crowns of many winter cereals and cool season grasses. Grayish-white mycelia spread in a roughly circular pattern as long as environmental conditions remain favorable (11).

Infected grasses become brown and matted and may be permanently damaged if the crowns are destroyed. Whenever the microenvironmental conditions become unfavorable to the pathogen, several strands of mycelia may fuse and form a sclerotium (8, 11). Newly-formed sclerotia then serve as the over-summering bodies of the disease. Under certain conditions during the cool wet weather of autumn, sclerotia may give rise to sporophores and basidiospores. A critical factor in the production of basidiospores is the exposure of sclerotia to ultraviolet (UV) radiation in the range of 2700-3250 Å (28, 39). The diffuse light of late autumn and early winter may, therefore, permit sporulation. Basidiospores are of minor significance in relation to disease incidence (7, 29).

The two environmental factors of major importance for sclerotia germination are temperature and humidity. Temperatures between 2-10°C and high humidity levels are necessary for sclerotia to germinate in large numbers (28). Light quality or intensity has no effect on sclerotia germination but adequate exposures to ultraviolet wavelengths may cause sporogenic rather than mycelio-genic germination. Heavy soils reduce disease incidence of Typhula incarnata on winter barley (29). It is suggested that this reduction is due to the increased competition for nutrients and the fungistatic factors which predominate in heavy soils. Unlike sclerotia of Typhula idahoensis, which do not infect wheat plants when buried in soil at depths greater than 1 cm (13), sclerotia of T. incarnata will infect winter barley at depths greater than 2 cm (29). Since T. incarnata

is able to infect roots and is less dependent on aerial tissues, its sclerotia are often found deeper in the soil and are potentially more susceptible to microorganismal activity.

The survival and perpetuation of Typhula spp. have been attributed to an unknown dormancy mechanism which allows adequate numbers of sclerotia to survive adverse environmental conditions (13, 14, 28). Dormancy is usually overcome during late autumn or winter. This type of dormancy has been described as constitutive dormancy which can be broken by some environmental stimuli not usually required for normal vegetative growth (9). The induction of dormancy of this type is the result of some innate property of the organism and not due to changes in environmental conditions. During dormancy the sclerotia are subject to predation by nematodes, earthworms, centipedes, snails, gall midge larvae, mites, bacteria and fungi (9). Reference to unknown microorganismal antagonism is made frequently in research on Typhula spp. (14, 21, 28). Successful attacks by nonpathogenic organisms on Typhula sclerotia could eliminate large portions of inoculum during the dormant stages of the disease cycle.

Disease Control

Whenever prevention of Typhula blight on turf is desired, chemical applications during late autumn and early winter generally provide adequate control. Economic considerations usually prevent chemical applications on any turf areas not highly valuable. If environmental laws permit, phenyl mercury formulations

are used with good results (34). Cadmium compounds and organo-chlorines have also provided excellent disease control. Relatively little work has been done to explore alternative means of control. Lebeau (27) was able to control damage by an unidentified low-temperature Basidiomycete and Typhula spp. by maintaining soil temperatures of 0°C with soil-heating cables. Host resistance has been reported as a viable means of minimizing damage to grains and fine turf. Significant host resistance to Typhula idahoensis, Typhula incarnata and Fusarium nivale was shown on selected varieties of winter wheat (5, 6). Vargas (48) has compared 56 Kentucky bluegrass varieties to determine their susceptibility and resistance to Typhula itoana and Fusarium nivale. Appreciable resistance does exist and breeding-improved species may improve resistance.

Naturally occurring microorganismal inhibition of sclerotia germination may be a significant factor in preventing disease development. Significant decreases in sclerotia numbers of Typhula idahoensis were reported when legumes were used as a cover crop (21). Apparently, bacteria associated with the legumes were capable of colonizing sclerotia surfaces and inhibiting germination. The authors suggested that certain crop rotations may act as biological control mechanisms. Both lipids and insoluble carbohydrates appear to be important storage compounds in Typhula spp. (44). Since Typhula is dormant during the warmer months and its inoculum potential is fixed, the greatest hazard to its survival exists while soil microbial activity is at its peak. Many soil-borne organisms are capable of utilizing these storage compounds as a nutrient source.

The Antagonist

Various types of parasitism, antagonism, and fungistasis between soil-borne pathogens and other common soil fungi have been reported (16, 17, 24, 25, 26, 31, 37, 42, 43, 52, 53, 54). Many researchers have found toxic filtrate compounds and antibiotics produced by fungi that are inhibitory to other fungi (3, 16, 49, 53). Trichoderma spp. are frequently reported as antagonists of soil-borne fungi (23, 26, 30, 37, 43). Since sclerotia are the main source of infection for Typhula spp., antagonism toward them is essential if reduction of inoculum potential is desired. Antagonism to sclerotia by Trichoderma has been reported (43, 54). Sclerotia formation of Rhizoctonia solani is inhibited when Trichoderma lignorum (Tode) Harz is present (43). The inhibition was attributed to the production of some unknown inhibitory chemicals by Trichoderma lignorum. When plots of blue lupines, tomatoes, and peanuts growing under natural field conditions were inoculated with Trichoderma harzianum, damage due to Sclerotium rolfsii was significantly reduced (54). This biocontrol technique is one of the few successful field trials employing fungal interaction.

One important problem that has made work with Trichoderma spp. more difficult is the confusion and inconsistency in the taxonomy of the genus. Several workers have attempted to describe the precise species with which they have worked (16, 54). Rifai (41) revised the nomenclature of the genus in 1969 and grouped several strains of each species into "species aggregates". Emphasis in identification is placed on the microscopic characteristics of the conidiophores, phialides and phialospores. Some credence may be placed on the odor of certain colonies (41).

Growth Requirements of *Trichoderma* Spp.

Optimum growing conditions for many species of *Trichoderma* are fulfilled by soils of temperate regions during warm seasons. Temperature, moisture, pH and nutrient requirements are not so specific as to be prohibitive when considering the fungus as a potentially useful antagonist. *Trichoderma viride* has been found in natural soils under moderately acid conditions (pH 5.0 to 6.0) and grows well in culture on acidified media. *Trichoderma lignorum* has no requirement for vitamins but requires a carbon and nitrogen source for spore germination (33). Any vitamin requirement is fulfilled through natural synthesis. Soil temperatures ranging from 15-25°C will sustain the growth of *Trichoderma* although spores will germinate in culture at temperatures as low as 2°C. Populations of Trichoderma spp. increase as cellulose amendments are added to soil (49). Sanford (43) noted that sclerotia production was not inhibited by *T. lignorum* in natural soils without additions of corn meal. It appears economically feasible soil amendments may enhance the usefulness of *Trichoderma* spp. as an antagonist.

Biological Control

Certain difficulties in establishing the existence of a viable organism for biological disease control may be overcome by defining biological control as developed in the context of these experiments. Biological control exists when a reduction in inoculum potential or infection centers of a pathogen occurs (23). This reduction is brought about directly or indirectly by other biological agencies. A reduction in sclerotia numbers is a fundamental step in attaining biological

control. Sclerotia of Typhula incarnata have not been used as test organisms in studies of Trichoderma antagonism. Sclerotia formation was inhibited in studies involving other organisms (43, 54).

Survival of sclerotia in the soil is dependent upon several factors. Moisture, temperature, aeration, soil reaction, soil organic matter and activity of microorganisms affect the longevity of sclerotia in the soil (9). The interaction of these factors is generally more important than any single factor. Sclerotia of many fungi are resistant to dessication and changes in soil temperature and are not affected by typical oxygen concentrations in soil (9). Additions of nitrogenous soil amendments may reduce the number of viable sclerotia in soil (19). Ammonia was the only compound to affect viability directly. Other compounds may have stimulated fungistatic action by increasing antagonistic activity at the sclerotia-soil interface. The sclerotia of Typhula incarnata are in a dormant and oversummering stage when air temperatures are greater than 10°C. Simultaneously, the great majority of potential antagonists, including Trichoderma spp., are initiating growth.

EXPERIMENT I

EFFECT OF TRICHODERMA SPP. ON TYPHULA INCARNATA SCLEROTIA ON ARTIFICIAL MEDIA (PDA)

Procedure

Two isolates of Trichoderma spp. were taken from soil in the Amherst, Massachusetts area. Identification of these species was made after colonies were grown on acidified potato-dextrose-agar (PDA) for three days at 20°C. Microscopic characteristics of the conidiophores, phialides and phialospores in conjunction with a key developed by Rifai (41) were used for species identification. One of the isolates was identified as Trichoderma konigii. The dark green spore mass, phialides grouped in whorls of four or five, and smooth-walled phialospores were the essential characters involved in identification. The second isolate had a pale green spore mass, phialides grouped in false whorls generally consisting of fewer than four, and phialospores with rough walls characteristic of T. viride. This species will be referred to as T. viride I since another culture of T. viride was obtained from the American Type Culture Collection (ATCC) and will be referred to as T. viride II. Any differences between these strains will then be noted separately. A fourth culture, T. harzianum, was also purchased from ATCC. All stock cultures of Trichoderma spp. and Typhula incarnata were grown on PDA (pH 5.7 to 6.0).

