Evaluation of *Talaromyces flavus* as a Biological Control Agent Against *Verticillium dahliae* in Potato

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ABSTRACT

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Talaromyces flavus was evaluated as a potential biological control agent of *Verticillium dahliae* on potato in field microplot studies by amending soil with various levels of microsclerotial inoculum of *V. dahliae* and various rates of pyrophyllite- (Pyrax) base pellets containing ascospores of *T. flavus*. *Verticillium*-free potato seed pieces were planted in test soils and the plants that developed were evaluated over time. Disease progress and yields were not consistently affected by applied rates of *T. flavus* pellet inoculum at any level of *V. dahliae*. Populations of *V. dahliae* recovered from soil or plant roots at harvest were also unaffected by rates of pellet inoculum of *T. flavus*. Recovery of *T. flavus* from rhizosphere soil clinging to potato root sections at harvest in both years of the study never exceeded 6.8% of the sections. Wheat bran-base pellet inoculum of *T. flavus* increased recovery of *T. flavus* sixfold to 10-fold from rhizosphere soil and 1,000-fold from nonrhizosphere soil compared with the Pyrax-base pellet inoculum. Despite this increase, there was still no significant effect on disease development, tuber yield, or percent recovery of *V. dahliae* from rhizosphere or nonrhizosphere soil at harvest.

Potato early dying is caused by the soilborne fungus *Verticillium dahliae* Kleb. Damage to potato (*Solanum tuberosum* L.) is greatly increased by coinfection with root-lesion nematodes, *Pratylenchus* spp. (19,22,29). Because these pathogens are cosmopolitan in many agricultural soils and few disease control options are currently available to growers, potato early dying is of worldwide importance in potato production (19,23), resulting in significant yield losses (5,29). In Ohio, documented losses from potato early dying have been as high as 30% (29).

At present, management of potato early dying is accomplished primarily by use of soil-applied pesticides and crop rotation. Although some soil fumigants (1,6,11,25,29) and systemic insecticide-nematicides (8,31) are effective against potato early dying, they are expensive, and significant environmental and safety concerns restrict their use. Effectiveness of crop rotation as a management option is controversial, in that both successes (12,17,18) and failures (3,9) have been reported.

Recent studies have shown some potential for biological control of *V. dahliae* with the fungus *Talaromyces flavus* (Klöcker) Stolk & Samson (anamorph: *Penicillium dangeardii* Pitt, usually reported as *P. vermiculatum* Dangeard). This fungus was first reported as an antagonist of *Rhizoctonia solani* Kühn (2), and more recently as an antagonist of *Sclerotinia sclerotiorum* (Lib.) de Bary (24,32) and *V. albo-atrum* Reinke & Berth. (10). Biological control of *V. dahliae* by *T. flavus* was first demonstrated in eggplant in 1982 in a study in which preplant soil drenches with ascospores of *T. flavus* decreased the incidence of Verticillium wilt and increased yields (21). In 1986, Davis et al (7) reported that use of a dry Pyrax (pyrophyllite dust, hydrous aluminum silicate, R. T. Vanderbilt Co., Norwalk, CT) seed-piece treatment amended with ascospores of *T. flavus*, when applied to potato seed pieces also treated with the fungicide thiabendazole, resulted in reduced potato early dying severity and increased yields in fumigated soil. Fravel et al (14) reported that Pyrax-base pellets amended with *T. flavus* applied broadcast to soil and incorporated to a depth of 15 cm reduced incidence of potato early dying in a field naturally infested with *V. dahliae*. They also found that *T. flavus* could be isolated from soil the year following a single application of the Pyrax pellet form of *T. flavus*.

Our studies were conducted in field microplots to evaluate the use of *T. flavus* as a biological control agent against *V. dahliae* on potato. Specific objectives were to study the effects of *T. flavus* on disease progress and yield reduction caused by *V. dahliae* in potato and to examine the effects of *T. flavus* on soil populations of *V. dahliae*.

MATERIALS AND METHODS

Preparation of inoculum. Inoculum of *V. dahliae* used throughout this study was prepared by growing the fungus at approximately 22 C on minimal medium (28) overlaid with cellophane. After 14–17 days, microsclerotia were harvested from plates, washed thoroughly three times to remove conidia and mycelium fragments, and incorporated into a dry, sterile-soil carrier. Details of this process have been reported (13).

Before use, the concentration of microsclerotia in inocula was assayed by dilution-plating on streptomycin sulfate-alcohol agar (26). Plates were incubated for 7–10 days at approximately 22 C, at which time colonies of *V. dahliae* were counted and the concentration of *V. dahliae* microsclerotia in the inoculum was calculated. Because of difficulty in uniformly incorporating small amounts of microsclerotial inoculum into test soils requiring low levels of *V. dahliae*, some microsclerotial inoculum was further diluted with additional dry, sterile-soil carrier (9:1, v/v) so that at least 20 g of inoculum were added to each 20-L aliquot of soil in all cases.

The isolate of *T. flavus* (isolate Tf1) used throughout this study was obtained from D. R. Fravel, USDA-ARS, Beltsville, MD. It was maintained on plates of potato-dextrose agar (PDA) and kept in the dark at 28–30 C. Under these conditions, *T. flavus* forms abundant ascospores within 4 wk following transfer onto fresh PDA (20,27).

Ascospores were harvested by adding a small amount of sterile, distilled, deionized water to seven 4-wk-old cultures of *T. flavus* and scraping the surface of each plate. The resulting suspensions were then combined in approximately 200 ml of sterile, distilled, deionized water and blended for 1.5–2.0 min at high speed in a sterile 250-ml metal blender bowl. The concentration of ascospores in the resulting suspension was determined with a hemacytometer.

