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Proliferation of Pythium nunn (Lifshitz et al.) in Tennessee soils and the effects of sugars, oils and culture filtrates of Trichoderma spp. on growth and reproduction of Pythium nunn and Pythium ultimum (Trow)

Darrell D. Hensley

To the Graduate Council:

I am submitting herewith a thesis written by Darrell D. Hensley entitled "Proliferation of Pythium nunn (Lifshitz et al.) in Tennessee soils and the effects of sugars, oils and culture filtrates of Trichoderma spp. on growth and reproduction of Pythium nunn and Pythium ultimum (Trow)." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Mark T. Windham, Major Professor

We have read this thesis and recommend its acceptance:

J. Graveel, L.F. Johnson, C.J. Southards

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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PROLIFERATION OF *PYTHIUM NUNN* (LIFSHITZ ET AL.) IN TENNESSEE SOILS AND THE EFFECTS OF SUGARS, OILS AND CULTURE FILTRATES OF *TRICHODERMA* SPP. ON GROWTH AND REPRODUCTION OF *PYTHIUM NUNN* AND *PYTHIUM ULTIMUM* (TROW)

A Thesis

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Darrell D. Hensley

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ABSTRACT

Pythium nunn (N3) a mycoparasite of other *Pythium* spp., was added to five Tennessee soils (Bruno, Bowdre, Fullerton, Morganfield, and Robinsonville) to study its ability to colonize these soils. Mycelial mats were mixed into the soils and N3's inoculum density was determined weekly during an eight week incubation period. Numbers of colony forming units (cfu) fluctuated from nondetectable levels to 1040 cfu's/g of soil in the second to fourth week of incubation and decreased to approximately 80 cfu's or lower in the fourth to eighth week. In a separate experiment, N3's ability to colonize Morganfield and Robinsonville soils was compared with it's ability to colonize Nunn sandy loam soil (a soil from Colorado from which it was originally isolated). Colony forming units of N3 were higher in the Tennessee soils than in the soil from Colorado after five weeks of incubation.

The effects of eight oils on growth and/or reproduction of *Pythium ultimum* (N1) and N3 were determined. Wheat germ oil with 20 or 3 g/l glucose or sucrose, consistently stimulated growth of N1 when compared to the other oils with sugar or controls. Codliver, wheat germ, sesame, and sunflower oils stimulated growth of N3. Several oils had varying effects on growth of both N1 and N3 when different sugars were used. Few or no sporangia of N3 formed in the

oil and sugar combinations. These two Pythium spp. apparently have different chemical requirements for growth and reproduction.

Isolates of Trichoderma koningii, T. harzianum, T. viride and T. pseudokoningii were tested for their abilities to inhibit growth and/or stimulate reproduction of N3 and N1. Culture filtrates of the isolates were sterilized by cold filtration or autoclaving, were seeded with agar plugs of N1 or N3. All filtrates tested inhibited growth of N1 and N3. Two isolates of T. viride inhibited growth and oospore formation of N1 to a greater extent than did the other Trichoderma species. There was no difference in inhibition of growth and oospore formation with autoclaved or cold filtrates. Stimulation of reproductive structures in N1 and N3 by filtrates of Trichoderma species was not observed. Inhibition of growth of N3 in filtrates of Trichoderma reduces the possibility of integrating P. nunn with Trichoderma as an effective biocontrol management system.

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PART I

CHARACTERISTICS OF *PYTHIUM NUNN* AND *PYTHIUM ULTIMUM*

I. TAXONOMIC COMPARISON

Pythium nunn Lifshitz et al. (order Peronosporales) produces extensively branched hyphae with delicate side branches. Sporangia develop acrogenously or intercalary, are spherical, oval, or lemon shaped, and fail to form zoospores when placed in water (7). Antheridia originate by monoclinous or diclinous development, are born apically on inflated or crooked antheridial stalks, and are lobulate, clavulate and rounded. One to five antheridia may be attached to an oogonium. Oogonia form acrogenously, are spherical, and have smooth and thin cell walls. Oospores are aplerotic and cell walls are thicker than cell walls of non-fertilized oogonia (7).

Pythium ultimum Trow shares many morphological characteristics with P. nunn but differs in pathogenicity. Pythium ultimum attacks a wide range of plant hosts (1,2,3,4,5,6) but P. nunn has not been observed to be a plant pathogen (7). Sporangia of P. ultimum are produced terminally (8) and usually germinate directly. Van der Platts-Niterink (8) and Waterhouse (9) indicated that some isolates of P. ultimum may form zoospores.

Pythium ultimum produces sac-like antheridia and usually one, but up to three antheridia may attach to each oogonium. Antheridia are commonly monoclinous, hypogynous and frequently straight. Oogonia are terminal, sometimes

intercalary and globose with smooth walls. Oospores form singlely and are aplerotic, globose and thick walled (8,9). Pythium nunn and P. ultimum can be separated morphologically by P. ultimum's sac-like antheridia and thicker walled oospores.

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PART II

PROLIFERATION OF *PYTHIUM NUNN* IN TENNESSEE SOILS

I. ABSTRACT

Pythium nunn (N3) a mycoparasite of other *Pythium* spp., was added to five Tennessee soils (Bruno, Bowdre, Fullerton, Morganfield, and Robinsonville) to study its ability to colonize these soils. Mycelial mats were mixed into the soils and N3's inoculum density was determined weekly during an eight week incubation period. Numbers of colony forming units (cfu) fluctuated from nondetectable levels to 1040 cfu's/g of soil in the second to fourth week of incubation and decreased to approximately 80 cfu's or lower in the fourth to eighth week. In a separate experiment, N3's ability to colonize Morganfield and Robinsonville soils was compared with it's ability to colonize Nunn sandy loam soil, (a soil from Colorado from which it was originally isolated). Colony forming units of N3 were higher in the Tennessee soils than in the soil from Colorado after five weeks of incubation.