Agar was made with filtrate from 200 g of potatoes boiled 15 minutes, 1.5% bacto-agar and 2.0% bacto-dextrose. The solution was autoclaved 15 min at 15 psi in 500 ml Erlenmeyer flasks. Streptomycin sulfate (100 µg/ml) was added after autoclaving. Pyrex petri plates (100mm X 20mm) were sterilized with dry heat (230°C) for several hours before use. Forty ml of PDA were poured in each plate and allowed to cool. Ten sclerotia of T. incarnata were placed in each plate; 20 plates in each treatment. The treatments were: (i) 10 sclerotia of T. incarnata and 1000-2000 spores of T. konigii streaked on the agar away from the sclerotia; (ii) T. incarnata and T. viride I; (iii) T. incarnata and T. harzianum; (iv) T. incarnata and T. viride II; (v) a control with no Trichoderma inoculation. The plates were then placed in an incubator for six days at 20°C. Mycelial growth of each species of Trichoderma spread throughout the plates and sporulation was abundant at the end of the incubation period. The sclerotia were then removed from the plates and surface sterilized for 90 seconds using a 1:1 solution of 95% ethyl alcohol and 5% sodium hypochlorite. Sclerotia were then rinsed for 30 sec in distilled, deionized water and blotted dry. They were then placed on freshly poured PDA plates and incubated for 30 days at 5°C. The numbers of viable sclerotia were recorded as they germinated. The sclerotia that were not subject to Trichoderma inoculation were also surface sterilized and served as the control for comparing sclerotia viability.

Results

All of the plates were totally overgrown with mycelium and spores of each Trichoderma spp. by the sixth day and most by the third day of incubation. Many of the sclerotia of T. incarnata were covered with the green spores typical of each species of Trichoderma. Some of the infected sclerotia were soft and easily crushed with the tip of a dissecting needle. Surface sterilization was often not sufficient to eliminate the growth of Trichoderma and after several days of incubation at 5°C the colonies could be seen moving out of the sclerotia. The results in Table 1 show that death of the infected sclerotia was almost totally complete while the uninoculated checks had excellent germination percentages. Statistical analysis of the data was not carried out due to the magnitude of the difference between the control and the treated plates and because of unequal variances associated with 100% infected treatments.

The most rudimentary step in the investigation of the antagonistic properties of an organism as they relate to biological control must occur on artificial media in the laboratory. The overwhelming success of this initial test suggested that a similar test performed under conditions more representative of those found in the field would indicate conclusively the merits of the antagonist.

TABLE 1

EXPERIMENT I

NUMBERS OF GERMINATING SCLEROTIA TREATED WITH

TRICHODERMA SPP. AFTER 30 DAYS INCUBATION

Treatments	Days			% Germination
	10	20	30	
Control	186	191	191	95.5
T. konigii	0	1	3	1.5
T. viride I	0	0	0	0.0
T. harzianum	0	0	0	0.0
T. viride II	0	0	0	0.0

Data represent total number of viable sclerotia (200=100%).

EXPERIMENT II

EFFECT OF TRICHODERMA SPP. ON TYPHULA INCARNATA SCLEROTIA ON NATURAL AND AMENDED SOILS

Procedure

Equal weights of loam and sand were combined to yield 3 Kg of sand-loam mix. One-half of this soil was wetted to field capacity and autoclaved at 121°C and 15 psi for one hour. Seventy-five grams of corn meal were then added to one-half (750 g) of the sterile soil and one-half (750 g) of the nonsterile soil. Fifty grams of unamended, nonsterile soil were added to each of 15 dry sterilized petri plates; equal weights of corn meal amended nonsterile soil, unamended sterile soil, and corn meal amended sterile soil were each added to 15 petri plates for a total of 60 plates. Five sclerotia of Typhula incarnata were placed in each plate. The treatments were as follows:

Unamended Nonsterile Soil

(i) Control - No Trichoderma inoculation

(ii) 1000-2000 spores of T. konigii in soil

(iii) 1000-2000 spores of T. viride I in soil

(iv) 1000-2000 spores of T. harzianum in soil

(v) 1000-2000 spores of T. viride II in soil

The same treatments were repeated in the amended nonsterile soil, unamended sterile soil and the amended sterile soil. Each treatment was replicated three times. The plates were then incubated 10 days at 20°C with nine hours of fluorescent light (800 foot-candles). At the end of the incubation period the sclerotia were removed from the plates and surface sterilized. They were then placed on freshly poured PDA plates and incubated for 30 days at 5°C. The numbers of viable sclerotia were recorded as they germinated. The entire experiment was repeated three times and data were collected for each repetition.

Results

Sterile vs. Nonsterile Soil

At the conclusion of the incubation period at 20°C many of the sclerotia in the treated plates were covered with spores of the respective Trichoderma spp. Some sclerotia were soft and easily crushed just as they were in the experiment on artificial media. Recolonization of the autoclaved soil by Trichoderma was very rapid and all the sclerotia were parasitized by the fifth day. The plates inoculated with T. harzianum seem to have the most prolific spore production on both sterile and nonsterile soils. Germination of sclerotia in the controls ranged from 73-100% and averaged 95% in the sterile soil treatments and 87.5% in the natural soil (Table 2). None of the sclerotia of T. incarnata germinated on sterile soil in the treated plates and only 5 of the 120 germinated on natural soils.

Amended vs. Nonamended

Both sets of treatments containing corn meal produced a considerably larger number of spores of every species of Trichoderma than the unamended treatments. On sterile soil, growth of Trichoderma was rapid and the entire plate was covered with green spores. Nonsterile soil treatments with corn meal added encouraged growth of other fungi present. Several plates were filled with Mucorales initially but at the conclusion of the incubation period Trichoderma appeared to dominate the plates. No other species of fungi was

isolated from the sclerotia after surface sterilization and incubation at 5°C. There were no significant differences between the amended and nonamended treatments.

TABLE 2

EXPERIMENT II

SERIES I

NUMBER OF VIABLE SCLEROTIA OF T. INCARNATA INOCULATED WITH TRICHODERMA SPP. ON STERILE SOIL, NATURAL SOIL, UNAMENDED SOIL AND AMENDED SOIL

	Treatments	Days 10	20	30	% Germination
			Sterile Soil		
No corn meal	Control	13	14	14	93.3
	T. konigii	0	0	0	
	T. viride	0	0	0	
	T. harzianum	0	0	0	
	T. viride II	0	0	0	
Corn meal added 5 g/50 g soil	Control	14	15	15	100
	T. konigii	0	0	0	
	T. viride I	0	0	0	
	T. harzianum	0	0	0	
	T. viride II	0	0	0	
			Natural Soil		
No corn meal	Control	11	13	13	93
	T. konigii	0	0	0	
	T. viride I	0	0	0	
	T. harzianum	0	0	0	
	T. viride II	0	0	0	
Corn meal added 5 g/50 g soil	Control	10	11	11	73
	T. konigii	0	0	0	
	T. viride I	0	0	0	
	T. harzianum	0	0	0	
	T. viride II	0	0	0	

Data represent total number of viable sclerotia (15 = 100%) after 30 days incubation at 5°C.

TABLE 3

EXPERIMENT II

SERIES II

NUMBER OF VIABLE SCLEROTIA OF T. INCARNATA INOCULATED WITH TRICHODERMA SPP. ON STERILE SOIL, NATURAL SOIL, UNAMENDED SOIL AND AMENDED SOIL

	Treatments	Days 10	20	30	% Germination
		Sterile Soil			
No corn meal	Control	12	15	15	100
	T. konigii	0	0	0	0
	T. viride I	0	0	0	0
	T. harzianum	0	0	0	0
	T. viride II	0	0	0	0
Corn meal added 5g/50g soil	Control	8	12	12	80
	T. konigii	0	0	0	0
	T. viride I	0	0	0	0
	T. harzianum	0	0	0	0
	T. viride II	0	0	0	0
		Natural Soil			
No corn meal	Control	11	13	13	87
	T. konigii	0	1	1	7
	T. viride I	0	0	1	7
	T. harzianum	0	0	0	0
	T. viride II	0	0	0	0
Corn meal added 5g/50g soil	Control	10	14	14	93
	T. konigii	0	0	0	0
	T. viride I	0	0	0	0
	T. harzianum	0	0	0	0
	T. viride II	0	0	0	0

Data represent total number of viable sclerotia (15-100%) after 30 days incubation at 5°C.

TABLE 4

EXPERIMENT II

SERIES III

NUMBER OF VIABLE SCLEROTIA OF T. INCARNATA INOCULATED WITH TRICHODERMA SPP. ON STERILE SOIL, NATURAL SOIL, UNAMENDED SOIL AND AMENDED SOIL

	Treatments	Days 10	Days 20	Days 30	% Germination
			Sterile Soil		
No corn meal	Control	15	15	15	100
	T. konigii	0	0	0	0
	T. viride I	0	0	0	0
	T. harzianum	0	0	0	0
	T. viride II	0	0	0	0
Corn meal added 5g/50g soil	Control	13	14	14	93
	T. konigii	0	0	0	0
	T. viride I	0	0	0	0
	T. harzianum	0	0	0	0
	T. viride II	0	0	0	0
			Natural Soil		
No corn meal	Control	12	13	13	87
	T. konigii	0	1	2	13
	T. viride I	0	0	0	0
	T. harzianum	0	0	0	0
	T. viride II	0	1	1	7
Corn meal added 5g/50g soil	Control	13	15	15	100
	T. konigii	0	0	0	0
	T. viride I	0	0	0	0
	T. harzianum	0	0	0	0
	T. viride II	0	0	0	0

Data represent total number of viable sclerotia (15=100%) after 30 days incubation at 5°C.