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Mycelial fragments were observed only rarely in these suspensions.

Sodium alginate pelletized ascospore inoculum of *T. flavus* was produced as described by Fravel et al (15), using a gravity flow apparatus consisting of a glass funnel attached with plastic tubing and glass "Y" connections to disposable glass Pasteur pipettes from which the lower 2–3 cm had been removed. For each batch of pellets, 10 g of sodium alginate were mixed with 450 ml of sterile, distilled, deionized water and a sufficient amount of ascospore suspension to result in a final concentration of 10^5 ascospores per gram of bulking agent, either Pyrax or finely ground wheat bran. The mixture was placed in a sterile glass blender and blended at a high speed for 2 min. It was poured into a sterile beaker and 100 g of a bulking agent and 450 ml of sterile, distilled, deionized water were added. This mixture was blended together by hand and then stirred continuously until used. Portions of the mixture were poured into the funnel of the apparatus and allowed to flow through the pipettes, where the mixture dripped into a 0.25 M solution of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), which served as the gelling agent. After about 500 ml of pellets had collected in the gelling solution, pellets were gathered, spread out on brown butcher paper, and allowed to air-dry for 24–36 hr. Dry pellets were gathered and stored at 25 C in a sterile screw-capped jar for up to 4 wk before use.

In 1987, as a further check on the concentration of *T. flavus* ascospores in pellets, 5 g of pellets were added to 45 ml of a sterile aqueous solution containing 1.18 g/L of potassium phosphate (8.7×10^{-3} M KH_2PO_4) and 4.25 g/L of sodium phosphate (3.0×10^{-3} M Na_2HPO_4) and stirred for 3–4 hr on a magnetic stir plate until the pellets had dissolved completely (15). The resulting suspension was diluted 10^{-2} to 10^{-5} with 9-ml aliquots of 0.1% water agar. Three to five 1-ml replicates of all dilutions were plated on *T. flavus* selective agar medium (TFA) (20). Plates were incubated 13–14 days at 28–30 C, and the number of colony-forming units per gram of pellets was calculated. This assay was performed immediately following pellet manufacture. All rates of Pyrax-base pellet inoculum used are reported on a kilogram-per-hectare basis, but were calculated for mixing into soil based on the surface area of the microplots used in these studies.

Preparation of potato planting stock. The potato cultivar Superior, which is highly susceptible to *V. dahliae*, was used throughout these studies. Seed pieces were cut from tubers produced by plants propagated from rooted cuttings taken from pathogen-free tissue cultures and grown in steam-disinfested soil under greenhouse conditions (30). At planting, sprouts ranged from 0.5 to 2.0 cm long. In all experiments, one seed piece was planted at a depth of 2–3 cm in each container.

Soil mixing. Nonfumigated Wooster silt loam (65% silt, 20% fine sand, 15% clay, 2% organic matter, pH 5.7–6.5, CEC 8–10) was gathered from cultivated fields near Wooster, OH, that had never been used for potato production. Before using, soil was passed through a screen with 1-cm openings to remove clods and debris. Pyrax-base pellet inoculum of *T. flavus* and microsclerotial inoculum of *V. dahliae* were then mixed with soil for 2–3 min in an electric cement mixer to achieve desired treatment combinations. After mixing, 50-g samples were taken from each batch of infested soil. Samples from like treatments were bulked and stored at 4 C in polyethylene bags until assayed for populations of *V. dahliae*.

Assays for *Verticillium* and *Talaromyces*. Soil populations of *V. dahliae* were assayed by wet-sieving, as described by Martin et al (22), at both mixing and harvest. A 10-g sample of soil was passed through a sieve with a pore size of 841 μm, then washed with tap water through nested sieves with pore sizes of 125 and 38 μm. The residue on each sieve was collected into separate 50-ml beakers, where residue and water totaled 10–20 ml. The contents of each beaker were then spread over 10 plates of sodium polygalacturonic acid agar (SPA) (22). Plates were then incubated at approximately 22 C for 13–17 days, after which the soil was washed from the agar surface. The number of colonies of *V. dahliae* formed in the medium were then counted on each plate and populations of microsclerotia per gram of soil were calculated and then transformed to natural logarithms to equalize variance.

Samples of field soil taken immediately after mixing with microsclerotial inoculum were stored at 4 C until assayed. Samples taken at harvest were placed on 15-cm-diameter unwaxed paper plates and allowed to air-dry for 1 mo at approximately 22 C. This was done to kill residual conidia and mycelial fragments of *V. dahliae* (4) so that colonies that developed on assay plates could be considered to have developed from microsclerotia.

Populations of *T. flavus* were assayed from both rhizosphere soil clinging to potato roots and nonrhizosphere soil gathered from microplots at harvest. Soils were stored in polyethylene bags at 4 C for up to 4–5 wk until assayed. Nonrhizosphere soil was passed through a sieve with a pore size of 841 μm to remove clods and debris, and then placed onto 15-cm-diameter unwaxed paper plates. Approximately 5 g of each sieved soil were collected into sterile test tubes, capped, and stored at 4 C for assay, usually within 1–4 days. Remaining soil on each plate was weighed, air-dried at approximately 22 C for 1 mo, and then reweighed to determine percent moisture. Soil samples were assayed for *T. flavus* by performing 10-fold serial dilution platings of 1-g aliquots of fresh soil onto TFA. Plates were incubated in polyethylene bags for 14 days in the dark at approximately 30 C, and then the number of *T. flavus* colonies were counted. Colonies ranged in size from 2 to 20 mm in diameter and were orange-yellow in color. Calculated populations were corrected for soil moisture to determine colony-forming units per gram of dry soil and then were transformed to natural logarithms to equalize variance.