II. INTRODUCTION

Pythium nunn Lifshitz et al. was originally isolated from a Nunn sandy loam soil in Colorado and identified as a mycoparasite of *P. ultimum* Trow, *P. vexans* deBary, *P. aphanidermatum* Edson, *Phytophthora parasitica* Dastur, *Phytophthora cinnamomi* Rands and *Rhizoctonia solani* Kuhn

(9). Pythium nunn has been used sucessfully to induce soil suppressiveness to plant pathogenic Pythium species (7,8) and has not been reported as a plant pathogen. Therefore Pythium nunn has the potential to be a useful biological control agent.

Lifshitz and Baker (5) made nonsuppressive soils with pH values of 7.3 suppressive to P. ultimum and P. vexans by the addition of P. nunn, but soils with pH value of 5.5 did not become suppressive. In soils with pH values of 7.3, P. nunn's populations increased dynamically, while populations of P. ultimum and P. vexans decreased. Pythium nunn was effective as an antagonist of fungi associated with seedling diseases in greenhouse situations (7,12,13). The ability of P. nunn to survive or function as an antagonist in Tennessee soils is unknown. This research was conducted to determine if P. nunn would survive and/or proliferate in selected Tennessee soils.

III. MATERIALS AND METHODS

Soils. Soils used in this study were surface (0-15 cm) samples selected to represent a range of textures (Table II-1)*. The field moist soils were air-dried passed through a

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* All tables and figures may be found in Appendix, Part II.

2 mm screen. Soil pH was determined on a 1:1 soil to water ratio (10) with an Orion model 501 pH meter (American Scientific Mcgaw, Il. 60085). The quantity of $CaCO_3$ needed to raise the pH to 7, was determined by Cation Exchange Capacity (CEC) (4). Acid soils were saturated with deionized distilled water, $CaCO_3$ added, and then were allowed to stand for seven days at 25 C. A 5 bar pressure plate extractor (Soil Moisture Equip. Corp., Santa Barbara, CA 93105) was used to determine field moisture holding capacity (-33 kPa) (3).

The soils were amended with 1% oatmeal (Quaker Oats Company, Chicago, IL. 60604-9001) weight/weight (w/w) and autoclaved for one hour on two consecutive days (12,13). Bean leaves (Phaseolus vulgaris L. `KY Wonder 295') were dried for 24 h at 60 C, crumbled and passed through a 1 mm mesh screen, and added to the soil at a rate of 0.3% (1 g of leaves/333 g of soil) at weekly intervals (7,12).

Culture of Pythium nunn. Pythium nunn (N3) (R. Baker, Colorado State University, Fort Collins 80523) was maintained on medium containing 1 g potato dextrose agar with 14 g of Bacto-agar per liter of distilled water. Agar plugs of N3 obtained with a 7 mm cork borer were placed in ten 250 ml Erlenmeyer flasks that contained 100 ml of potato dextrose broth. The cultures were incubated at 27 C for two weeks on a reciprocal shaker (Eberbach Corporation, Ann Arbor, Michigan 48104) and mycelial mats of P. nunn were

harvested after two weeks. Mycelial mats were added to soil at the rate of 2 mats/2000 g soil and the soil was then mixed thoroughly. Soil moisture was adjusted to -33 kPa by the addition of sterile distilled water with a sterile pipette and thoroughly mixed. Each soil was divided into 4 portions (333 g of soil each) and each portion was placed into an alcohol-sterilized plastic 355 ml cup. The cups were covered with Saran wrap (Dow Inc., Indianapolis, IN 46268-0511) and incubated at 27 C or 16C. All soils were incubated for eight weeks. Quantity of P. nunn was determined at the beginning of each experiment and at weekly intervals by plating dilutions of soil on Pythium selective media (2).

Pythium nunn in Tennessee and Colorado soils. The Morganfield silt loam, Robinsonville sandy loam (Tennessee soils) and a Nunn sandy loam (from Colorado), were passed through a 2 mm mesh screen, placed in 50x50 mm clay pots and amended with dried bean leaves at 0, 2, 3, 4, and 5 weeks. Pythium nunn was added to the soils as described above and the treatments were replicated 6 times. Soils were maintained at -33 kPa with sterile distilled water and population densities of P. nunn were determined at 0, 2, 3, 4, and 5 weeks.

IV. RESULTS

Colony forming units fluctuated during the 8 week incubation period from nondetectable levels to 1040 cfu's/g of soil, and then decreased. Saprophytic activity of P. nunn at 27 C for all soils was highest at 3, 4 and 5 weeks (Fig. II-1). In soils incubated at 16 C, greater saprophytic increases were observed at the second week and cfu's decreased to low levels by the third week (Fig. II-2).

In an experiment comparing Nunn soil with two Tennessee soils, Robinsonville soil had the highest cfu's of N3 after 4 weeks of incubation (Fig. II-3), and Nunn soil had the lowest population densities. After five weeks of incubation cfu's in Nunn soil declined almost to zero, but remained relatively high in two Tennessee soils (Fig. II-3).

V. DISCUSSION

A combination of abiotic and/or biotic factors may have caused a reduction in cfu's of N3 at 6 and 3 weeks of incubation at 27 C and 16 C, respectively. Pythium species have been reported to be sensitive to antagonism by soil microflora (5,11). These microflora may have contaminated soil samples when bean leaves were added to the soil. The inhibition of sporangial germination of P. splendens Braun in Kohala soil was found to be associated with microbial

activity (6). Soil moisture adjustments to -33 kPa may have indirectly influenced N3 population levels by optimizing conditions for antagonistic development and anaerobic conditions may have occurred resulting in buildup of organic acids (1).