TABLE 5

COMPOSITE OF SERIES I, II AND III

NUMBER OF VIABLE SCLEROTIA OF T. INCARNATA INOCULATED WITH TRICHODERMA SPP. ON STERILE SOIL, NATURAL SOIL, UNAMENDED SOIL AND AMENDED SOIL

	Treatments	Days			% Germination
		10	20	30	
		Sterile Soil			
No corn meal	Control	40	44	44	98
	T. konigii	0	0	0	0
	T. viride I	0	0	0	0
	T. harzianum	0	0	0	0
	T. viride II	0	0	0	0
Corn meal added 5 g/50 g soil	Control	35	41	41	91
	T. konigii	0	0	0	0
	T. viride I	0	0	0	0
	T. harzianum	0	0	0	0
	T. viride II	0	0	0	0
		Natural Soil			
No corn meal	Control	34	39	39	87
	T. konigii	0	2	3	7
	T. viride I	0	0	1	2
	T. harzianum	0	0	0	0
	T. viride II	0	1	1	2
Corn meal added 5 g/50 g soil	Control	33	40	40	88
	T. konigii	0	0	0	0
	T. viride I	0	0	0	0
	T. harzianum	0	0	0	0
	T. viride II	0	0	0	0

Data represent total number of viable sclerotia (45 = 100%).

EXPERIMENT III

PRODUCTION OF ANTIBIOTICS AND ANTAGONISM AT 9.5°C

Procedure

In order to determine whether any of the four Trichoderma spp. used in this study were producing any antibiotics which would inhibit mycelial growth or sclerotia production of T. incarnata, a laboratory experiment examining the respective growth rates of each fungus at mutually tolerable temperatures was devised. Five 100 mm petri plates containing one sclerotium each served as the control for T. incarnata. Five plates each of Trichoderma konigii, T. viride I, T. harzianum and T. viride II inoculated with 100-500 spores of the respective species served as controls also. A single sclerotium of T. incarnata was placed at one side of each of 20 plates and each species of Trichoderma was placed at the opposite side of the plates with five plates used for each species. There was a total of 45 plates which were incubated at 9.5°C for 18 days under a 14-hr dark/8-hr light cycle of 200 ft-c.

After five days the radial growth in each plate was recorded. Measurements of mycelial growth were then taken every second day for the next 12 days. The numbers of sclerotia produced by T. incarnata were also recorded.

Results

The colony radii of T. incarnata and Trichoderma spp. grown individually and in competition with each other appear in Table 3. Final average growth rates are also listed in Table 3. A comparison of the growth rate of T. incarnata growing individually and in competition with Trichoderma spp. appears in Figures 2 and 3. The useful comparisons in this experiment were those between T. incarnata grown alone and with each Trichoderma spp., respectively. There were no significant differences between any of these groups. However, growth of each Typhula colony ceased upon contact with mycelium of Trichoderma spp. and was subsequently overgrown with Trichoderma. Production of sclerotia was almost totally inhibited by Trichoderma while in the control plates sclerotia were produced. Sporulation was abundant in all plates containing Trichoderma spp. and all attempts to reisolate the test host were unsuccessful.

The system of simultaneous growth of these two fungi is a highly unlikely natural phenomenon since optimum growing temperatures for each organism are quite disparate. Since the overwhelming majority of microorganismal activity occurs during the warmer months, antagonistic activities directed at Typhula spp. must, by necessity, be directed at dormant sclerotia.

TABLE 6

EXPERIMENT III

MEASUREMENTS OF COLONY RADII OF TYPHULA INCARNATA AND TRICHODERMA SPP. GROWN INDIVIDUALLY AND IN COMPETITION AT 10°C

Colonies	Days							Average mm/day
	5	7	9	11	13	15	17	
T. incarnata	7	11	12	13	19	20	22	1.29
	1	3	8	11	13	14	20	1.27
	8	11	12	14	17	25	28	1.64
	3	6	12	12	16	22	32	1.88
	0	3	7	9	14	19	28	1.64
Average	4	7	10	12	14	18	26	
T. konigii	0	12	55	62	67	73	90	5.29
	2	8	14	18	24	44	90	5.29
	2	8	15	20	28	35	65	3.82
	2	16	30	32	43	51	90	5.29
	3	9	12	12	15	38	65	2.64
Average	2	10	25	29	35	48	80	
T. viride I	4	6	15	17	25	33	53	3.11
	3	6	15	19	26	36	61	3.58
	2	6	15	18	26	37	55	3.23
	3	5	14	15	22	33	58	3.41
	3	8	13	15	20	31	40	2.35
Average	3	6	14	17	24	34	53	

Data represent radial growth in millimeters.

TABLE 6 continued

EXPERIMENT III

MEASUREMENTS OF COLONY RADII OF TYPHULA INCARNATA AND TRICHODERMA SPP. GROWN INDIVIDUALLY AND IN COMPETITION AT 10°C

Colonies	Days							Average mm/day
	5	7	9	11	13	15	17	
T. harzianum	2	10	14	19	26	39	48	2.82
	2	8	13	18	23	39	50	2.94
	9	12	21	24	30	43	45	2.64
	6	11	17	23	31	44	57	3.35
	2	8	15	19	24	45	90	5.29
Average	4	12	16	20	27	42	58	
T. viride II	6	13	35	40	48	49	66	3.88
	10	15	26	29	35	47	54	3.17
	10	12	23	27	31	43	49	2.88
	11	16	24	28	35	43	52	3.06
	9	15	26	27	29	50	90	5.29
Average	9	14	27	30	35	46	62	
T. incarnata - T. konigii	3-2	7- 8	12-12	13-15	21-16	25-22	25-25	1.47-1.47
	2-1	5- 5	7-10	10-11	13-14	22-20	26-28	1.53-1.64
	1-2	3-11	9-14	6-14	9-16	10-22	11-25	0.65-1.47
	1-3	3-13	4-16	6-21	6-25	15-39	18-57	1.06-3.35
	0-1	1- 6	3-12	4-20	6-23	14-25	16-28	0.94-1.64
Average	1-2	4- 8	7-13	8-16	11-19	17-25	19-32	

Data represent radial growth in millimeters.

TABLE 6 continued

EXPERIMENT III

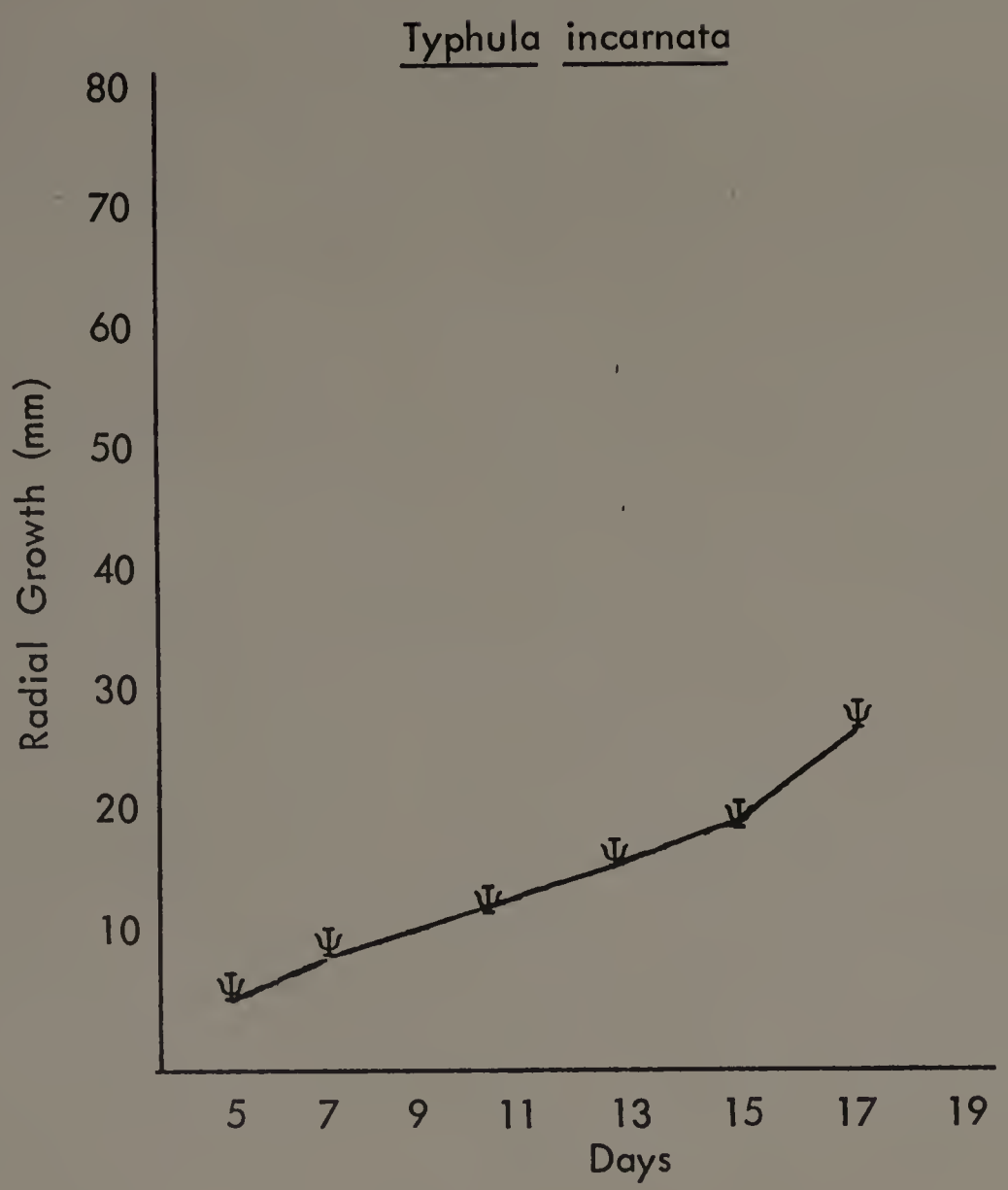
MEASUREMENTS OF COLONY RADII OF TYPHULA INCARNATA AND TRICHODERMA SPP. GROWN INDIVIDUALLY AND IN COMPETITION AT 10°C