Colonization of rhizosphere soil by *T. flavus* was determined from root systems gathered from individual potato plants. Roots were hand-separated from nonrhizosphere soil and 25 2-cm-long root sections with adhering soil were cut at random from each root system, plated on TFA (five sections per plate), and incubated in the dark at 28–30 C for 14 days. Following incubation, each set of five plates was examined for typical orange-yellow colonies of *T. flavus*. Each set was scored for the number of root sections with one or more associated colonies of *T. flavus* and recorded as a percentage of total root sections examined. Percentage data were transformed using the arc sine square root transformation to normalize distribution before further statistical analyses were performed.

Evaluation of disease progress and tuber yields. Beginning at onset of symptoms, each test plant was visually rated weekly until harvest on a scale of 0–3, where 0 = no visible symptoms; 1 = some chlorosis, especially in older leaves; 2 = general chlorosis coupled with some necrosis and wilting; and 3 = severe wilting or death (30). Based on these ratings, areas under disease progress curves (AUDPC) were calculated by the method of trapezoids using days of the year as the X axis (13). Values of AUDPC were then standardized to a 0–3 scale by dividing the calculated areas by the total length of each rating period. The standardized area is referred to as AUDPC throughout the manuscript. Standardization of the areas was done to aid in interpretation of the area data by relating it to the visual rating scale. At harvest, tubers were gathered from each plant and fresh weights were recorded.

Field microplot studies. Methodologies for using clay-tile field microplots (25 cm diameter × 30 cm long) in potato early dying research in Ohio have been detailed elsewhere (22,30). In all cases, 15 replicate microplot tiles, each containing a single potato plant, were used per treatment.

Microplot tiles were planted on 6 June 1986 and 2 June 1987. Soil in each tile was fertilized with approximately 10 g of 10-20-20 NPK granular fertilizer at 31

days after planting in 1986, and 37 days after planting in 1987. Fertilizer was spread around the base of each plant and worked into the upper 2–3 cm of soil. Insects, primarily potato leafhoppers (*Empoasca fabae* Harris), were controlled by three applications each year of azinphos-methyl (Guthion) at the rate of 0.42 kg a.i./ha. In 1987, the insecticide carbaryl (Sevin) was also applied at the rate of 1.34 kg a.i./ha for control of the tarnished plant bug (*Lygus lineolaris* Plaisot de Beauvois). The fungicide chlorothalonil (Bravo 500) was applied twice in 1987 at the rate of 1.02 kg a.i./ha to control foliar early blight (*Alternaria solani* Sorauer). Due to hot, dry weather in 1987, each microplot was irrigated with 600 ml of water (equivalent to 12.0 mm of rain) 14 days after planting, and 2,000 ml (equivalent to 40.0 mm of rain) 60 and 84 days after planting.

Disease ratings were begun 56 and 70 days after planting in 1986 and 1987, respectively, and continued weekly for the next 5–6 wk until harvest. Disease ratings were used to calculate AUDPC values for each treatment as described. Microplots were harvested 97 and 101 days after planting in 1986 and 1987, respectively. Each microplot tile was removed from the ground and the contents of each were placed into separate metal baskets. Fresh weights of tubers were recorded and the root system and a sample of nonrhizosphere soil from each tile were gathered into separate polyethylene bags and stored at 4–5 C for 2–14 days. Replicates of each treatment combination were selected at random and rhizosphere soil was assayed for *T. flavus* and nonrhizosphere soil was assayed for populations of both *T. flavus* and *V. dahliae*. After appropriate transformations, data were analyzed by linear regression. A significance level of $P = 0.05$ was used in all analyses.

The 1986 field experiment was designed to examine the effects on potato early dying of various rates of Pyrax-base pellets, amended or unamended with ascospores of *T. flavus*, added to soil infested with various levels of *V. dahliae*. Data were collected on potato early dying symptom development, tuber yield, colonization of rhizosphere soil by *T. flavus*, and final nonrhizosphere soil populations of both fungi.

Treatments used in this study were factorial combinations of 0, 25, and 100 microsclerotia of *V. dahliae* per gram of soil and either 0, 0.06, 0.3, 1.5, or 7.5 g/microplot (0, 12, 61, 305, or 1,523 kg/ha broadcast) of Pyrax-base pellet inoculum of *T. flavus*. Control microplots contained 1.5 or 7.5 g/tile of Pyrax-base pellets that were not amended with ascospores of *T. flavus*. Rates of Pyrax-base pellets were chosen to cover a broad range of potential application rates. Levels of microsclerotia of *V. dahliae* were chosen to simulate amounts found

Table 1. Area under disease progress curves (AUDPC) and tuber yields of individual potato plants grown in 1986 in field microplots containing soil infested at planting with various levels of microsclerotia of *Verticillium dahliae* and/or rates of Pyrax-base pellets amended with ascospores of *Talaromyces flavus*

V. dahliae (microsclerotia per gram of soil)	*T. flavus* pellets (kg/ha)					
	0	12	61	305	1,523	P[a]
			AUDPC[b]			
0	0.85	1.12	1.13	1.02	0.98	0.615
25	1.36	1.25	1.13	1.07	1.22	0.868
100	1.27	1.48	1.30	1.46	1.57	0.106
P[a]	0.042	0.034	0.111	0.002	0.000	
			Yield[c]			
0	573	555	599	597	591	0.804
25	547	556	480	529	574	0.585
100	450	569	523	569	589	0.187
P[a]	0.054	0.884	0.538	0.812	0.961	

[a] P = linear trend significance level for the change in AUDPC or yield with increasing rates of one organism at a constant rate of the other.
[b] Area under disease progress curves calculated from six weekly evaluations of each plant on a 0–3 visual rating scale and then adjusted to the scale by dividing the total area by the length of the rating period. Each value is the average of 15 replicate plants.
[c] Average fresh weight of tubers in grams per plant of 15 replicate plants, 97 days after planting.