Colony forming units of N3 increased in Tennessee soils, and N3 grew better in Morganfield and Robinsonville soils than it did in the Nunn sandy loam soil (Fig. II-3). These population densities of N3 in Tennessee soils were to similar levels reported for Colorado soils (12,13). An increase in cfu's of N3 at weeks 3 and 4 (Fig. II-1 and II-3) and weeks 1 and 2 in soil incubated at 16 C (Fig. II-2) may have been due to N3's ability to utilize the bean leaves. Population reductions of N3 near the end of incubation period may have been due to an increase in antagonistic microflora populations which caused lyses of N3 hyphae. The decline in N3 populations in Nunn soil after five weeks is different from reports by Paulitz and Baker (12,13) which stated that population densities of N3 gradually increased after 55 days of incubation in raw and steamed Nunn soil. More research must be undertaken to determine how to stabilize maximum P. nunn populations in Tennessee soils.

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APPENDIX

PART II

Table II-1

Properties of soils used in this investigation.

Soil Series	Subgroup	pH	CEC	$CaCO_3$ added[y]	Water content -33 kPa
			-cmol(+)/kg-	-g-	-%-
Bruno, s[z]	Typic Udifluvent	5.8	3.87	0.45	6.1
Bowdre, c	Aquic Hapludoll	5.9	21.34	4.00	30.6
Robinsonville, sl	Typic Udifluvent	7.0	-	-	13.4
Morganfield, sil	Typic Udifluvent	7.2	-	-	12.9
Fullerton, c	Typic Paleudult	5.0	6.74	6.00	22.0
Nunn, s	Aridic Argiustoll	7.2	-	-	13.0

[x] Cation exchange capacity obtained from sum of exchangeable Ca, Mg, Na, K, and H.

[y] Grams of $CaCO_3$/kg of soil to adjust pH to 7.

[z] s=sand, sil=silt loam, c=clay, sl=sandy loam

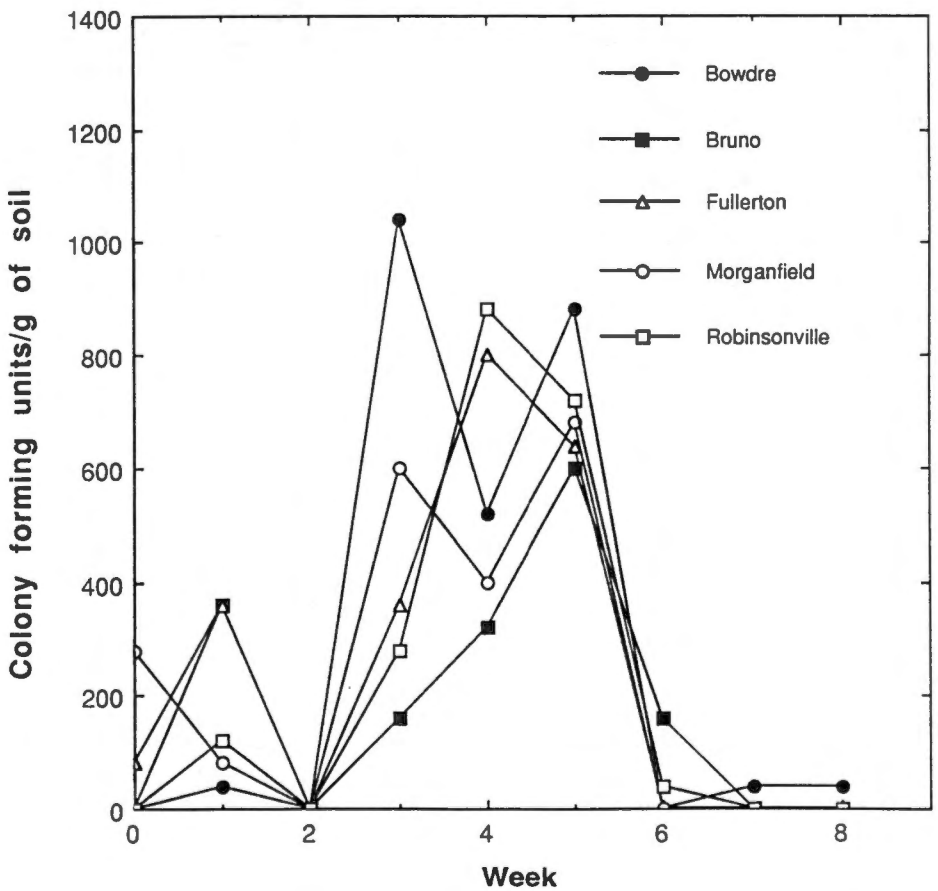

Figure II-1. Population densities of P. nunn when incubated at 27 C and -33 kPa soil moisture potential for 8 weeks in five soils.

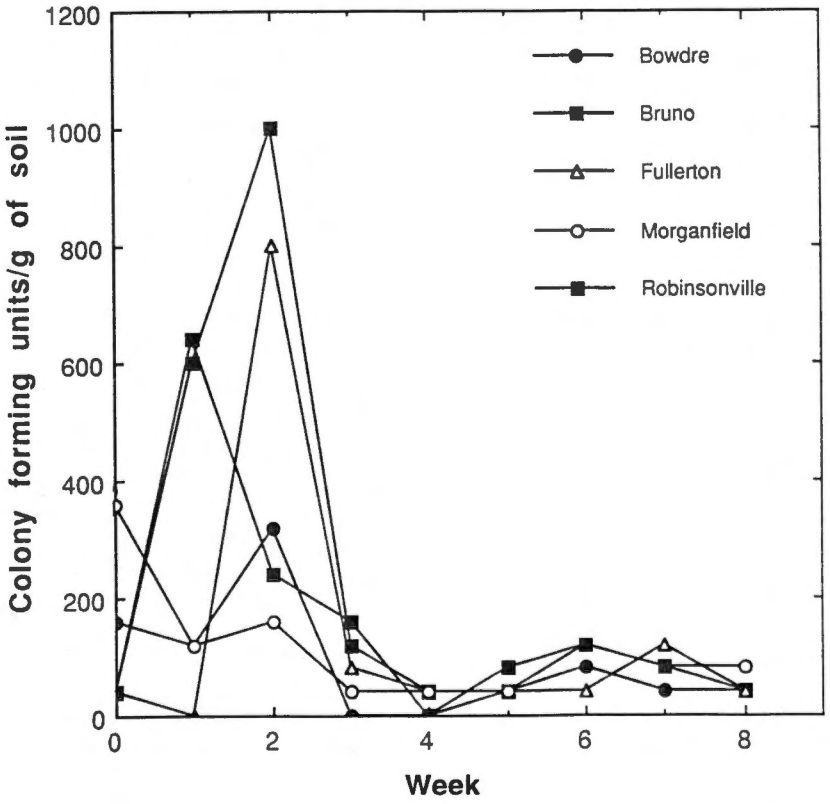

Figure II-2. Population densities of P. nunn when incubated at 16 C and -33 kPa soil moisture potential for 8 weeks in five soils.