Colonies	Days							Average mm/day
	5	7	9	11	13	15	17	
T. incarnata - T. viride I	1- 1	6- 6	10-15	12-16	16-22	25-35	28-43	2.53-1.64
	2- 1	10- 5	12-12	17-18	18-25	27-38	29-40	2.35-1.70
	3- 1	10- 3	10-20	11-22	12-57	15-70	15-80	4.70-0.88
	5- 1	9- 4	11-17	14-21	17-30	21-41	26-53	1.53-3.11
	2- 1	9- 8	11-14	14-20	20-29	27-39	29-50	2.94-1.70
Average	2- 1	9- 5	11-15	14-20	16-32	23-46	25-54	
T. incarnata - T. harzianum	0- 2	2- 8	4-16	5-22	8-26	15-41	21-52	3.06-1.28
	2- 2	5-10	8-16	13-24	17-28	23-42	25-60	3.51-1.47
	1- 1	4- 4	7-20	8-26	12-33	21-47	20-61	3.58-1.27
	2- 2	6- 6	11-10	14-22	17-25	25-60	25-80	4.70-1.47
	3- 8	6- 7	13-15	16-21	18-27	26-40	29-53	3.11-1.70
Average	1- 3	4- 7	9-15	11-23	14-28	22-46	24-61	
T. incarnata - T. viride II	4- 8	7-17	11-21	14-25	16-30	20-32	25-35	2.06-1.47
	5- 8	8-19	13-30	15-33	20-41	21-47	30-55	3.23-1.76
	3-10	9-16	11-25	16-26	18-30	28-40	30-47	2.76-1.76
	5- 9	6-16	10-25	10-30	10-40	10-46	20-49	2.88-1.27
	5- 4	11-13	14-22	16-22	22-23	22-61	25-61	3.58-1.47
Average	4- 8	8-14	12-24	14-27	17-32	20-45	26-49	

Data represent radial growth in millimeters.

FIGURE 1

MEASUREMENTS OF COLONY RADII OF TYPHULA INCARNATA AND TRICHODERMA SPP. GROWN INDIVIDUALLY AND IN COMPETITION AT 9.5° C



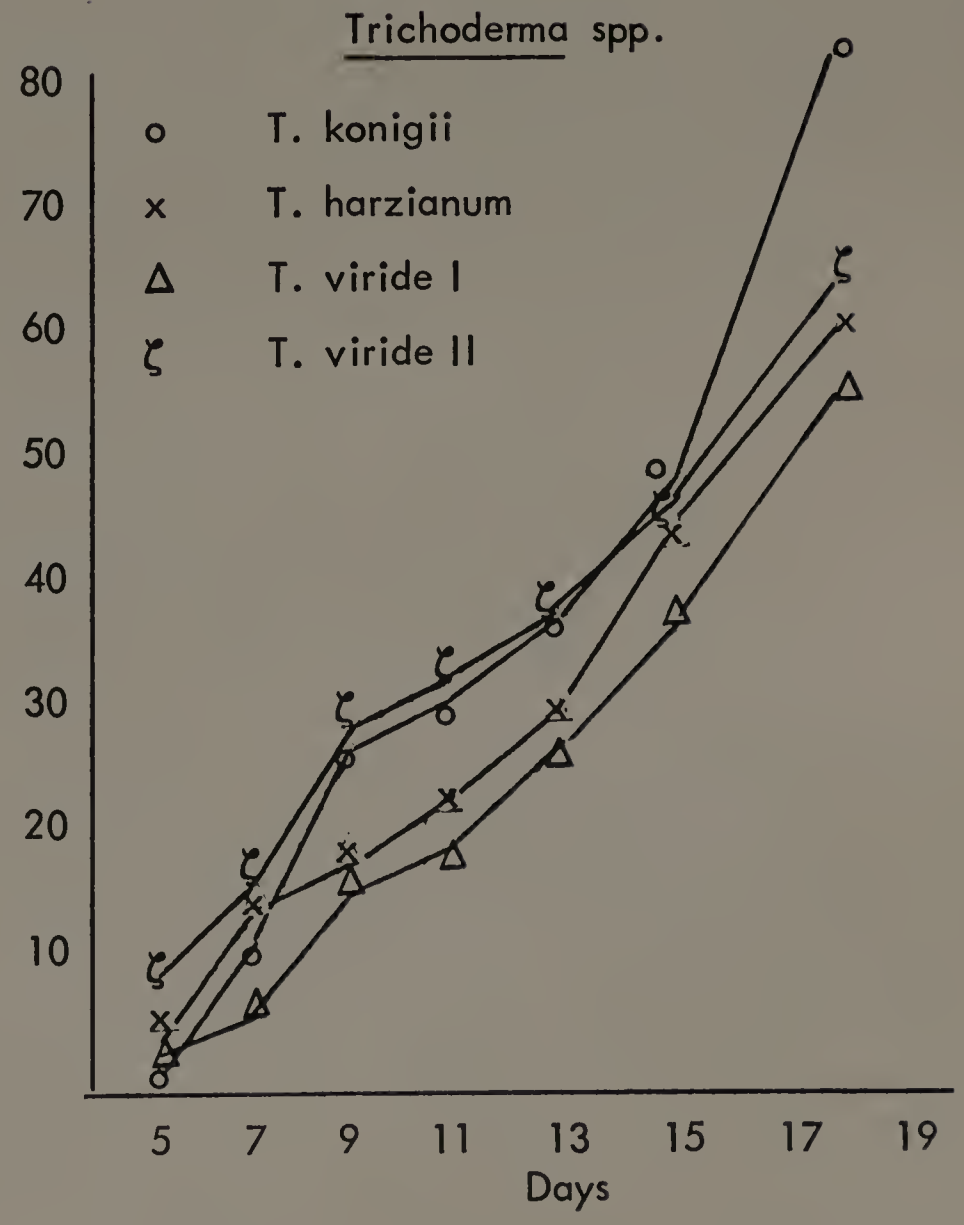
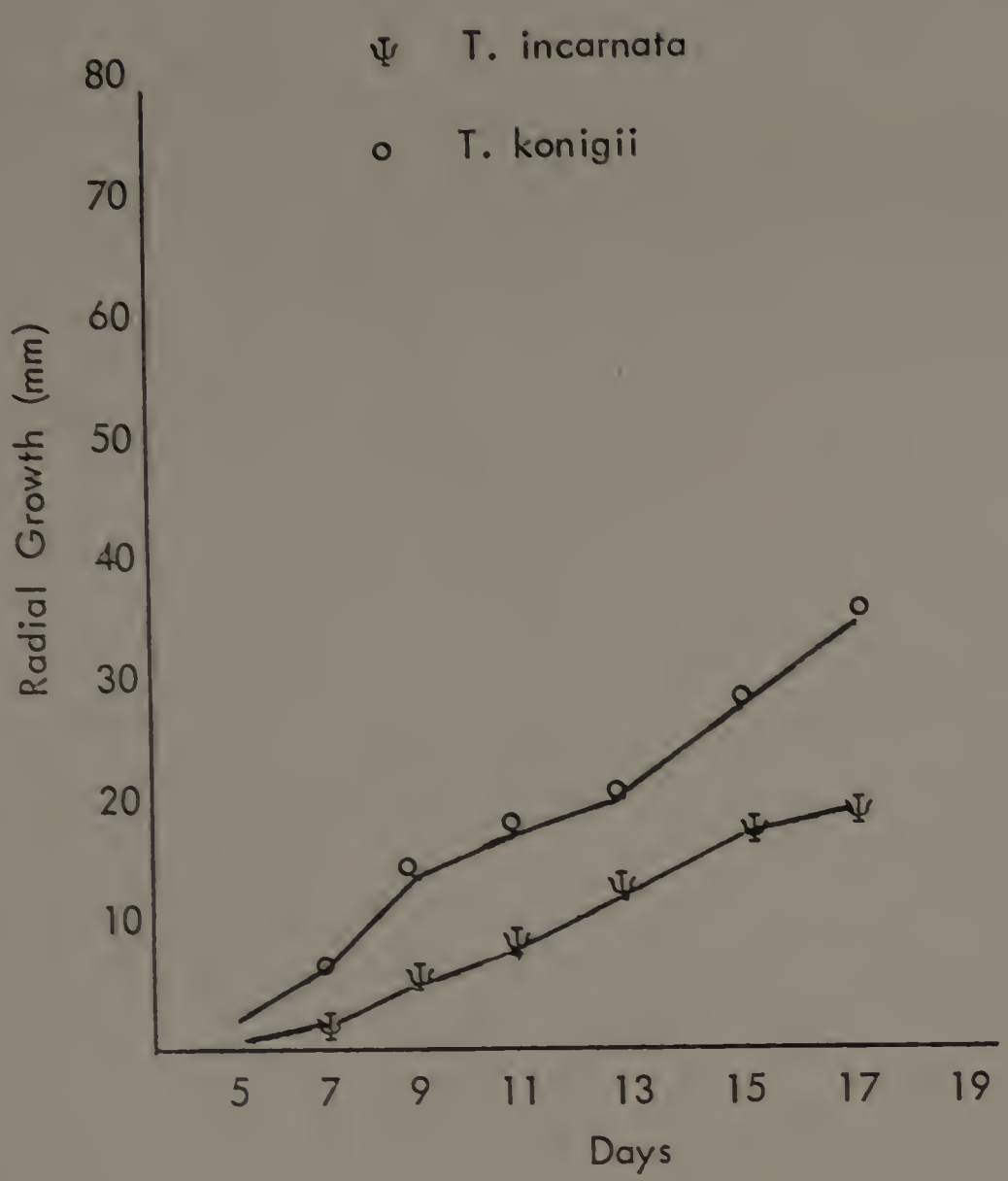