Table 2. Area under disease progress curves (AUDPC) and tuber yields of individual potato plants grown in 1986 in field microplots containing soil infested at planting with various levels of microsclerotia of *Verticillium dahliae* and/or rates of unamended Pyrax-base pellets

V. dahliae (microsclerotia per gram of soil)	Unamended pellets (kg/ha)			
	0	305	1,523	P[a]
		AUDPC[b]		
0	0.85	1.11	1.01	0.602
25	1.36	1.21	1.09	0.121
100	1.27	1.49	1.71	0.003
P[a]	0.042	0.003	0.000	
		Yield[c]		
0	573	657	523	0.233
25	547	474	496	0.648
100	450	461	333	0.014
P[a]	0.054	0.019	0.003	

[a] P = linear trend significance level for the change in AUDPC or yield with increasing rates of one organism at a constant rate of the other.
[b] Area under disease progress curves calculated from six weekly evaluations of each plant on a 0–3 visual rating scale and then adjusted to the scale by dividing the total area by the length of the rating period. Each value is the average of 15 replicate plants.
[c] Average fresh weight of tubers in grams per plant of 15 replicate plants, 97 days after planting.

Table 3. Average populations of *Verticillium dahliae* assayed from soil[a] from field microplots in 1986 at harvest of potato plants grown for 97 days

V. dahliae (microsclerotia per gram of soil)	Amended pellets (kg/ha)						Unamended pellets (kg/ha)			
	0	12	61	305	1,523	P[b]	0	305	1,523	P[b]
0	0.53[c]	0.60	1.77	0.32	0.64	0.654	0.53[c]	0.93	0.14	0.587
25	2.21	2.70	1.74	2.31	2.53	0.765	2.21	0.82	0.81	0.205
100	1.81	2.50	3.28	3.37	3.33	0.568	1.81	1.94	2.18	0.737
P[b]	0.568	0.134	0.206	0.023	0.049		0.568	0.009	0.174	

[a] Soil infested at planting with various levels of microsclerotia of *V. dahliae* and/or rates of Pyrax-base pellets amended with ascospores of *Talaromyces flavus* or unamended.
[b] P = linear trend significance level for the change in *V. dahliae* populations with increasing rates of one organism at a constant rate of the other.
[c] *V. dahliae* microsclerotia (ms), reported as $\log_e(ms+1)/g$ of soil, determined by wet-sieving air-dried field soil and plating onto sodium polygalacturonic acid media. Each value is the average of five soil samples, each collected from one of five replicate microplots.

in uninfested and moderately and heavily infested fields.

Harvest assays for root colonization by *T. flavus* were performed on all plants as previously described. Five replicate microplots from each treatment were selected at random and assayed for final populations of *V. dahliae* in nonrhizosphere soil as before. From these, three replicate plants from the treatments involving pellets amended with ascospores of *T. flavus* and the untreated controls were selected and assayed for final soil populations of *T. flavus*.

Areas under the disease progress curve and tuber yield data from the three rates of unamended pellets at the zero level of microsclerotia of *V. dahliae* were analyzed by one-way analysis of variance ($P = 0.05$) and least significant differences (LSD) were calculated.

The 1987 field microplot study was conducted in a similar manner, except that treatments involving Pyrax-base pellets unamended with ascospores of *T. flavus* were omitted. Two treatments were also included using wheat bran-base pellets to compare the inert Pyrax bulking agent with one that could serve as a food base for *T. flavus*.

Treatment combinations used in this study were all factorial combinations of 0, 0.41, 0.83, 1.23, and 1.65 g/microplot (0, 84, 168, 252, and 336 kg/ha broadcast) of Pyrax-base pellets amended with ascospores of *T. flavus* and either 0, 25, or 70 microsclerotia of *V. dahliae* per gram of soil. Also included were treatment combinations of 1.65 g/microplot (336 kg/ha broadcast) of wheat bran-base pellets amended with ascospores of *T. flavus* with either 0 or 70 microsclerotia of *V. dahliae* per gram of soil.

In addition to the assays performed in 1986, five replicates of each treatment were selected at random and assayed for the presence of *V. dahliae* in the roots at harvest. This was done by plating 10 2-cm-long root sections, which had been washed with a stream of tap water to remove most soil, onto plates of SPA. Following incubation at approximately 24 C for 14 days, plates were examined for the presence of microsclerotia of *V. dahliae*. Percentages of roots infected were calculated and the data transformed as before. Comparisons between wheat bran-base pellets, Pyrax-base pellets, and untreated controls were made by performing a one-way analysis of variance ($P = 0.05$) and calculating LSDs. Data from treatments of the two rates of *V. dahliae* infestation were analyzed separately.

RESULTS

The 1986 field microplot study was designed to examine the effects of various rates of Pyrax-base pellets on development of potato early dying disease. Pellets, amended or unamended with ascospores of *T. flavus*, were incorporated into soil infested with one of three levels of microsclerotia of *V. dahliae* (Tables 1–4). Analysis of AUDPC data indicated that, in most cases, there were significant increases in disease resulting from increasing levels of microsclerotia of *V. dahliae*. However, increasing rates of pellets containing *T. flavus* had no effect on disease development (Table 1). These results are similar to those for unamended pellets, except that at the highest rate of *V. dahliae* there was a significant trend toward higher AUDPC with higher rates of unamended pellets (Table 2).