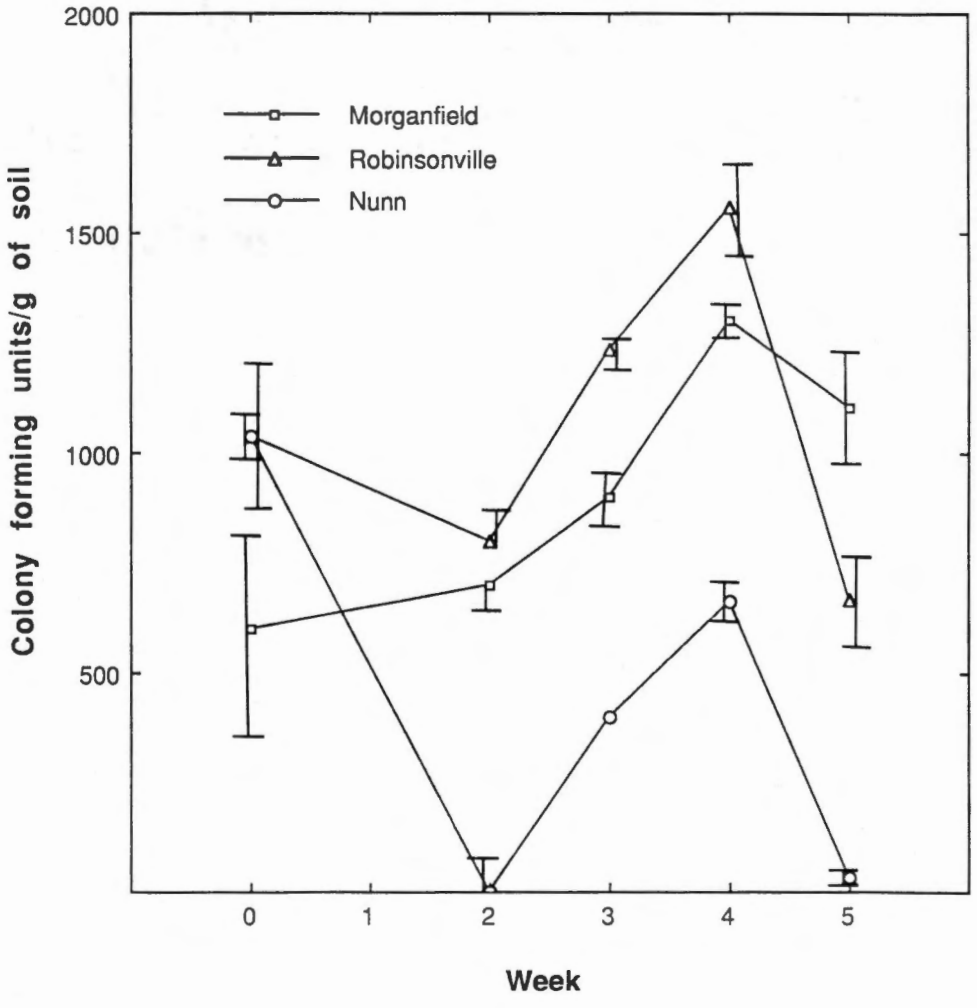

Figure II-3. Population densities of P. nunn when incubated at 16 C and -33 kPa soil moisture potential in three soils. Standard error is indicated by a vertical line.

PART III

A COMPARISON OF OIL AND SUGAR AMENDMENTS ON GROWTH AND REPRODUCTION OF *PYTHIUM NUNN* AND *PYTHIUM ULTIMUM* IN LIQUID CULTURE

I. ABSTRACT

The effects of eight oils on growth and/or reproduction of Pythium ultimum (N1) and Pythium nunn (N3) were determined. Wheat germ oil with 20 or 3 g/l glucose or sucrose, consistently stimulated growth of N1 when compared to the other oils with sugar or sugar controls. Codliver, wheat germ, sesame, and sunflower oils increased growth of N3 when compared other oils, and sugar controls. Several oils had varying effects on growth of both N1 and N3 when different sugars were used. Few or no sporangia of N3 formed in the oil and sugar combinations. This suggest that these two Pythium spp. apparently have different chemical requirements for growth and reproduction.

II. INTRODUCTION

Some Pythium species grow and reproduce readily on several types of media (3,10) while other species require specific chemicals for optimum growth or formation of reproductive structures (10). If essential chemicals are missing from the nutrient substrate, growth rates of some Pythium species may be reduced (2,6) and abnormally shaped reproductive structures may be formed (3,4,5). Aqueous garden pea (Pisum sativa) extract (10) and cholesterol (1,7,14) stimulated growth and sexual reproduction of

several Pythium species. Other reports (1,5) indicated that sterol was required by P. myriotylum Drechsler and P. periplocum Drechsler for normally shaped oospores and sporangia, respectively.