FIGURE 2

MEASUREMENTS OF COLONY RADII OF TYPHULA INCARNATA AND TRICHODERMA SPP. GROWN INDIVIDUALLY AND IN COMPETITION AT 9.5° C



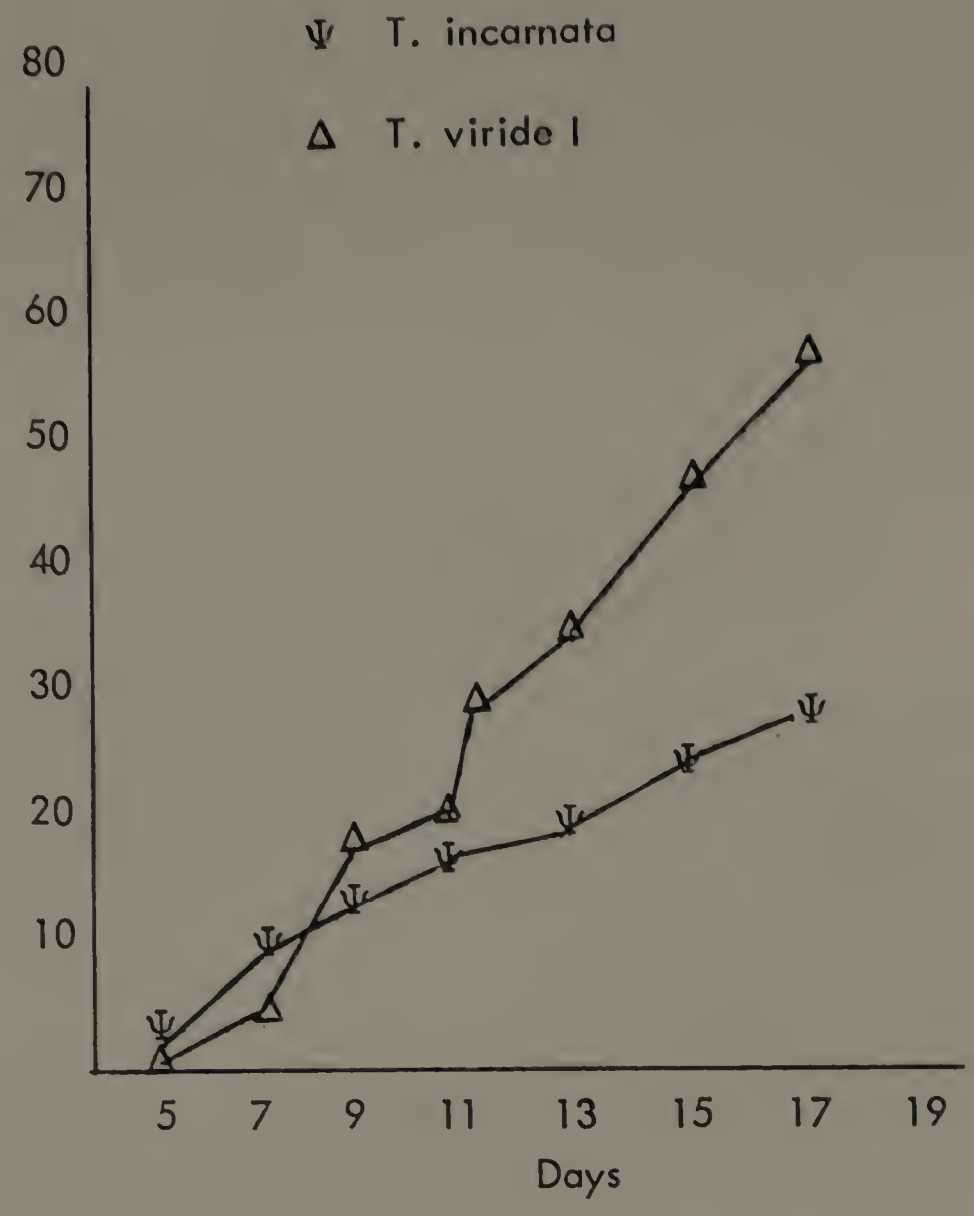
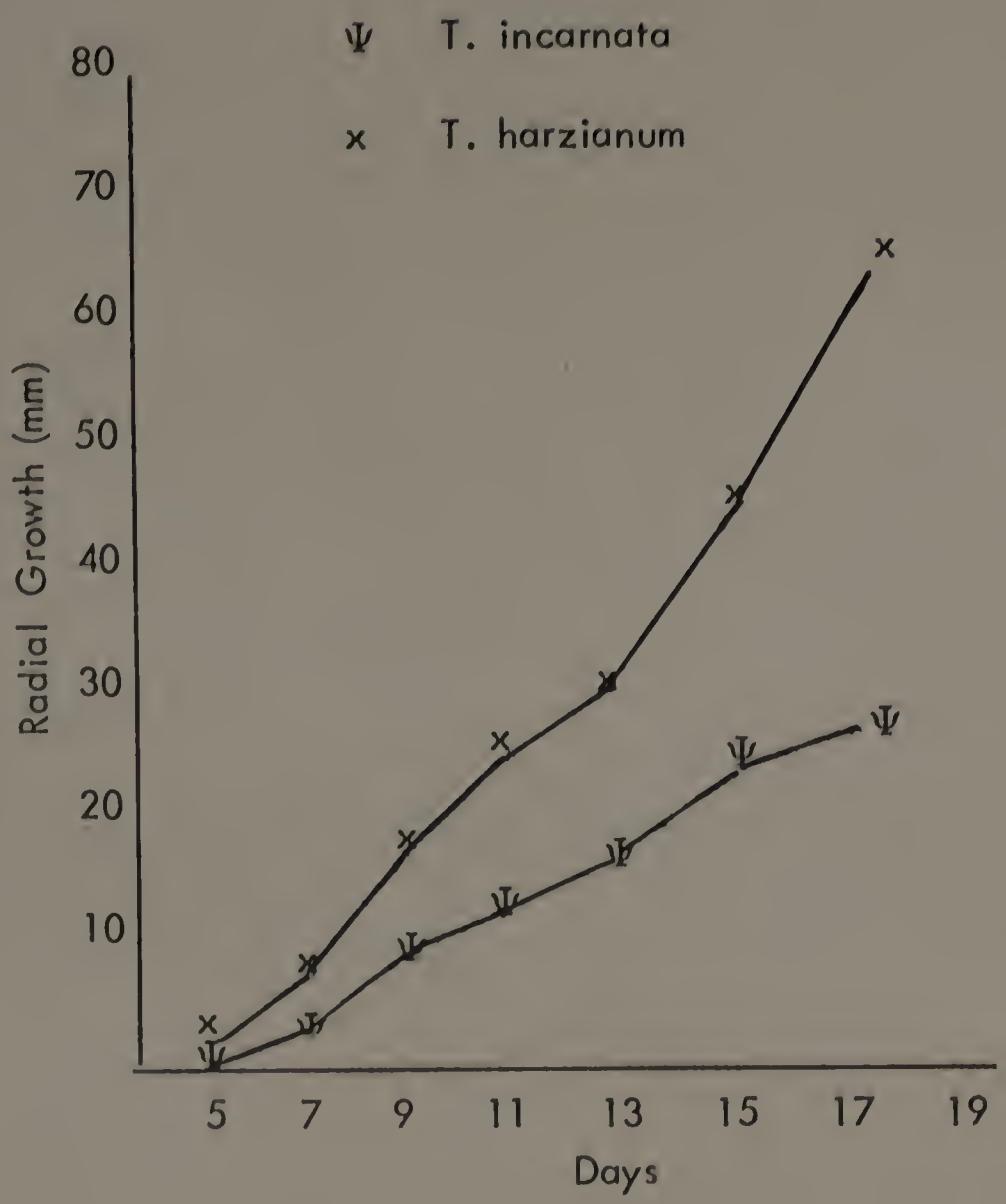
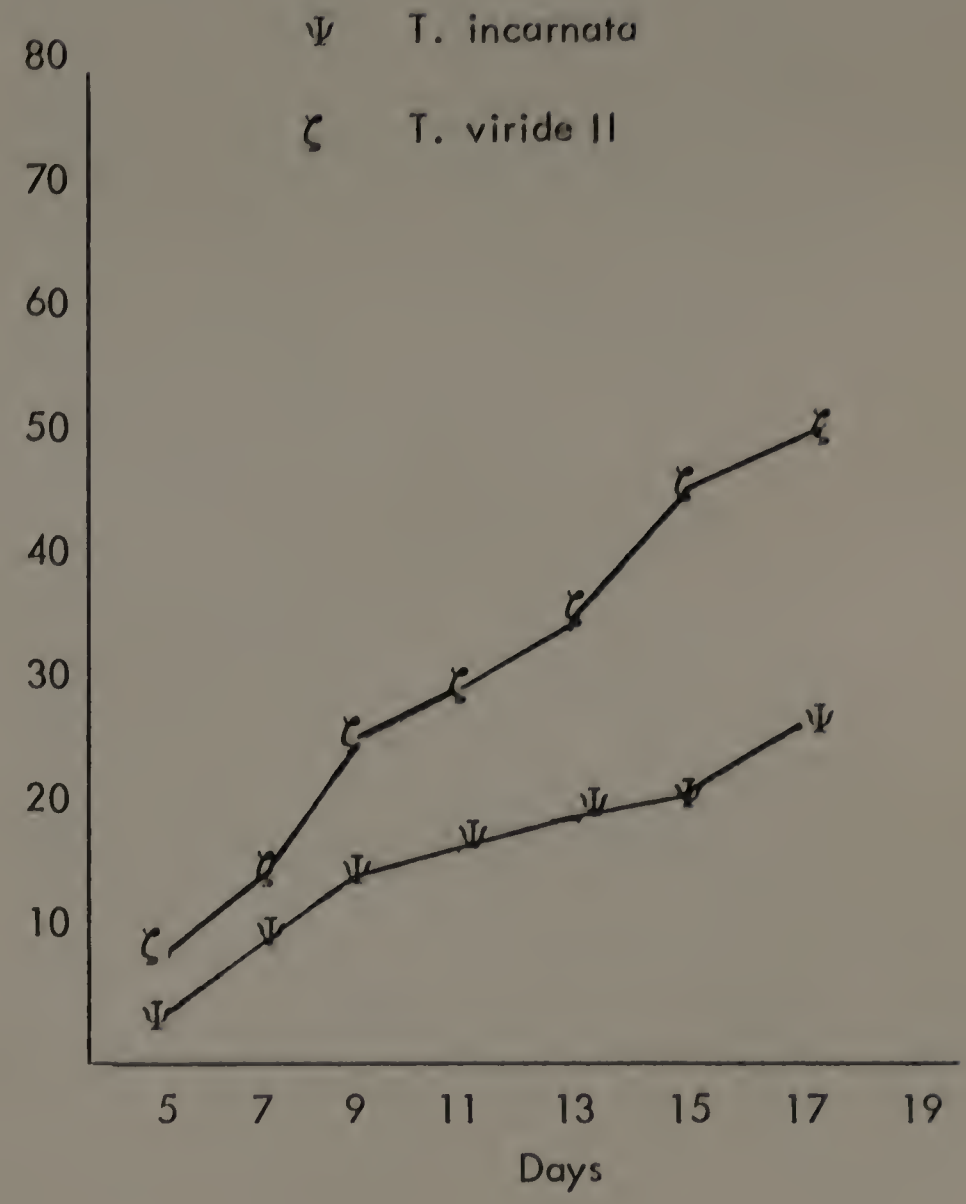


