Biological Control of Verticillium Wilt of Eggplant in the Field

J. J. MAROIS, Research Plant Pathologist, Soilborne Diseases Laboratory, Plant Protection Institute, ARS, USDA, Beltsville, MD 20705; S. A. JOHNSTON, Assistant Extension Specialist in Plant Pathology, Cook College, New Jersey Agricultural Experiment Station, Rutgers Research and Development Center, Bridgeton 08302; M. T. DUNN, Research Associate, Department of Botany, University of Maryland, College Park, 20742; and G. C. PAPAVIZAS, Research Plant Pathologist, Soilborne Diseases Laboratory, Plant Protection Institute, ARS, USDA

ABSTRACT

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 Disease 66:1166-1168.

Thirty-four isolates of soilborne fungi comprised of 12 genera and 15 species were tested under greenhouse conditions against *Verticillium dahliae*, causal agent of Verticillium wilt of eggplant (*Solanum melongena*). Six isolates of the soilborne fungi that reduced disease in greenhouse experiments were tested in two fields under different production systems. One isolate of *Talaromyces flavus* reduced disease by 76 and 67% in fields 1 and 2, respectively. Yield was also increased by 18 and 54% (by weight) and 22 and 71% (by number of fruit) in fields 1 and 2, respectively. This is the first report of *T. flavus* being used to control a plant disease caused by *Verticillium* sp.

Verticillium wilt of eggplant (*Solanum melongena* L.), caused by *Verticillium dahliae* Kleb., may destroy entire fields of eggplant in areas highly infested with the pathogen. Crop rotation is not effective in reducing losses from the disease because the broad host range of the pathogen includes many important vegetable crops, and the pathogen persists as microsclerotia (ms) in the absence of known hosts for many years (7). Soil fumigants, which delay the onset of symptoms, are presently used to reduce disease severity in fields infested with the pathogen. Because of the possible adverse effects upon the environment and high costs of fumigants, and the difficulty of crop rotation, the possibility of controlling Verticillium wilt of eggplant with fungal antagonists and reduced amounts of fumigant was investigated.

Eggplant was utilized as a model crop system because of the particular production practices associated with it. In New Jersey, where 25% of the eggplants in the United States are produced (1), plants are started in the greenhouse in 10-cm-diameter pots 8–10 wk before planting in the field. This system enables one to add high populations of beneficial microorganisms to the growth medium (potting mix) of the young plants before their exposure to soilborne pathogens. Also, the high concentration of organic matter in the potting mix (1:1, peat-vermiculite mixture) could provide a suitable substrate for the further establishment of beneficial microorganisms.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

Accepted for publication 21 April 1982.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Initial screening. Thirty-four isolates of soilborne fungi comprising 12 genera and 15 species were selected for their ability to survive in soil and tested for their potential to control Verticillium wilt of eggplant. The fungi were *Aspergillus alutaceus* Berk. & Curt., *Chaetomium lentum* van Warmelo, *Chloridium virescens* (Pers.) W. Gams & Hol.-Jech., *Codinaea heteroderae* Morgan-Jones, *Eupenicillium javanicum* (van Beyma) Stolk & Scott, *Fusarium* sp., *Gliocladium virens* Miller, Giddens & Foster, *Paecilomyces lilacinus* (Thom) Samson, *Penicillium funiculosum* Thom, *Pseudogymnoascus roseus* Raillo, *Talaromyces flavus* (Klöcker) Stolk & Samson, *T. trachyspermus* (Shear) Stolk & Samson, *Trichoderma hamatum* (Bon.) Bain, *T. harzianum* Rifai, and *T. viride* Pers.

For preliminary greenhouse tests, eggplant seeds (cv. Classic) were planted in 10-cm-diameter pots, and five pots were treated with one test fungus. The test fungi were grown for 2 wk on potato-dextrose agar (PDA; Difco, Detroit, MI 48201), and the colony surface was then washed with 5 ml of autoclaved-distilled water. Ten milliliters of the resulting conidial or ascospore suspension, which contained approximately 10^5 conidia or ascospores per ml, was added to each of five pots. The application of the test fungi was repeated 0, 15, 22, and 59 days after seeding. The pots were maintained in a greenhouse compartment at 25 C under natural daylight conditions.