Other compounds have been found to affect growth and reproduction of Pythium species. Calcium enhanced formation of sporangia, oogonia, and oospores of P. debaryanum de Bary, P. ultimum Trow and P. irregulare Buisman (16). Zinc, manganese, copper, and molybdenum stimulated growth and oogonial formation of P. graminicola Subramaniam (9). The effects of nineteen sugars were also studied, and glucose was reported to be the optimum carbon source for growth and sexual reproduction of P. acanthicum Drechsler (2). The objective of this research was to determine if amendments of oils and sugars to a nutrient medium would enhance the growth and/or reproduction of P. ultimum (N1) and P. nunn Lifshitz et al. (N3).

III. MATERIALS AND METHODS

The effects of eight oils and two sugars on growth and reproduction of Pythium ultimum B-6 (ATCC # 56081) and P. nunn (R. Baker, Colorado State University, Fort Collins, 80523) were determined. Ten petri dish replicates were made for each oil, sugar and Pythium combination. Data were

taken from five of the replications at 14 and 21 days for the remaining five. Stock cultures of N1 and N3 were maintained on 1 g potato dextrose agar with 14 g of Bacto-agar per liter of distilled water and incubated at approximately 23 C. Agar plugs containing Pythium spp. were obtained with a 7 mm cork borer and placed into petri dishes containing test solution.

Preparation of test solutions. Oils used in these experiments were obtained from Natural & Organic Foods, 7025 Kingston Pike, Knoxville, Tn. and included wheat germ oil (Solgar Co. Inc., Lynbrook, N.Y. 11563); olive oil (James Plagniol Extravirgin); codliver oil (Twin Laboratories, Inc. Ronkonkoma, N.Y. 11779); and peanut, soy, corn, sesame and sunflower oils (Hain Pure Food Co., Inc. Los Angeles, Ca. 90061). Two sugars (glucose and sucrose) were compared on an equal weight basis, 20 g/l or 3 g/l. Oils were added to the test solutions at the rate of 10.4 g/l. The basal medium was similar to the one used by Hendrix (6) and contained 1.5 g/l $NaNO_3$, 1.0 g/l KH_2PO_4, 0.5 g/l $MgSO_4 \cdot 7H_2O$, 1.11 g/l $CaCl_2$ and 2 µg/l of thiamine hydrocholoride. After sterilization, 20 ml of the growth medium were poured into each petri dish (9 cm diameter) and an agar plug of N1 or N3 was added. The cultures were incubated at 16 C in total darkness.

Data collection. Colony diameter of N3 was measured as the mean of two diameters of each colony. Mycelium of N1

completely covered the petri dish after 14 day incubation and growth of N1 was measured by visual observation with a density index of 1 to 8, with 8 having denser growth. Sporangia were counted (20 fields) at 200X magnification with a phase contrast microscope (Bausch & Lomb, Rochester, New York, 14603). Mycelial mats were collected on previously weighed Whatman No. 1 filter paper (Whatman Ltd., Maidstone, England) with suction, dried at 60 C for 24 h, and weighed.

Statistical analysis. Petri dishes were arranged in growth chambers in a completely randomized design. Data were analyzed by an analysis of variance procedure (PROC ANOVA) of the Statistical Analysis System (SAS) (12) and two factors with two levels of sugar and eight oils and a control for each sugar were used. Mean separation tests were performed according to Student-Newman-Keuls tests (13). There was no significant difference between the 14 and 21 days of growth and these were analyzed as one test.

IV. RESULTS

Effects of oils and sugars on Pythium ultimum. There did not appear to be a significant difference between glucose and sucrose (in the controls without oils) for

supporting growth (thallus weight) of N1 (Table III-1)*. Also, in the controls without oil, better growth was obtained with 20 g/l of either sugar than with the 3 g/l sugar concentration (Table III-1).

Wheat germ oil was outstanding in its ability to support growth of N1. When growth was measured by thallus weight, significantly better growth was obtained with wheat germ oil than any other oil on media containing 20 g/l of glucose or sucrose. However olive oil with 3 g/l glucose significantly supported better growth than other oils with 3 g/l of either sugar (Table III-1). When measured by density, wheat germ oil supported better growth on media with 20 g/l glucose and supported significantly better growth on media with 3 g/l of either sugar (Table III-1).

There was no outstanding "poor oil". All of the oils supported significantly better growth of N1 over the controls when a low concentration (3 g/l) of either sugar was used (Table III-1). However, in media containing 20 g/l of either sugar, several of the oils did not increase growth over the controls without oil (Table III-1).

Effects of oils and sugars on Pythium nunn. There did not appear to be a significant difference between glucose

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* All tables may be found in Appendix, Part III.

and sucrose (in the controls without oils) for supporting growth of N3. In the controls without oil, better growth was obtained with 20 g/l of either sugar than with 3 g/l (Table III-2).

Sunflower and codliver oils with 20 g/l sucrose significantly supported better growth of N3 than other oils with 20 g/l of sugar when thallus weight was measured. These were not significantly greater than sunflower and codliver oils with 20 g/l glucose (Table III-2). When thallus weigths were measured, peanut, olive, sesame, and soy oils with 3 g/l of either sugar supported significantly better growth than the controls without oil (Table III-2).

Sesame oil with 20 g/l glucose significantly supported better growth than other oils or sugars without oils when measured by colony growth. Wheat germ oil with 3 g/l of either sugar supported better growth than the other oils or sugars without oils (Table III-2).

Codliver and wheat germ oils with 3 g/l of either sugar did not increase thallus weight of N3 over the controls without oil (Table III-2). When measuring thallus weight, media containing 20 g/l of either sugar, several oils did not increase growth of N3 (Table III-2). In media containing 20 or 3 g/l of either sugar, several oils did not increase growth of N3 (Table III-2).

V. DISCUSSION

Both oils and sugars have been shown to stimulate growth and/or reproduction of Pythium (2,5). Wheat germ oil with 20 or 3 g/l of glucose or sucrose increased colony diameter and thallus weight of N1 and these findings were similar to the effects of wheat germ oil on thallus weight of P. graminicola and P. vexans (8). Variation of colony diameter and thallus weight did occur and possibilities for varying effects of oils on Pythium may have been due to the fatty acid chains and/or the sterol content of the oils (3). Another reason for variations in diameter of colonies and thallus weight in the oil and sugar combinations may have been due to the ability of the species to catabolize the sugar and/or oils.