Tuber yields for plants grown in soil treated with amended pellets were unaffected by increasing levels of *V. dahliae* when pellets were present (Table 1). At the zero rate of pellets, however, increasing levels of *V. dahliae* did have a significant negative influence on yield. There were no significant effects on yield associated with increasing rates of pellets at any level of *V. dahliae* (Table 1). At all rates of unamended pellets, tuber yields declined with increasing levels of *V. dahliae*. At the highest level of *V. dahliae*, yields declined with increasing rates of unamended pellets, but no significant trends were observed at other levels of *V. dahliae* (Table 2).

In the absence of *V. dahliae*, unamended pellets had little effect on potato plants. One-way analysis of variance indicated that unamended pellets at the zero level of *V. dahliae* had no significant effect on yield. With regard to AUDPC, however, application of the 305 kg/ha rate of unamended pellets resulted in an AUDPC significantly higher than the zero rate, while the 1,523 kg/ha rate resulted in an AUDPC that did not differ significantly from that at the zero rate.

Analysis of nonrhizosphere soil taken from microplots at harvest indicated that populations of *V. dahliae* in infested soil ranged from five to 27 microsclerotia per gram of air-dried soil. Increasing rates of pellets amended or unamended with *T. flavus* had no effect on recovery of populations of *V. dahliae* (Table 3). In both cases, populations of *V. dahliae* recovered were generally in proportion to initial levels of infestation, regardless of whether or not added pellets contained *T. flavus* (Table 3). A small, naturally occurring population of *V. dahliae* ranging from zero to five microsclerotia

Table 4. Average populations of *Talaromyces flavus* assayed from soil[a] from field microplots in 1986 at harvest of potato plants grown for 97 days

V. dahliae (microsclerotia per gram of soil)	*T. flavus* pellets (kg/ha)					P[b]
	0	12	61	305	1,523	
0	7.21[c]	6.05	6.12	5.96	6.96	0.207
25	5.81	5.83	6.09	6.12	6.27	0.572
100	6.16	6.21	6.88	5.34	5.55	0.105
P[b]	0.931	0.663	0.019	0.020	0.076	

[a] Soil infested at planting with various levels of microsclerotia of *Verticillium dahliae* and/or rates of Pyrax-base pellets amended with ascospores of *T. flavus*.
[b] P = linear trend significance level for the change in *T. flavus* populations with increasing rates of one organism at a constant rate of the other.
[c] *T. flavus* populations, reported as $\log_e(\text{cfu}+1)$/g of soil, determined by dilution-plating onto *T. flavus* selection agar media. Each value is the average of three soil samples, each collected from one of three replicate microplots.

Table 5. Area under disease progress curves (AUDPC) and tuber yields of individual potato plants grown in 1987 in field microplots containing soil infested at planting with various levels of microsclerotia of *Verticillium dahliae* and/or rates of Pyrax-base pellets amended with ascospores of *Talaromyces flavus*

V. dahliae (microsclerotia per gram of soil)	*T. flavus* pellets (kg/ha)					P[a]
	0	84	168	252	336	
			AUDPC[b]			
0	0.59	0.72	0.59	0.49	0.57	0.607
25	1.02	0.77	0.87	1.47	1.32	0.007
70	1.72	1.64	1.31	1.51	1.17	0.012
P[a]	0.000	0.000	0.000	0.000	0.061	
			Yield[c]			
0	418	516	415	429	398	0.592
25	422	455	510	455	366	0.641
70	362	341	382	456	322	0.637
P[a]	0.299	0.003	0.193	0.770	0.168	

[a] P = linear trend significance level for the change in AUDPC or yield with increasing rates of one organism at a constant rate of the other.
[b] Area under disease progress curves calculated from five weekly evaluations of each plant on a 0–3 visual rating scale and then adjusted to the scale by dividing the total area by the length of the rating period. Each value is the average of 15 replicate plants.
[c] Average fresh weight of tubers in grams per plant of 15 replicate plants, 101 days after planting.

per gram of air-dried soil was also detected in uninfested soil (Table 3).

Populations of *T. flavus* recovered from nonrhizosphere soil at harvest were not affected in any consistent manner by initial levels of *V. dahliae* infestation or by increasing rates of Pyrax-base pellet inoculum of *T. flavus* (Table 4). Percent recovery at harvest of *T. flavus* from potato root sections grown in this microplot study were very low, ranging from 0 to 1.4%.

Table 6. Average populations of *Verticillium dahliae* assayed from soil from field microplots and average percent recovery of *V. dahliae* from root sections collected in 1987 at harvest from individual potato plants grown for 101 days in field microplots containing soil infested at planting with various levels of microsclerotia of *V. dahliae* and/or rates of Pyrax-base pellets amended with ascospores of *Talaromyces flavus*

V. dahliae (microsclerotia per gram of soil)	*T. flavus* pellets (kg/ha)					
	0	84	168	252	336	P^a
	Soil populations[b]					
0	0.13	0.07	0.05	0.09	0.75	0.118
25	3.29	1.32	0.88	4.07	2.71	0.509
70	2.91	3.12	3.45	3.23	3.40	0.310
P^a	0.030	0.000	0.000	0.016	0.015	
	Percent recovery from roots[c]					
0	0.6	0.0	0.0	1.6	0.0	1.00
25	24.3	7.3	13.7	12.3	27.9	0.746
70	16.6	25.4	25.9	6.0	20.8	0.783
P^a	0.276	0.009	0.071	0.664	0.144	

[a] P = linear trend significance level for the change in percent roots infected with *V. dahliae* with increasing rates of one organism at a constant rate of the other.
[b] *V. dahliae* microsclerotia (ms), reported as $\log_e(ms+1)/g$ of soil, determined by wet-sieving air-dried field soil and plating onto sodium galacturonic acid media. Each value is the average of five soil samples, each collected from one of five replicate microplots.
[c] Each value is the average percent recovery of *V. dahliae* from 10 washed, 2-cm-long root sections collected from each of five replicate plants.