Fifty-nine days after seeding, the plants were transplanted into 20-cm-diameter pots that contained field soil artificially infested with 10 ms of *V. dahliae* per gram of air-dried soil. The ms were obtained from 5-wk-old cultures grown on PDA in total darkness at room temperature (23–26 C). The pathogen cultures were mixed in a Sorvall Omni-Mixer for 2 min and collected on a 200-mesh sieve (75 mm) to separate the ms from mycelial fragments and conidia. The ms were placed in 100 ml of water, and the concentration was determined with an eosinophil counter, an apparatus similar to a standard hemacytometer except that the depth of the counting field is 0.2 rather than 0.1 mm; the greater depth allows for uniform dispersal of the ms. Dilutions of ms were mixed into the soil to distribute the inoculum as uniformly as possible. The 20-cm-pots were placed in a greenhouse bench, and the percentage of plants with foliar symptoms was determined weekly. The final disease assessment was made 9 wk after transplanting when the plants were 17 wk old. Those antagonists that reduced disease 80–100% in the preliminary greenhouse tests were tested further in the field.

Field trials. The first experiment was done in a field located at the USDA Agricultural Research Center at Beltsville, MD. The plot, which contained Westphalia fine sandy loam (pH 7.0, measurement obtained from a 1:2 suspension of soil in 0.01 M calcium chloride), had been used for several years for tomato breeding and variety testing. The natural population density of *V. dahliae* at the time of planting was 3.4 ms/g of soil, as determined by a wet sieving and soil dilution plate technique developed by Green and Papavizas (6). Black polyethylene mulch (2 mil) was placed over 75-cm-wide beds. Holes 6 cm in diameter were cut in the center of the mulch at 30-cm intervals. Sixty grams of a resin-coated, slow-release fertilizer (18-6-12, Osmocote, Sierra Chemical Co., Milpitas, CA 93035) and 50 ml of an antagonist conidial or ascospore suspension (10^4 conidia or ascospores per milliliter) were placed in each transplant hole. Eight-week-old eggplants (Classic) infested with the antagonists as in the greenhouse experiments were transplanted from 10-cm pots into the prepared holes.

The second experiment was done in a field located at the Rutgers Research and Development Center in Bridgeton, NJ, on a plot with an Aura loam soil (pH 6.5). Each 6-m row of plants in the field was infested artificially with *V. dahliae* by adding 30 ml of a blended suspension of the pathogen prepared from a 4-wk-old colony grown in potato-dextrose broth and 100 g of 4-wk-old cultures grown on autoclaved beet (*Beta vulgaris* L.) seeds. The inoculum was immediately incorporated into the soil to a depth of 10 cm and then fumigated with 1,3-dichloropropene and related chlorinated C_3 hydrocarbons + 17% trichloronitromethane (chloropicrin) hereafter designated as 1,3-D+T (Telone C17, Dow Chemical Co., Midland, MI 48640) at 66 L/ha. The fumigant was sealed into the soil by dragging a board over the area immediately after fumigation. The 1,3-D+T was applied at 66 L/ha to test the feasibility of utilizing reduced rates of fumigant. The reduced rate may have increased the efficacy of the antagonist by reducing pathogen inoculum or by providing a less competitive soil environment for the antagonist to become established. The recommended rate to control Verticillium wilt of eggplant is 280 L/ha.

Eggplants (cv. Harris Special Highbush) were infested with the antagonists as in the greenhouse experiments except that antagonists also were added at time of planting by incorporating 50 g of 2-wk-old colonies grown on autoclaved cornmeal into the planting furrow of each 6-m-long plot of 10 plants. The population density of *V. dahliae* at time of planting, 2 wk after fumigation, was 2.8 ms/g of soil, as determined by wet sieving (6). Treatments were replicated four times in a randomized complete block design.