Sporangia formation of N3 was least affected by oils when compared to the affects of oils on colony growth. The low numbers of sporangia formed by N3 in the oil and sugar combinations may have been due to a high carbon to nitrogen ratio (C/N) (2). The lack of oospore formation by N1 may have resulted from inhibition due to the high sugar and oil levels used in these experiments (Dr. L. F. Johnson - personal communication). Schlosser and Gottlieb (14) reported that P. ultimum mycelium catabolized glucose faster and produced more carbon dioxide in the presence of sterol than mycelium grown without a sterol. Production of carbon

dioxide may have reduced growth and/or sporulation of Pythium (11,15). This research indicated Pythium ultimum and P. nunn differ in their requirement of sugar and substances found in oil for maximum growth and/or reproduction.

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APPENDIX

PART III

TABLE III-1

Effects of two sugars and eight oils on growth of Pythium ultimum in a mineral medium. Measurements were the means of 14 and 21 days of growth.

		Thallus weight[x] (g/colony)		Density of growth[xy]	
Sugar	Oil	20g/l[z]	3g/l[z]	20g/l[z]	3g/l[z]
		----------g----------			
Glucose	Control	0.030 ef	0.022 d	3.00 d	1.40 g
	Soy	0.032 ef	0.124 bc	3.00 d	3.00 f
	Olive	0.073 cd	0.279a	5.00 bc	7.00 b
	Wheat germ	0.171a	0.147 bc	7.00a	8.00a
	Codliver	0.071 cd	0.083 c	3.10 d	5.00 d
	Peanut	0.024 f	0.174 b	4.50 bcd	4.50 de
	Sesame	0.064 cde	0.153 bc	5.00 bc	5.00 d
	Sunflower	0.096 c	0.135 bc	3.00 d	4.00 def
	Corn	0.055 cdef	0.110 bc	4.00 cd	4.50 de
Sucrose	Control	0.023 f	0.019 d	3.00 d	1.40 g
	Soy	0.088 cd	0.167 bc	4.00 cd	3.50 ef
	Olive	0.064 cde	0.129 bc	5.00 bc	6.00 c
	Wheat germ	0.136 b	0.138 bc	6.10ab	8.00a
	Codliver	0.083 cd	0.087 bc	6.00ab	5.00 d
	Peanut	0.050 def	0.139 bc	5.50 bc	4.00 def
	Sesame	0.059 cdef	0.105 bc	5.50 bc	3.00 f
	Sunflower	0.094 c	0.105 bc	5.00 bc	4.00 def
	Corn	0.071 cd	0.119 bc	4.00 cd	4.50 de

[x] Means followed by the same letter for each column are not significantly different (p=0.05) according to Student-Newman-Keuls. Mean of 5 replicates.

[y] Density of colony growth measured on an index of 1-8, 8=greatest density.

[z] Concentration of sugar used.

TABLE III-2

Effects of two sugars and eight oils on thallus weight and colony growth of Pythium nunn in a mineral medium. Measurements were the means of 14 and 21 days of growth.

		Thallus weight[x] (g/colony)		Colony growth[xy]	
Sugar	Oil	20g/l[z]	3g/l[z]	20g/l[z]	3g/l[z]
		----------g----------		----------cm----------	
Glucose	Control	0.012 e	0.008 f	1.00 h	0.10 e
	Soy	0.021 de	0.070abc	2.22 g	1.50 cd
	Olive	0.023 cde	0.063 bcd	4.75 b	1.22 d
	Wheat germ	0.034 bcd	0.027 ef	4.40 bc	2.60ab
	Codliver	0.049ab	0.033 def	4.85 b	2.20 bc
	Peanut	0.018 de	0.079abc	1.97 g	0.52 de
	Sesame	0.031 cd	0.098a	5.85a	1.45 cd
	Sunflower	0.041abc	0.065abc	1.99 g	0.50 de
	Corn	0.026 cde	0.090ab	2.20 g	1.27 d
Sucrose	Control	0.017 de	0.009 f	0.80 h	0.10 e
	Soy	0.034 bcd	0.073abc	3.70 cde	0.73 de
	Olive	0.025 cde	0.088abc	3.40 def	1.50 cd
	Wheat germ	0.033 bcd	0.023 f	4.05 bcd	3.20a
	Codliver	0.053a	0.027 ef	5.00 b	1.35 d
	Peanut	0.024 cde	0.079abc	3.55 cdef	0.58 de
	Sesame	0.034 bcd	0.062 bcd	2.70 fg	0.50 de
	Sunflower	0.050a	0.054 cde	4.60 b	0.50 de
	Corn	0.035 bcd	0.063 bcd	2.86 efg	0.82 de

[x] Means followed by the same letter for each column are not significantly different (p=0.05) according to Student-Newman-Keuls. Mean of 5 replicates.

[y] Diameter of the colony was measured as the mean of two diameters of each colony.

[z] Concentration of sugar used.

TABLE III-3

Effects of two sugars and eight oils on sporangia formation of Pythium nunn in a mineral medium. Measurements were the means of 14 and 21 days of growth.

		Sporangia[x]	
Sugar	Oil	20g/l[y]	3g/l[y]
Glucose	Control	8.1ab	0.2 b
	Soy	2.9 cd	2.9 b
	Olive	10.0a	1.7 b
	Wheat germ	0.5 d	4.5 b
	Codliver	0.2 d	0.0 b
	Peanut	0.8 d	1.4 b
	Sesame	0.0 d	0.6 b
	Sunflower	2.3 cd	5.6 b
	Corn	3.2 cd	1.5 b
Sucrose	Control	4.3abc	0.8 b
	Soy	2.9 cd	5.5 b
	Olive	7.2abc	2.1 b
	Wheat germ	0.6 d	10.7a
	Codliver	0.0 d	0.0 b
	Peanut	0.6 d	4.3 b
	Sesame	1.3 d	0.4 b
	Sunflower	0.2 d	2.5 b
	Corn	2.6 cd	3.0 b

[x] Sporangia/20 fields.