Table 7. Average populations of *Talaromyces flavus* assayed from soil[a] from field microplots in 1987 at harvest of potato plants grown for 101 days

V. dahliae (microsclerotia per gram of soil)	*T. flavus* pellets (kg/ha)					
	0	84	168	252	336	P^b
0	1.54[c]	1.95	2.16	2.45	2.52	0.240
25	1.20	2.40	1.89	2.30	3.77	0.011
70	1.21	2.12	2.64	2.92	2.47	0.100
P^b	0.083	0.902	0.538	0.532	0.746	

[a] Soil infested at planting with various levels of microsclerotia of *Verticillium dahliae* and/or rates of Pyrax-base pellets amended with ascospores of *T. flavus*.
[b] P = linear trend significance level for the change in *T. flavus* populations with increasing rates of one organism at a constant rate of the other.
[c] *T. flavus* populations, reported as $\log_e(cfu+1)/g$ of soil, determined by dilution-plating onto *T. flavus* selective agar media. Each value is the average of five soil samples, each collected from one of five replicate microplots.

Table 8. Comparative effects of Pyrax- or wheat bran-base pellets amended with ascospores of *Talaromyces flavus* added to soil in field microplots in the presence or absence of microsclerotia of *Verticillium dahliae* on subsequent populations of *T. flavus* in the soil at harvest and the percent recovery of *T. flavus* from unwashed root sections of potato plants grown in field microplots in 1987 for 101 days

Pellet type[a]	Percent recovery from unwashed root sections		Populations in soil at harvest	
	Not infested[b]	Infested	Not infested	Infested
Pyrax base	5.1[c]	0.4	2.53[d]	2.47
Wheat bran base	33.8	49.5	9.52	9.42
Untreated control	0.0	0.0	1.54	1.21
$LSD_{0.05}$	12.4	4.9	1.63	1.47

[a] Pellets applied at the rate of 336 kg/ha.
[b] Soil infested or not infested with *V. dahliae* before planting. *V. dahliae* applied at the rate of 70 microsclerotia per gram of soil.
[c] Each value is the average percent recovery of *T. flavus* from 25 unwashed, 2-cm-long root sections collected from each of five replicate plants.
[d] *T. flavus* populations, reported as $\log_e(cfu+1)/g$ of soil, determined by dilution-plating onto *T. flavus* selective agar media. Each value is the average of five soil samples, each collected from one of five replicate microplots.

In the 1987 field microplot study, the effects of five rates of Pyrax-base pellets amended with ascospores of *T. flavus* incorporated into soil infested with one of three levels of microsclerotia of *V. dahliae* on various aspects of potato early dying disease were examined (Tables 5–7). Dilution-plating of dissolved pellets showed the concentration of *T. flavus* ascospores to be 3.9×10^5 and 2.0×10^5 per gram of Pyrax-base and bran-base pellets, respectively. Analysis of disease progress data indicated no consistent relationship of AUDPC with increasing rates of pellets amended with *T. flavus* (Table 5). Increasing levels of *V. dahliae*, however, resulted in consistently increased disease at all rates of pellets (Table 5). Tuber yields were not affected by addition of *T. flavus* at any level of *V. dahliae*. Increasing levels of *V. dahliae* affected yield only at the 84 kg/ha rate of pellets (Table 5).

Nonrhizosphere soil populations of *V. dahliae* at harvest ranged from two to 59 microsclerotia per gram of air-dried soil. Final nonrhizosphere soil populations were significantly related to initial infestation levels, despite the presence of low levels of naturally occurring *V. dahliae* detected in uninfested soil, which ranged from zero to one microsclerotia per gram of air-dried soil (Table 6). Populations of *V. dahliae* in nonrhizosphere soil at harvest were not affected by increasing rates of Pyrax-base pellets containing *T. flavus* (Table 6). The average percent recovery of *V. dahliae* from potato root sections assayed at harvest was clearly higher in plants grown in soil infested with *V. dahliae*, but was not consistently related to original infestation level (Table 6). Increasing rates of Pyrax-base pellets containing *T. flavus* also had no effect on the percent recovery of *V. dahliae* from roots (Table 6).

Infestation levels of *V. dahliae* had no influence on populations of *T. flavus* assayed from soil at harvest (Table 7). There were trends toward increased recovery of *T. flavus* with increasing rates of pellet inoculum, but this trend was significant only at the 25 microsclerotia level of *V. dahliae* (Table 7). Percent recovery of *T. flavus* at harvest from potato root sections taken from microplots was again low, ranging from 0 to 6.8%.

Pyrax-base pellets containing ascospores of *T. flavus* were compared with wheat bran-base pellets containing ascospores of *T. flavus* and an untreated control in the 1987 field microplot study. Wheat bran-base pellets resulted in a sixfold to 10-fold increase in percent recovery of *T. flavus* from potato root sections and a 1,000-fold increase in percent recovery of the fungus from nonrhizosphere soil at harvest, compared with Pyrax-base pellets (Table 8). In spite of the improved establishment of *T.*

flavus with wheat bran-base pellets, there were still no significant differences in percent recovery of *V. dahliae* from roots or soil at harvest, in AUDPC, or in tuber yield between either wheat bran-base or Pyrax-base pellets.

DISCUSSION

The isolate of *T. flavus* used in these studies failed to provide effective biological control of potato early dying disease when applied as Pyrax-base alginate pellets. In both the 1986 and 1987 field microplot studies, disease development (AUDPC) was not affected in a consistent manner by increasing rates of pelletized inoculum of *T. flavus*. In fact, in most cases, AUDPC were completely unaffected by increasing pellet rates. The only exception to this was with unamended pellets at the highest level of *V. dahliae* where there was a significant trend toward increased AUDPC with increased pellet rates. We do not, however, attach much importance to this observation, because this trend did not occur at other levels of *V. dahliae*.