Disease incidence and yield were determined weekly in each field. Disease severity was determined by the percentage of plants showing symptoms. Yield was determined by harvesting all fruit more than 15 cm in length and recording the total number and weight from each plot of 10 plants.

RESULTS

Of the 34 fungal isolates tested in the greenhouse, six reduced the incidence of Verticillium wilt of eggplant to 0–20%, as compared with the 90% disease incidence in the controls. The other 28 fungi did not decrease disease and were not tested further. The six isolates tested in the field were *Aspergillus alutaceus, Gliocladium virens, Paecilomyces lilacinus, Talaromyces flavus, Trichoderma harzianum*, and *T. viride*. Only one isolate, *Talaromyces flavus*, reduced Verticillium wilt of eggplant and increased yield in both test fields.

At the field in Beltsville, yield of eggplant was increased 18% by number of fruit and 22% by weight of fruit when *T. flavus* was added to the soil as a biological control agent (Table 1), significant at the 5% level. The weekly yield from the treated plants was always greater than the nontreated plants (Fig. 1). Disease was less in the *T. flavus*-treated plots than in the nontreated plots during the entire harvest season (Fig. 2). Nine weeks after planting, the percentage of disease in the antagonist-treated plants was 12.5% compared with 52.5% in the control, a reduction of 76% ($P = 0.05$) (Table 1).

In Bridgeton, yield was increased 55% by number of fruit and 71% by weight of fruit when *T. flavus* was added to soil (Table 2). As in the Beltsville field, disease was always less in the antagonist-treated plots than in the control plots (Fig. 3). Although there was no difference ($P = 0.05$) between the yield of the unfumigated control and the plot treated with 1,3-D+T at 66 L/ha, the plot treated with 280 L/ha (the recommended rate for control of Verticillium wilt of eggplant) had greater yield ($P = 0.05$) than any of the other treatments (Table 2). Disease incidence was similar in the *T. flavus*-treated plots that were fumigated previously with 1,3-D+T at 66 L/ha and the plots only fumigated at 280 L/ha (Fig. 3). The antagonist plus the lower rate of 1,3D+T did not control the disease completely, but it delayed disease progress the same as the recommended chemical control. Disease incidence 9 wk after planting was reduced 67% ($P = 0.05$) in the *T. flavus*-treated plots, as compared with the plots that received only 66 L/ha of 1,3-D+T (Table 2).

A. alutaceus and *Trichoderma viride* decreased disease ($P = 0.05$) in the Beltsville field (Table 1); *Paecilomyces lilacinus* and *T. viride* decreased disease ($P = 0.05$) in the Bridgeton field (Table 2). None of the antagonists, except *Talaromyces flavus*, increased yield ($P = 0.05$).

DISCUSSION

Talaromyces flavus was an effective biological control agent in reducing Verticillium wilt of eggplant and in increasing yield as determined by both numbers and weight of fruit. This is the first report of *T. flavus* being used to control a plant disease caused by *Verticillium* spp. Boosalis (3) found that *Penicillium vermiculatum* Dangeard (= *T. flavus*) was a parasite of *Rhizoctonia solani* Kühn; however, disease control was obtained only in autoclaved soil. The only example of control of *Verticillium* spp. with biological agents was that with *Trichoderma lignorum* Tode (= *T. viride*) on cotton (*Gossypium hirsutum* L.) and eggplant in Russia (4). The *Talaromyces*

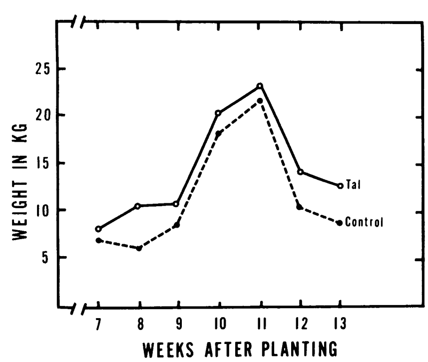

Fig. 1. Relationship of yield (weight in kg) of eggplant to time (weeks after planting) in plots treated with *Talaromyces flavus* (Tal) and control plots in field 1 in Beltsville, MD.