[y] Means followed by the same letter in each column are not significantly different (p=0.05) according to Student-Newman-Keuls. Mean of 5 replicates.

PART IV

EFFECTS OF CULTURE FILTRATES OF *TRICHODERMA* SPP. ON GROWTH AND REPRODUCTION OF *PYTHIUM NUNN* AND *PYTHIUM ULTIMUM*

I. ABSTRACT

Isolates of Trichoderma koningii, T. harzianum, T. viride and T. pseudokoningii were tested for their abilities to inhibit growth and/or stimulate reproduction of Pythium nunn (N3) and Pythium ultimum (N1). Culture filtrates of the isolates, sterilized by cold filtration or autoclaving, were seeded with agar plugs of N1 or N3. All filtrates tested inhibited growth of N1 and N3. Two isolates of T. viride inhibited growth and oospore formation of N1 to a greater extent than did the other Trichoderma species. There was no difference of inhibition of growth and oospore formation with autoclaved or cold filtrates. Stimulation of reproductive structures in N1 and N3 by filtrates of Trichoderma species was not observed. Inhibition of growth of N3 in filtrates of Trichoderma reduces the possibility of integrating P. nunn with Trichoderma as an effective biocontrol management system.

II. INTRODUCTION

Metabolites of Trichoderma spp. are composed of various compounds which have different effects on many genera of fungi (4,5,10,13,17,26). Some of these metabolites include trichoviridin (25), dermadin (25) and other volatile

(9,13,14,24) and non-volatile (8,10) compounds. The effects of these metabolites on Pythiaceous fungi include inhibition of growth and increased formation of sexual reproductive structures.

Metabolites of different isolates of Trichoderma vary in their effects on growth of fungi. Dennis and Webster (8,9) reported that metabolites of Trichoderma viride Pers. ex Fries isolate 1 inhibited growth of Pythium ultimum Trow but not Pyronema domesticum Sow. Sacc, and metabolites from T. viride isolate 16 had no effect on P. ultimum but inhibited growth of Pyronema domesticum. Weindling (28) tested culture filtrates of T. lignorum Tode and reported that filtrates inhibited growth of Rhizoctonia solani Kuhn and other fungi. Others (2,17) have indicated that T. viride metabolites inhibited growth of Seiridium cardinale Sutton & Gibson, and five Aspergillus species. Barton (1) reported that three isolates of T. viride differed in their ability to produce antibiotics which inhibited growth of Pythium mamillatum Meurs. De oliveira et al. (10) reported that antibiotics produced by T. harzianum Rifai were inhibitory to Sclerotium cepivorum Berk. Other substances such as volatile products of Trichoderma isolates PRL 2343 and 2616 had no apparent effect on sexual reproduction of Phytophthora or Pythium (13).

Trichoderma spp. produced compounds have been used to induce sexual reproduction in Phytophthora and Pythium (3,4,5,13,20). Haskins and Gardner (13) reported that oospores were produced by Pythium acanthicum Drechsler and Pythium catenulatum Matthews when the fungi were grown in a medium containing killed mycelium of Trichoderma. Oospore production of Phytophthora cinnamomi Rands was enhanced when the fungus was incubated in T. viride infested soil (21) or when cultured with T. koningii Oudemans (20). Trichoderma viride has been observed to induce development of sexual structures in normally sterile isolates of Phytophthora (3). Trichoderma koningii, T. piluliferum Webster & Rifai, T. polysporum Link ex Pers. and T. viride have been reported to stimulate production of sexual structures in Phytophthora cambivora Buisman, P. cinnamomi, and P. palmivora Butler (4,5). The objectives of this research were (1) to determine if metabolites from isolates of four Trichoderma spp. inhibit growth of Pythium ultimum and Pythium nunn and (2) to determine if filtrates of selected isolates of Trichoderma induce sexual reproduction in P. ultimum and P. nunn.

III. MATERIALS AND METHODS

Origin of fungal cultures. P. nunn (ATCC #20693) and P. ultimum B-6 (ATCC #5608) were obtained from American Type

Culture Collection, Rockville, Maryland. Trichoderma koningii T8 (12) and T. harzianum T12 (12) were obtained from Dr. G. Harmon, (Geneva, NY). Trichoderma viride isolate A26-10-3 [A26], A345-10-1 [A345], A286-10-1 [A286], T. pseudokoningii AM4-10-1 [AM4], and T. harzianum 15-12-9 [15] (15) were obtained from Dr. L. F. Johnson, Knoxville, TN. Fungal cultures were maintained on medium containing 1 g PDA with 14 g of Bacto-agar per liter of distilled water.

Preparation of filtrates. Ten grams of cornmeal were simmered in 1 liter of distilled water for 1 h at 85 C. The broth was strained and filtered through cheese cloth and Whatman No. 1 filter paper, divided into 100 ml aliquots, and poured into 250 ml flasks. After sterilization a 7 mm diameter plug of Trichoderma was added to each flask and cultures were incubated for one week. Filtrates of Trichoderma were obtained by separating the broth from thalli of Trichoderma with a sterile 0.45 µm Gelman filter and filter apparatus (Gelman Sciences Inc., Ann Arbor Michigan 48106). Half of the filtrate from each isolate of Trichoderma was autoclaved. Seven mls of autoclaved or cold filtrates were placed in a 5.5 cm diameter petri dish. A 7 mm diameter plug of P. nunn (N1) or P. ultimum (N3) was added to each dish. Five petri dish replicates were prepared for each Trichoderma isolate/heat

treatment/Pythium spp. combination. Pythium cultures were incubated at 16 C in total darkness for 21 days. Sterile corn meal media cultures were used as controls.