FIGURE 3

MEASUREMENTS OF COLONY RADII OF TYPHULA INCARNATA AND TRICHODERMA SPP. GROWN INDIVIDUALLY AND IN COMPETITION AT 9.5° C





EXPERIMENT IV

GREENHOUSE - GROWTH CHAMBER STUDY

Procedure

Penncross creeping bentgrass (Agrostis palustris) was seeded in the field on Walpole fine sandy loam and maintained at 1/2-inch mowing height throughout the growing season. In November, 4-inch turf plugs were taken from the plots and transplanted in 5-inch plastic pots. The remaining volume in the pots was filled with additional sandy loam. The pots were then maintained in the greenhouse until needed.

Sclerotia of T. incarnata used in this experiment were obtained by growing the fungus on a 7:1 asceptic mixture of ground oats and corn meal moistened with a 2% dextrose solution. This procedure produced sclerotia in abundant quantities required for pot inoculations. Fifty to one hundred sclerotia per pot were used in each treatment requiring inoculation. All Trichoderma spp. used in this experiment were produced as in previous experiments.

In order to effectively reduce the inoculum potential of T. incarnata, Trichoderma spp. must proliferate sufficiently to attack most of the dormant sclerotia. Since corn meal amendments have enhanced the growth of Trichoderma spp. (43) and since it is potentially an economical and practical soil amendment, it was used in selected treatments in this experiment. To avoid physical contact

between pathogen and antagonist during pot inoculations a 3-day interval was used between sclerotia planting and Trichoderma inoculations. There were 12 treatments (A-L) with 4 pots per treatment. Treatments A-D served as various types of controls so that infected pots could be indexed against check pots. Specifically, Treatment A was a greenhouse check with no sclerotia added. Treatment B was a greenhouse check with sclerotia added. Treatments C and D were brought from the greenhouse, placed at 5°C and inoculated with mycelia and sclerotia, respectively. In treatments E-H, 1000-2000 spores of T. viride I (E), T. konigii (F), T. harzianum (G) and T. viride II (H) were mixed with a small volume of sterile sand and sprinkled over each pot. Sufficient water was applied to each pot to wash all sand to the soil surface. Three days later, 25 to 50 sclerotia of T. incarnata were placed in each pot. In treatments I-L the same procedure was followed as in E-H with the addition of 5g of corn meal to each pot in each of the 4 treatments. The pots were then incubated at ambient greenhouse temperatures for 14 days. They were watered as needed and the turf was maintained at 1/2-inch clipping height.

At the end of the greenhouse incubation period the pots were placed in growth chambers at 5°C for a two-week hardening period. Light intensity was adjusted to 400 ft-c for 8 hr daily during this period. All pots were then placed in polyethylene lined plywood boxes patterned after the snow mold cabinets of Cormack and Lebeau (10). Water was added to the boxes to maintain high humidity. The pots were held for 8 weeks at 5°C. The percentage of diseased area per pot was recorded at the conclusion of 8 weeks. All pots were then

removed to the greenhouse for a two-week recovery period and fresh and dry clipping weights of each treatment were recorded at the conclusion of the recovery period.

Results

Turf inoculated with mycelia, ground oats and corn meal (Treatment C) was most heavily damaged and had a significantly lower clipping weight total than the other treatments (Table 7). Infection of turf in this treatment was not caused by sclerotia germination, however, and the damage to turf showed only that the turf was in a susceptible condition. The highest clipping weight total appeared in the untreated control as expected. None of the pots inoculated with sclerotia showed any significant differences in relation to damaged turf area. Some of the variation in clipping weights between treatments could be attributed to the variability occurring during collection of the clippings.

Upon examination of sclerotia at the conclusion of the experiment, none of them appeared to have germinated. Some of the sclerotia were covered with spores of Trichoderma spp. and were no longer viable. Several sclerotia had given rise to basidia that were apparently sterile when examined under the microscope. Humidity levels in the snow mold cabinets ranged from 65-90% during the course of the experiment and should not have been a factor acting against the germination of sclerotia.

All the pots incoulated with Trichoderma spp. and sclerotia of T. incarnata had no visible signs of injury to the turf. However, the only treatment that had serious damage to topgrowth was inoculated with mycelium. The potential value of Trichoderma spp. as antagonists under natural conditions was not determined conclusively. The reason for failure of these treatments to develop typical symptoms of gray snow mold is not clear. Possible problems with technique will be discussed in the following sections.