Our results are consistent with Fravel et al (14), in which no significant decrease in wilt incidence was achieved in an Idaho field study by the use of Pyrax-base pellet inoculum of *T. flavus* in the first year. In the second year, however, wilt incidence was reduced in plants grown in soil treated the previous year with *T. flavus*, but to which no additional inoculum had been added. In another Idaho study, Davis et al (7) found that *T. flavus* applied to potato seed pieces as a Pyrax-base dust did not reduce wilt incidence in nonfumigated soil. Lack of control of *V. dahliae* in potato reported in these studies is in contrast to greenhouse and field studies with eggplant by Marois et al (21), who found that in unfumigated soil an aqueous drench of ascospores of *T. flavus* was able to significantly reduce disease incidence caused by *V. dahliae*.

Although *T. flavus* did not decrease disease development in either year of our studies, yield data in 1986 were initially encouraging. That year, tuber yields were not decreased by *V. dahliae* with any rate of *T. flavus*. This pattern, however, was not seen in the 1987 field microplot study. Reasons for the differences between years are unclear. Because addition of Pyrax-base pellet inoculum of *T. flavus* at planting did not increase percent recovery of *T. flavus* from roots or populations in nonrhizosphere soil at harvest, it is unlikely that the absence of yield reduction due to *V. dahliae* in 1986 could be attributed to added *T. flavus* inoculum. The fact that a yield reduction was observed in 1987, even with greatly increased soil populations of *T. flavus* from wheat bran-base pellets, further confirms this theory. The possibility of higher populations of *V. dahliae* in the controls as the reason for the difference is eliminated by the observation that populations of *V. dahliae* recovered from soil at the end of the 1986 season were, in most cases, higher where pellets of *T. flavus* were added at planting. This result is also not related to the use of the Pyrax carrier itself, because at all rates of unamended pellets there were significant reductions in yield with increasing rates of *V. dahliae*.

At 0 and 25 microsclerotia per gram of soil levels of *V. dahliae*, rates of unamended pellets had no effect on tuber yields. At the 100 microsclerotia per gram level of *V. dahliae*, there was a trend of decreasing yields with increasing pellet rates. We do not attach much importance to this single observation, however, given that it is not consistent with results observed at other levels of *V. dahliae*. Based on these observations, we conclude that in our studies application of *T. flavus* in Pyrax-base pellets at planting had no consistent effect on subsequent tuber yields at any level of *V. dahliae*. This is in agreement with field results on potato by Fravel et al (14).

Our studies indicate that the inclusion of a food base with inoculum of *T. flavus* may be critical. In these tests, wheat bran-base pellet inoculum resulted in considerably increased colonization of both roots and soil by *T. flavus* when compared with Pyrax-based pellets. Papavizas et al (27) found that, under laboratory conditions in the absence of a plant, the addition to soil of wheat bran-base pellets containing ascospores of *T. flavus* resulted in significantly higher soil populations of *T. flavus* as compared with those achieved with Pyrax-base pellet inoculum. Based on these results, it seems that the inclusion of a food base with *T. flavus* inoculum is essential to affect significant colonization of rhizosphere and nonrhizosphere soil by this fungus.

In our studies, we could detect no consistent effect of this isolate of *T. flavus* on *V. dahliae*. Soil populations of *V. dahliae* at harvest were not influenced by initial applications of *T. flavus* inoculum in any consistent manner, even though soil assays showed that *T. flavus* was present at harvest. Even wheat bran-base pellet inoculum, which resulted in very high soil populations of *T. flavus* at harvest, had no significant effect on populations of *V. dahliae* in soil at harvest or on recovery rate of *V. dahliae* from potato roots as compared with other treatments.

One possible explanation as to why *T. flavus* had no effect on *V. dahliae* in our studies might be that the two fungi must both be present in the soil for some period of time before *T. flavus* can be effective as a biological control agent. This explanation is supported by Fravel et al (14), who found that while there was no reduction in disease incidence in the first year of their field study, there was in the second year. Further support for this theory is provided by Isaac (16), who found that the fungus *Blastomyces luteus* Cost. and Roll. was able to reduce incidence of disease caused by *V. dahliae* in tomatoes only when both fungi were applied to soil at least 4 mo before planting. Studies by Marois et al (20) do not support this. They found that under laboratory conditions in the absence of a plant, germination of microsclerotia of *V. dahliae* was reduced by 32–41% after only 2 wk incubation in soil infested with *T. flavus*.

Results of our work, and that of others, suggest need for further research. Comparative efficacy studies of several isolates of *T. flavus* may be useful, as well as studies with various application methods, food bases, and preplant incubation periods. Ecological studies of these microorganisms in soil may also be useful in understanding interactions occurring in this system.