Colony diameters were measured in each plate and oospores were counted in 10 fields (200x magnification) with a phase contrast microscope (Bausch and Lomb, Rochester, New York, 14603). Data were analyzed by an analysis of variance procedure (PROC ANOVA) of the Statistical Analysis System (SAS) (22). Mean separation tests were performed according to Student-Newman-Keuls test (18). There was no significant difference in inhibition of growth with heated or cold filtrates and results were combined and analyzed as one test. The experiment was repeated.

IV. RESULTS

Effect of culture filtrates of Trichoderma on Pythium nunn. The corn meal broth control had the greatest colony growth (Table IV-1)*. No growth of N3 occured in filtrates of T. viride A286 (Table IV-1). Little growth was observed in filtrates of A345, AM4, A26, and T8 (Table IV-1). Isolate T12 and 15 inhibited growth less than other

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* All tables may be found in Appendix, Part IV.

filtrates (Table IV-1). No oospores or sporangia were observed in any filtrate of Trichoderma or corn meal broth control.

Effect of culture filtrates of Trichoderma on Pythium ultimum. Culture filtrates of T. viride A345 and A286 completely inhibited growth of N1 (Table IV-2). All filtrates significantly inhibited growth and oospore formation of N1 (Table IV-2). No oospores were formed in filtrates of A345 or A286.

V. DISCUSSION

Haskins and Gardner (13) suggested that inducement of sexual structures in P. acanthicum in filtrates of Trichoderma spp. was due to fullfillment of it's sterol requirement. Sterol is present in corn oil (12) and sterol in corn meal may have been responsible for sexual reproduction in N1. However, no oospores were produced in the corn meal broth by N3.

The inhibition of growth of the Pythium spp. may have been due to Trichoderma utilizing necessary nutrients in the broth or producing Pythium growth inhibiting compounds (16). Variation in growth inhibition of Pythium spp. in filtrates from the different Trichoderma spp. may have been due to some isolates producing higher contrations of an inhibitor or producing different inhibitors. Growth inhibiting

antibiotics have been noted by other researchers (16,25) and several have been identified (8,25). Dermadin and trichoviridin are metabolic products of strains of T. koningii and are known to inhibit some species of bacteria (25).

There was no difference in the inhibitory effects of autoclaved and cold filtrates of Trichoderma, indicating that these were not broken down by heat. These results were similar to Papavizas et al. (19) where isolates of Trichoderma spp. were exposed to ultraviolet radiation, and produced heat-stable metabolites toxic to Sclerotium cepivorum. However, Lifshitz et al. (16) reported that seven filtrates of Trichoderma spp. inhibited growth of a Pythium sp. and 1:10 diluted four-day-old-filtrates or autoclaved filtrates did not inhibit linear growth of the Pythium sp. Trichoderma harzianum inhibited radial growth of Sclerotium cepivorum but inhibitory activity was considerably reduced after steam sterilization (10). Lifshitz et al. (16) and De Oliveira et al. (10) may have been working with extracts containing volatile compounds of Trichoderma spp. since inhibitory affects were nullified after steam sterilization or after a four day period.

Dennis and Webster (8) suggested that growth medium and/or pH of medium may have an affect on antibiotic

production. Since the Trichoderma isolates used by these other workers were grown on potato dextrose broth (10) or on 2% malt broth (16), the effects reported in the present study could have been different because of the different substrate used (corn meal broth).

Production of toxic metabolites by Trichoderma spp. may possibly be used in biological control of P. ultimum. However, inhibition of growth of P. nunn with both cold filtrates and autoclaved filtrates of Trichoderma reduces the possibility of integrating P. nunn with Trichoderma as an effective biocontrol management system.

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APPENDIX

PART IV

Table IV-1

Growth of *Pythium nunn* in filtrates of *Trichoderma* spp.

Isolate	Colony diameter[z] after 21 days
	-cm-
Control	5.00a
T. harzianum T12	1.70 b
T. harzianum 15	1.30 c
T. koningii T8	0.64 d
T. pseudokoningii AM4	0.57 d
T. viride A26	0.45 de
T. viride A345	0.17 de
T. viride A286	0.00 e

[z] Means followed by the same letter are not significantly different (p=0.05) according to Student-Newman-Keuls. Values listed are the means of 20 culture replicates and colony diameter is the mean of two measurements of each colony.

Table IV-2

Growth of *Pythium ultimum* in filtrates of *Trichoderma* spp.

Isolate	Colony diameter[xy] after 21 days	Oospores[x]
	-cm-	-10 fields-
Control	5.50a	206.00a
T. viride A26	2.05 b	13.80 c
T. harzianum T12	1.95 b	34.10 c
T. harzianum 15	1.47 bc	98.05 b
T. pseudokoningii AM4	1.40 bc	23.50 c
T. koningii T8	0.70 cd	91.30 b
T. viride A286	0.00 d	0.00 c
T. viride A345	0.00 d	0.00 c

[x] Means followed by the same letter in each column are not significantly different (p=0.05) according to Student-Newman-Keuls. Values listed are the means of 20 culture replicates.

[y] Colony diameter is the mean of two measurements of each colony.

VITA

Darrell Donaldson Hensley was born in Memphis, Tennessee on March 16, 1961. He attended Harding Academy and graduated from Germantown High School in 1979. He attended Memphis State University and the University of Tennessee at Martin and received a B. S. degree in Agriculture Science from The University of Tennessee at Martin in 1984. After a brief period working at Memphis Meat Packing Company (John Morrell Inc.) he accepted a position as an Extension Assistant with the Entomology and Plant Pathology Department of The University of Tennessee Agricultural Extension Service. In 1986, Mr. Hensley accepted a graduate research assistantship at The University of Tennessee, Knoxville and began study toward a Master of Science degree in Plant Pathology. He reached that goal in December 1988.