EXPERIMENT IV

LIST OF TREATMENTS

A No sclerotia added - Maintained in greenhouse with treated pots

B 25-50 sclerotia/pot - Maintained in greenhouse with treated pots

C 25 g mycelia, ground oats and corn meal inoculated - Placed at 5°C in growth chamber

D 25-50 sclerotia/pot - Placed at 5°C in growth chamber

E 25-50 sclerotia/pot + 1000-2000 spores of T. viride I

F 25-50 sclerotia/pot + 1000-2000 spores of T. konigii

G 25-50 sclerotia/pot + 1000-2000 spores of T. harzianum

H 25-50 sclerotia/pot + 1000-2000 spores of T. viride II

I 25-50 sclerotia/pot + 1000-2000 spores of T. viride I + 5 g corn meal/pot

J 25-50 sclerotia/pot + 1000-2000 spores of T. konigii + 5 g corn meal/pot

K 25-50 sclerotia/pot + 1000-2000 spores of T. harzianum + 5 g corn meal/pot

L 25-50 sclerotia/pot + 1000-2000 spores of T. viride II + 5 g corn meal/pot

TABLE 7

FRESH AND DRY CLIPPING WEIGHTS OF TURF INOCULATED WITH TRICHODERMA SPP. AND/OR TYPHULA INCARNATA

Pot No.	Treatments (grams)											
	A	B	C	D	E	F	G	H	I	J	K	L
	Fresh Weights*											
1	1.51	0.97	0.70	2.11	2.16	1.51	1.32	1.62	1.43	1.62	1.79	2.09
2	1.78	2.41	0.37	1.71	1.71	0.70	1.73	1.78	1.19	1.80	1.60	2.41
3	1.91	1.22	0.81	1.21	2.01	1.42	1.73	1.61	1.28	1.09	2.21	1.23
4	2.58	1.16	0.71	0.70	1.59	1.22	1.50	1.41	1.18	0.70	1.18	1.14
Total	7.78	5.76	2.59	5.73	7.47	4.85	6.28	6.42	5.08	5.21	6.78	6.87
	Dry Weights											
1	0.30	0.20	0.15	0.31	0.37	0.31	0.21	0.33	0.28	0.15	0.17	0.19
2	0.39	0.51	0.02	0.28	0.32	0.12	0.39	0.32	0.27	0.31	0.35	0.44
3	0.41	0.30	0.13	0.20	0.52	0.25	0.23	0.38	0.22	0.29	0.41	0.24
4	0.56	0.27	0.12	0.11	0.32	0.30	0.35	0.35	0.22	0.16	0.36	0.23
Total	1.66	1.28	0.48	0.90	1.53	0.98	1.18	1.38	0.99	0.91	1.29	1.10
	d	bcd	a	ab	cd	abc	bcd	bcd	abc	ab	bcd	bcd

* Data represent weight of clippings at 1/2" clipping height.

Clippings were taken after a 20-day recovery period in the greenhouse.

Means in each column followed by a letter in common are not statistically significant at the 5% level according to the Duncan's New Multiple Range Test.

TABLE 8

PERCENT DISEASED AREA OF TURF INOCULATED WITH TRICHODERMA SPP. AND/OR TYPHULA INCARNATA AFTER 60 DAYS INCUBATION AT 5°C

Reps.	Treatment											
	A	B	C	D	E	F	G	H	I	J	K	L
1	0	0	10	50	0	0	0	0	0	0	0	0
2	0	0	0	70	0	0	0	0	0	0	0	0
3	0	0	10	60	0	0	0	0	0	0	0	0
4	0	0	5	95	0	0	0	0	0	0	0	0

Data represent visual estimates of damaged area.

EXPERIMENT V

BIOCONTROL IN THE FIELD

Procedure

The experiment was conducted on a 60 X 45 foot area of a golf green consisting of Penncross creeping bentgrass mowed at 5/16-inch. The area was under a normal golf green maintenance schedule throughout the growing season. The area received a total of 5 lbs N/1000 sq.ft. divided equally among five monthly fertilizations beginning in May. The green was thinned with a Ryan's Ren-O-Thin in May and topdressed in May, June and September. Application of treatments was done during the final topdressing.

The experimental area was developed to determine the antagonistic effects of Trichoderma spp. on sclerotia of T. incarnata when spores of each species were applied to plots in a topdressing mixture of 1:1 sand and loam. The effects of corn meal, nitrogen and phosphorus plus potassium amendments were also incorporated into this experiment. The 60 X 45 foot area was divided into twenty 15 X 9 foot blocks. Each block was then divided into 3 X 3 foot plots, making 15 plots within each block, for a total of 300 treatment sites. At the time the field experiment was initiated, only two species of Trichoderma were available, T. viride I and T. konigii. All comparisons were made using only these two species. Five holes approximately 1 cm deep were made with an 8 mm cork

borer in each 3 X 3 foot plot except the controls. A corresponding 8 mm biscuit of PDA and Trichoderma spores were cut from petri plates and placed in each hole in the appropriate treatments. No attempt was made to estimate the number of spores in each 8 mm section. The bentgrass plugs were reinserted in the holes and five sclerotia of T. incarnata were placed close to the soil surface of each plug and on the control plots.

All of the respective amendments were spread evenly over the entire 3 X 3 ft. plot. The treatments were as follows:

A Check

B Trichoderma konigii + Typhula incarnata (25 sclerotia)

C T. konigii

D T. konigii + Corn meal

E T. konigii + Corn meal + Typhula incarnata (25 sclerotia)

F T. viride 1 + Typhula incarnata (25 sclerotia)

G T. viride 1 + Corn meal

H T. viride 1

I T. viride 1 + Corn meal + Sclerotia

J T. viride 1 + Corn meal (watered in)

K T. konigii + Corn meal (watered in)

L T. konigii + N (1 lb/1000 NH_4NO_3)

M T. viride 1 + N (1 lb/1000 NH_4NO_3)

N T. konigii + P and K (10 lbs/1000 0-20-20)

O T. viride 1 + P and K (10 lbs/1000 0-20-20)

The experimental area was then maintained as a typical golf green through the remainder of the growing season. No maintenance other than mowing and irrigating was performed on the area. After all snow cover had melted and diseased areas had ceased activity, the severity of disease incidence and the percentage infected area in each plot was recorded.

Results

The experimental area was covered with a minimum of 1 inch of snow for a total of 92 days between 1 November 1972 and 1 April 1973. The first snow fell on 15 November and the last snow melted by 15 March. Temperatures were slightly above average for the winter months and precipitation was higher than normal and mostly in the form of rain. The area was lined out on 15 March in order to record the percentage of diseased area in the respective plots. A visual estimate of the diseased area was made in each of the 3 X 3 foot plots. The overall damage done by Typhula spp. was severe. When the experimental area was viewed in its entirety, none of the 3 X 3 foot plots were noticeably healthier than others. The very large number of infection centers which developed to 3-20 cm in diameter suggested that production of basidiospores during late autumn was unusually heavy.

Statistical analysis of the data by Duncan's New Multiple Range Test at the 5% level showed significance between plots treated with Trichoderma viride I plus corn meal and the remaining treatments. The least damaged treated plot averaged 66% diseased area as compared to the most heavily damaged plots which averaged 87% diseased area. Data for each plot appear in Table 6. From a practical viewpoint, none of the treatments yielded satisfactory results. In order for the antagonism of Trichoderma spp. to be used as a biological control mechanism, any infection caused by Typhula spp. in excess of 5% damaged turf in any given area would negate the value of

Trichoderma spp. Since disease incidence in the field trial was considerably higher than this arbitrary figure, statistical significance between treatments is of little practical worth.

EXPERIMENT V

TREATMENTS APPLIED TO FIELD EXPERIMENT - SEPTEMBER 1972

A Check

B *Trichoderma konigii* + *Typhula incarnata* (25 sclerotia)

C *T. konigii*

D *T. konigii* + Corn meal (25g)

E *T. konigii* + Corn meal + *Typhula incarnata* (25 sclerotia)

F *T. viride* I + *Typhula incarnata* (25 sclerotia)

G *T. viride* I + Corn meal

H *T. viride* I

I *T. viride* I + Corn meal + *Typhula incarnata* (25 sclerotia)

J *T. viride* I + Corn meal (water applied)

K *T. konigii* + Corn meal (water applied)

L *T. konigii* + N (1 lb/1000 NH_4NO_3)

M *T. viride* I + N (1 lb/1000 NH_4NO_3)

N *T. konigii* + P and K (10 lbs/1000 0-20-20)

O *T. viride* I + P and K (10 lbs/1000 0-20-20)

TABLE 9

PERCENT DISEASED AREA OF 3' X 3' PLOTS OF PENNCROSS CREEPING BENTGRASS TREATED WITH TRICHODERMA SPP. (TREATMENTS LISTED ON PAGE 41)