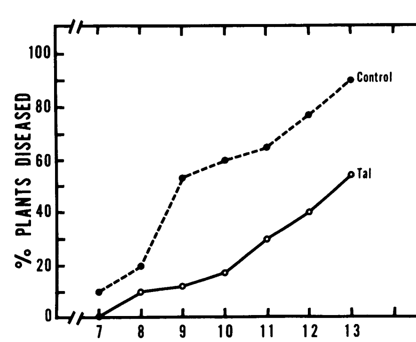

Fig. 2. Relationship of percentage of eggplants showing symptoms of Verticillium wilt (% plants diseased) to time (weeks after planting) in plots treated with *Talaromyces flavus* (Tal) and control plots in field 1 in Beltsville, MD.

Table 1. Effect of selected fungal antagonists on yield and Verticillium wilt of eggplant (cv. Classic) in a naturally infested field in Beltsville, MD

Treatment	Yield of fruit[w] Number	Yield of fruit[w] Weight (kg)	Diseased plants (%)[x]
No antagonist (control)	75.5	17.7	52.5
Aspergillus alutaceus[y]	67.0	17.0	2.5*[z]
Gliocladium virens	68.5	17.2	30.0
Paecilomyces lilacinus	83.7	20.3	30.0
Talaromyces flavus	89.5*	21.7*	12.5*
Trichoderma harzianum	74.2	18.4	52.5
T. viride	74.7	19.8	20.0*

[w] Average yield of four replicates of 10 plants each harvested weekly for 7 consecutive weeks.
[x] Average percentage of diseased plants from four replicates of 10 plants each 9 wk after planting in the field.
[y] Each antagonist was harvested from 2-wk-old cultures grown on petri plates of potato-dextrose agar and applied before transplanting as a conidial or ascospore suspension.
[z] * = Significantly ($P = 0.05$) different from the control according to Dunnett's procedure.

Table 2. Effect of selected fungal antagonists on yield and Verticillium wilt of eggplant (cv. Special Highbush) in an artificially infested field in Bridgeton, NJ

Treatment[w]	Yield of fruit[x]		Diseased plants (%)[y]
	Number	Weight (kg)	
Control: 1,3-D+T (66 L/ha)	19.7	9.2	52.5
Aspergillus alutaceus + 1,3-D+T (66 L/ha)	22.0	10.2	60.0
Gliocladium virens + 1,3-D+T (66 L/ha)	22.7	10.3	42.5
Paecilomyces lilacinus + 1,3-D+T (66 L/ha)	24.7	11.6	22.5*[z]
Talaromyces flavus + 1,3-D+T (66 L/ha)	30.5*	15.8*	17.5*
Trichoderma harzianum + 1,3-D+T (66 L/ha)	20.5	10.4	45.0
T. viride + 1,3-D+T (66 L/ha)	27.7	14.8	20.0*
1,3-D+T (280 L/ha)	42.0*	21.6*	20.0*
1,3-D+T (0 L/ha)	18.5	8.7	77.5

[w] Each antagonist was harvested from 2-wk-old cultures grown on potato-dextrose agar and applied before transplanting as a conidial or ascospore suspension. The antagonist was also applied at plant time by adding 50 g of 2-wk-old colonies grown on autoclaved cornmeal per plot. The plots were fumigated with 1,3-dichloropropene and related chlorinated C_3 hydrocarbons + 17% trichloronitromethane (1,3-D+T).

[x] Average yield of four replicates of 10 plants each harvested weekly for 7 consecutive weeks.

[y] Average percentage of diseased plants from four replicates of 10 plants each 9 wk after planting in the field.

[z] * = Significantly ($P = 0.05$) different from the control according to Dunnett's procedure.