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LITERATURE CITED

1. Ben-Yephet, Y., Siti, E., and Frank, Z. 1983. Control of *Verticillium dahliae* by metam-sodium in loessial soil and effect on potato tuber yields. Plant Dis. 67:1223-1225.
2. Boosalis, M. G. 1956. Efect of soil temperature and green-manure amendment of unsterilized soil on parasitism of *Rhizoctonia solani* by *Penicillium vermiculatum* and *Trichoderma* sp. Phytopathology 46:473-478.
3. Busch, L. V. 1973. Effect of some cropping practices on survival of *Verticillium*. (Abstr.) Am. Potato J. 50:381-382.
4. Butterfield, E. J., and DeVay, J. E. 1977. Reassessment of soil assays for *Verticillium dahliae*. Phytopathology 67:1073-1078.
5. Davis, J. R. 1981. Verticillium wilt of potato in Southeastern Idaho. Univ. Idaho Curr. Inf. Ser. 564. 4 pp.
6. Davis, J. R. 1985. Approaches to control of potato early dying caused by *Verticillium dahliae*. Am. Potato J. 62:177-185.
7. Davis, J. R., Fravel, D. R., Marois, J. J., and Sorensen, L. H. 1986. Effect of soil fumigation and seed piece treatment with *Talaromyces flavus* on wilt incidence and yield, 1983. Biol. Cult. Tests 1:18.
8. Davis, J. R., Jones, W. B., and Santo, G. S. 1981. Evaluation of aldicarb for Verticillium wilt supression of potato: *Verticillium dahliae* stem colonization and relationship to nematode populations. (Abstr.) Phytopathology 71:104.
9. Davis, J. R., and McDole, R. E. 1979. Influence of cropping sequences on soil-borne populations of *Verticillium dahliae* and *Rhizoctonia solani*. Pages 399-405 in: Soil-Borne Plant Pathogens. B. Schippers and W. Gams, eds. Academic Press, London.
10. Dutta, B. K. 1981. Studies on some fungi isolated from the rhizosphere of tomato plants and the consequent prospect for the control of Verticillium wilt. Plant Soil 63:209-216.
11. Easton, G. D., Nagle, M. E., and Bailey, D. L. 1974. Fumigants, rates and application methods affecting Verticillium wilt incidence and potato yields. Am. Potato J. 51:71-77.
12. Easton, G. D., Nagle, M. E., and Bailey, D. L. 1975. Residual effect of soil fumigation with vine burning on control of Verticillium wilt of potato. Phytopathology 65:1419-1422.

13. Francl, L. J., Rowe, R. C., Riedel, R. M., and Madden, L. V. 1988. Effects of three soil types on potato early dying disease and associated yield reduction. Phytopathology 78:159-166.
14. Fravel, D. R., Davis, J. R., and Sorensen, L. H. 1986. Effect of *Talaromyces flavus* and metham on Verticillium wilt incidence and potato yield, 1984–1985. Biol. Cult. Tests 1:17.
15. Fravel, D. R., Marois, J. J., Lumsden, R. D., and Connick, W. J., Jr. 1985. Encapsulation of potential biocontrol agents in an alginate-clay matrix. Phytopathology 75:774-777.
16. Isaac, I. 1954. Studies in the antagonism between *Blastomyces luteus* and species of *Verticillium*. Ann. Appl. Biol. 41:305-310.
17. Joaquim, T. R., Smith, V. L., and Rowe, R. C. 1988. Seasonal variation and crop rotation effects on population of *Verticillium dahliae* Kleb. in Ohio potato field soils. Am. Potato J. 65:439-447.
18. Keyworth, W. S. 1952. Verticillium wilt of potatoes in Connecticut 1951. Plant Dis. Rep. 36:16-17.
19. Krikun, J., and Orion, D. 1979. Verticillium wilt of potato: Importance and control. Phytoparasitica 7:107-116.
20. Marois, J. J., Fravel, D. R., and Papavizas, G. C. 1984. Ability of *Talaromyces flavus* to occupy the rhizosphere and its interaction with *Verticillium dahliae*. Soil Biol. Biochem. 16:387-390.
21. Marois, J. J., Johnston, S. A., Dunn, M. T., and Papavizas, G. C. 1982. Biological control of Verticillium wilt of eggplant in the field. Plant Dis. 66:1166-1168.
22. Martin, M. J., Riedel, R. M., and Rowe, R. C. 1982. *Verticillium dahliae* and *Pratylenchus penetrans*: Interactions in the early dying complex of potato in Ohio. Phytopathology 72:640-644.
23. McKeen, C. D. 1971. The etiology of Verticillium wilt and the overwintering of *Verticillium albo-atrum* and *V. dahliae* in southwestern Ontario. (Abstr. 23). Proc. Int. *Verticillium* Symp. 1st. Commonw. Mycol. Inst., Wey College, Kent, England.
24. McLaren, D. L., Huang, H. C., and Rimmer, S. R. 1986. Hyparasitism of *Sclerotinia sclerotiorum* by *Talaromyces flavus*. Can. J. Plant Pathol. 8:43-48.
25. Nachmias, A., and Krikun, J. 1985. Verticillium wilt of potato in Israel. Am. Potato J. 62:201-205.
26. Nadakavukaren, M. J., and Horner, C. E. 1959. An alcohol agar medium selective for determining *Verticillium* microsclerotia in soil. Phytopathology 49:527-528.
27. Papavizas, G. C., Fravel, D. R., and Lewis, J. A. 1987. Proliferation of *Talaromyces flavus* in soil and survival in alginate pellets. Phytopathology 77:131-136.
28. Puhalla, J. E., and Mayfield, J. E. 1974. The mechanism of heterokaryotic growth in *Verticillium dahliae*. Genetics 76:411-422.
29. Rowe, R. C., Davis, J. R., Powelson, M. L., and Rouse, D. I. 1987. Potato early dying: Causal agents and management strategies. Plant Dis. 71:482-489.
30. Rowe, R. C., Riedel, R. M., and Martin, M. J. 1985. Synergistic interactions between *Verticillium dahliae* and *Pratylenchus penetrans* in potato early dying disease. Phytopathology 75:412-418.
31. Schultz, O. E., and Cetas, R. C. 1977. Evaluation of granular nematicides for control of "early maturity wilt" of potatoes in New York state. Proc. 1977 Br. Crop Prot. Conf. Pests Dis. 2:491-498.
32. Su, S. J., and Lew, L. S. 1980. Three parasitic fungi on *Sclerotinia sclerotiorum* (Lib.) DeBary. Plant Prot. Bull. (Taiwan, ROC) 22:253-262.