Blocks	Treatments														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	90	75	100	100	85	95	90	95	90	90	95	75	65	95	80
2	95	90	88	85	75	80	90	95	88	85	90	80	80	81	80
3	96	90	92	80	95	62	85	99	90	92	70	99	90	90	95
4	97	90	95	98	91	90	90	99	95	98	99	95	95	98	96
5	85	95	80	90	90	65	95	60	80	90	85	80	98	95	85
6	70	65	55	60	68	95	88	65	50	60	86	95	73	82	90
7	87	55	75	80	90	85	70	90	90	75	85	92	90	94	92
8	98	91	87	88	50	92	96	89	96	40	85	94	99	90	90
9	80	90	80	85	40	65	95	90	40	50	30	70	85	100	50
10	95	75	80	55	88	90	70	40	65	75	70	80	92	85	95
11	55	65	65	55	50	83	90	88	60	50	85	85	60	60	82
12	92	95	99	99	95	95	55	98	92	40	85	90	100	85	50
13	20	70	25	85	95	30	100	95	80	30	90	40	55	95	100
14	50	20	25	50	40	30	28	10	10	15	20	25	29	60	45
15	95	100	95	92	90	88	95	100	95	92	99	98	92	85	85
16	80	75	90	35	75	70	86	90	97	55	60	60	90	88	50
17	80	80	80	90	80	70	55	80	75	75	65	65	90	95	90
18	70	65	60	60	50	30	95	50	75	55	40	25	95	85	50
19	88	90	90	100	90	99	93	98	99	99	95	100	88	85	96
20	90	100	85	95	75	90	92	70	60	55	90	80	95	98	95
Avg.	80	78	77	79	75	75	82	80	76	66	76	76	83	87	79
	ab	ab	ab	ab	ab	ab	b	ab	ab	a	ab	ab	b	b	ab

Means in each column followed by a letter in common are not statistically significant at the 5% level according to the Duncan's New Multiple Range Test.

DISCUSSION AND CONCLUSIONS

There has been considerable discussion concerning the difficulties of species identification within the genus Trichoderma (1, 17, 41, 54). A lack of continuity by taxonomists makes some of the work done with Trichoderma spp. difficult to repeat because of confused characteristics associated with many species. Perhaps more importantly, the variability of performance by strains within each species in relation to antagonistic properties or production of antibiotics has caused many of the inconsistencies. Every attempt should be made to test as many isolates for antagonism as possible. The concept of "species aggregates", developed by Rifai (41), allows for a flexible and organized system of classification and was adopted for use in these experiments.

The most persistent obstacles to overcome during development and implementation of a biological control system for a soil-borne plant pathogen are those occurring during the transition from laboratory to field conditions. Trichoderma spp. have been reported as antagonists to the mycelia, spores, or sclerotia of many different fungi (2, 17, 31, 53, 54). Laboratory results conclusively show the ability of any of the four species of Trichoderma tested to parasitize and destroy the sclerotia of Typhula incarnata on artificial media (PDA) and natural and amended soils. A preliminary test to determine the antibiotic activity of Trichoderma spp. toward Typhula did not suggest inhibition

of mycelial growth on the agar used under the existing conditions. The final colony diameters of T. incarnata growing with each species of Trichoderma were essentially equal to the final control colony diameter. There was apparently no production of growth inhibiting compounds by Trichoderma spp. and cessation of growth of Typhula colonies was only induced by the parasitism of mycelia and sclerotia by each of the Trichoderma spp. as evidenced by the complete domination by Trichoderma spp. in the agar plates in which both species of fungi were inoculated. Since the germination of sclerotia of T. incarnata is dependent on temperatures below 10°C, the likelihood of any simultaneous development of colonies of each organism is remote. Therefore, the antagonism of Trichoderma spp. toward the mycelia of T. incarnata has virtually no practical significance. The oversummering dormant sclerotia of T. incarnata lends itself most readily to attack by common soil inhabiting microorganisms. The cultural practices employed during golf course management to golf greens could potentially stabilize the populations of soil microflora and enhance the development of antagonistic fungi. The nutritional and environmental requirements of Trichoderma seem to be flexible enough to enable successful incorporation of this organism into golf green soils. Since the predominate turfgrass managed on golf greens in the cool-humid regions of the United States is Agrostis spp. whose cultural requirements are favored by a slightly acid soil, the prospects of establishing Trichoderma spp. in these soils is favorable. Normal maintenance practices on golf greens include irrigation to maintain sufficient moisture, frequent fertilizations to stimulate

growth, and soil aeration to alleviate compaction. Any or all of these practices can enhance the stabilization of common soil fungi populations by eliminating adverse radical environmental changes in the soil.

The greenhouse and field experiments (IV and V) did not produce any positive results for several potential reasons. Upon close examination of sclerotia inoculated on Penncross creeping bentgrass turf in pots in the greenhouse and growth chambers, it was cpparent that none of them had germinated and infected the turf or produced fertile basidia. Infections and damage did occur when mycelia were spread on the turf. Sporulation did not take place in the growth chambers because the proper exposure to light (2700 - 3200 Å) did not occur. Sclerotia did not germinate to cause direct infections of the turf for some unknown reason. A reliable technique for infecting turf with sclerotia of Typhula incarnata must be developed before any progress can be made toward biocontrol under simulated field conditions. Field results did not indicate any effects the antagonist may have had on sclerotia present. Damage to the entire experimental area was severe. Inoculation of spores of Trichoderma spp. was carried out in September 1972. Soil and air temperatures decreased soon after inoculation and it is not likely that Trichoderma spores were able to germinate and proliferate. An effective test of the efficacy of this organism as an antagonist of Typhula should be carried on throughout an entire growing season to maximize the spread and development of the antagonist in the soil. Applications of spores of Trichoderma spp. in conjunction with top-dressing and/or fertilization programs on golf greens during the growing season would greatly enhance the incidence of contact between antagonist and pathogen.

Any antagonistic or parasitic activity would be completed before sclerotia were exposed to conditions stimulating the production of basidiospores. The lack of success using species of Trichoderma as antagonists in these experiments could be caused by the inadequate period of favorable environmental conditions in the field.

One difficulty encountered in establishing a stable population of soil-inhabiting fungi in golf green soil mixtures is the effect of applications of fungicides to control warm-season turf diseases. The fungicides may seriously inhibit the degree to which nonpathogenic antagonists such as Trichoderma spp. could survive in soil. However, it has been reported that Trichoderma spp. are antagonistic toward other soil-borne turf pathogens of the warm season (2, 43, 52). Such antagonism could potentially reduce the number of fungicide applications required to prevent some of these diseases.

It would seem that an experimental field program examining factors controlling population levels of soil fungi on turf areas managed as golf greens would aid greatly in the development of antagonistic fungi as biological control organisms. The most encouraging laboratory results are of little immediate use to the turfgrass manager in the field unless they eventually provide a beneficial service to him. The importance of minimizing the need of chemical formulations from an aesthetic and economic view in the maintenance of recreational turf areas provides one goal to work toward in attempts to better understand biological processes and make them work for the professional in the field.

SUMMARY

Typhula incarnata Lasch ex. Fries is a serious turf pathogen causing Gray Snow Mold on valuable turf areas, such as golf greens, during the winter months. Several chemical formulations have proven valuable in preventing infections of the disease. However, some of these formulations may become unavailable for use due to their potential hazards to man and the environment. This investigation was undertaken to explore the antagonistic activity of Trichoderma spp., a common soil fungus, against sclerotia of T. incarnata for its use as a practical biocontrol agent.

Two species of Trichoderma, T. konigii and T. viride I, were isolated from soils in the Amherst, Massachusetts area. Two additional species, T. harzianum and T. viride II, were purchased from the American Type Culture Collection. Laboratory experiments examining the antagonism of Trichoderma spp. to sclerotia of T. incarnata showed that sclerotia were parasitized and did not germinate after sufficient incubation. A preliminary test of antibiotic production by Trichoderma spp. at 10°C did not show any inhibition of mycelial growth of T. incarnata, although colonies of T. incarnata were physically overrun with mycelia of Trichoderma spp.

A greenhouse experiment attempting to simulate the naturally occurring process of antagonism was performed whereby spores of Trichoderma spp. were inoculated into pots containing Penncross creeping bentgrass. After sufficient time for germination of the spores and parasitization of sclerotia of T. incarnata, the pots were placed in growth chambers at 5°C and incubated for 60 days to allow healthy sclerotia to infect the turf. Results of this experiment were inconclusive because sclerotia of T. incarnata failed to germinate and infect the turf.

A field study designed to examine the ability of spores of Trichoderma spp. to colonize soil under golf green turf and effectively reduce the severity of snow mold infection by destroying dormant sclerotia was performed. Spores of T. konigii and T. viride I were mixed with small volumes of sand and spread on selected plots during September 1972. Visual observations to determine the percentage of each plot damaged by snow mold organisms were made in March 1973. The entire experimental area (60' X 45') was severely damaged. No treatment reduced infections a sufficient amount. It is possible that the late season inoculation of Trichoderma spores prevented the development of extensive colonies required to parasitize sclerotia of T. incarnata.

Laboratory results conclusively indicated that each of the four species of Trichoderma was antagonistic toward sclerotia of T. incarnata. The viability of the sclerotia was drastically reduced after exposure to Trichoderma spp. Greenhouse and field experiments did not suggest that this phenomenon was occurring under the conditions of each experiment.

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