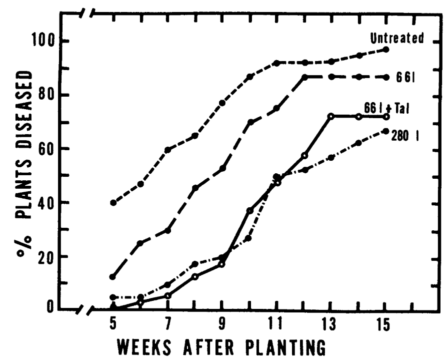

Fig. 3. Relationship of percentage of eggplants showing symptoms of Verticillium wilt (% plants diseased) in nontreated control plots, plots treated with 1,3-dichloropropene and related chlorinated C_3 hydrocarbons + 17% trichloronitromethane (chloropicrin) at 66 L/ha (66 L), plots treated with 66 L/ha and *Talaromyces flavus* (66 L + Tal), and plots treated with 280 L/ha (280 L) to time (weeks after planting) in field 2 in Bridgeton, NJ.

flavus isolate used in this study was obtained from sclerotia of *Sclerotinia minor* Jagger buried in soil at Beltsville. This species appears to be a common soil colonizer and was isolated from most soils studied (5).

Talaromyces flavus was effective in both fumigated and nonfumigated soils. It apparently became established in the pot mix, which was about 50% peat, at high enough levels to affect the activities of the pathogen. Although the actual mode of action has yet to be determined, it is clear that the antagonist reduced disease incidence without addition of a food base. However, because of the experimental design, it could not be determined whether fumigation at the lower rate (66 L/ha) increased the efficacy of the antagonist.

A. alutaceus reduced disease more than any other isolate in the Beltsville field, but this treatment also had the lowest yield. Furthermore, the treatment had little effect in the Bridgeton field. Apparently, *A. alutaceus* affects eggplants adversely under certain conditions, but it also reduces Verticillium wilt. These results emphasize the fact that biological control organisms are affected by different systems and that even though disease severity may be reduced, a corresponding increase in yield may not be observed.

By utilizing the actual soil environment rather than petri plates to screen for antagonists, the selection procedure was very effective. The disadvantages of using petri plates when studying microbial interactions were summarized by Baker and Cook (2). Even though it is not uncommon for organisms that give good disease control in the greenhouse to fail to do so in the field, the chances of success are increased greatly if the actual field situation is simulated as closely as possible in greenhouse experiments. Important parameters that must be considered are method of antagonist application, pathogen inoculum density in the soil, and the time period the plants are grown in the infested soil.

Talaromyces flavus applications can be implemented readily into present production systems. The antagonist can be applied while watering the seedlings in the greenhouse; the antagonist grows readily on several media; and the inoculum from one 2-wk-old colony on PDA is enough to treat 100 plants at the rates used in these experiments.

Further tests will be performed to refine the delivery system and to investigate methods of mass growing of antagonist inoculum. Experiments are planned to test the efficacy of *T. flavus* in controlling other plant diseases and to determine the variation that different isolates of *T. flavus*, either natural isolates or isolates genetically manipulated in the laboratory, may have regarding disease control.

ACKNOWLEDGMENT

We would like to thank James B. Amerault for technical assistance.

LITERATURE CITED

1. Anonymous. 1978. Agricultural Statistics. U.S. Dep. Agric. 605 pp.
2. Baker, K. F., and Cook, R. J. 1974. Biological Control of Plant Pathogens. W. H. Freeman and Company, San Francisco. 433 pp.
3. Boosalis, M. G. 1956. Effect 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.
4. Buimistru, L. D., and Shteinberg, M. E. 1978. Study of rhizosphere mycoflora of eggplants as affected by the application of *Trichoderma lignorum*. Izv. Akad. Nauk Mold. SSR Ser. Biol. Khim. Nauk 1978:87-88.
5. Dunn, M. T., and Lumsden, R. D. 1981. Fungi associated with sclerotia of *Sclerotinia minor* buried in various soils. (Abstr.). Phytopathology 71:766.
6. Green, R. J., Jr., and Papavizas, G. C. 1968. The effect of carbon source, carbon to nitrogen ratios, and organic amendments on survival of propagules of *Verticillium albo-atrum* in soil. Phytopathology 58:567-570.
7. Schreiber, L. R., and Green, R. J., Jr. 1962. Comparative survival of mycelium, conidia, and microsclerotia of *Verticillium albo-atrum* in mineral soil. Phytopathology 52:288-289.