Detrimental Effects of Sublethal Heating and *Talaromyces flavus* on Microsclerotia of *Verticillium dahliae*

E. C. Tjamos and D. R. Fravel

First author: Department of Plant Pathology, Agricultural University of Athens, Votanikos 11855, Athens, Greece; and second author: Biocontrol of Plant Diseases Laboratory, USDA, ARS, Beltsville, MD 20705.

This research was supported in part by BARD Grant No. US-1740-89, the United States-Israel Binational Agricultural Research and Development Fund.

We thank L. Kennaly and R. Nuclo for technical assistance and J. Chittams, University of Maryland and USDA, ARS Statistical Consulting Service, for assistance with comparison of disease progress curves.

Accepted for publication 27 December 1994.

ABSTRACT

Tjamos, E. C., and Fravel, D. R. 1995. Detrimental effects of sublethal heating and *Talaromyces flavus* on microsclerotia of *Verticillium dahliae*. Phytopathology 85:388-392.

Microsclerotia of *Verticillium dahliae* were not heated or heated in water with one of three regimes to simulate soil solarization. Regime 1 consisted of 31 C for 10 h followed by 35 C for 14 h. Regime 2 consisted of 33 C for 10 h followed by 36 C for 14 h. Regime 3 consisted of 35 C for 10 h followed by 38 C for 14 h. Heating for 1–5 days with regime 1 did not affect the final number of microsclerotia that germinated but did affect the rate at which they germinated. Additional days of heating with regime 2 or 3 affected both the number of germinated microsclerotia and the rate at which they germinated. Of the microsclerotia that germinated, colonies arising from heated microsclerotia formed significantly fewer melanized microsclerotia and melanization of these new microsclerotia was significantly slower than for those originating from the nonheated microsclerotia. When microsclerotia were heated as above, placed in nylon mesh envelopes, and buried in raw soil with or without the biocontrol agent *Talaromyces flavus*, there was a synergistic interaction between the heating and *T. flavus* resulting in increased mortality of the microsclerotia. Colonies arising from surviving, previously heated microsclerotia were slower growing than colonies from nonheated microsclerotia and were slower to form melanized microsclerotia. Sublethal heating and *T. flavus* acted additively to suppress Verticillium wilt of eggplant. Opportunities for combining sublethal heating with thermophilic biocontrol agents are discussed.

Dead fungal propagules placed in natural soil are degraded quickly by microbes. Living propagules, especially if they are dormant, are resistant to microbial degradation. Microsclerotia of *Verticillium dahliae* Kleb. can survive in a dormant state for up to 30 yr in agricultural fields. In some cases, propagules are not killed, but instead are weakened or stressed by sublethal heat or chemical treatment. These weakened propagules exhibit an impaired ability to defend themselves from attack by other microbes (7,19).

Solarization has been used to reduce losses to Verticillium wilt of tomato, olive, artichoke, *Prunus* spp., and cotton (14,15,23–26, 28). In some cases, the reduction of inoculum of *V. dahliae* in solarized soil was accompanied by a concomitant increase in populations of biocontrol fungi such as *Talaromyces flavus* (Klöcker) A. C. Stolk & R. A. Samson (25,27,28). Since the effects of solarization on populations of *V. dahliae* often last for 1–3 yr following a single solarization event, some of the beneficial effects of solarization may be due to the activities of thermotolerant antagonists (13,25,27,28).

Talaromyces flavus, which has demonstrated promise in control of Verticillium wilt on eggplant and potato (4,20,21), is thermotolerant (2). Heating at 70 C for 1 h is used to break ascospore dormancy (16). Thus, *T. flavus* is a logical choice for integration of biocontrol with solarization.

Verticillium dahliae overwinters in plant debris or soil as microsclerotia. These propagules are able to survive for several years under adverse environmental conditions in the absence of host plants (9,18,31). Fumigation, steaming, and solarization have been used extensively to lower populations of viable microsclerotia in soil and to reduce crop losses caused by *V. dahliae* (28). Microsclerotia of *V. dahliae* are sensitive to moist heat. At a constant temperature of 36 C or greater for 41 days this treatment will result in at least 90% mortality of microsclerotia in soil (12,22,23). Sublethal heating, however, may weaken the resting structures of plant pathogenic fungi, resulting in alterations of vital biochemical processes of the pathogen, thus allowing them to be attacked by soil microbes (6,7).

This research was undertaken to determine heating regimes necessary to weaken microsclerotia of *V. dahliae*, parameters useful in assessing weakening in *V. dahliae*, and whether or not these weakened propagules are more vulnerable to biocontrol by *T. flavus*. This information can be used to improve, augment, or develop more efficient integrated control of *V. dahliae*.

MATERIALS AND METHODS

Isolates. Isolate V-1 of *V. dahliae* (21) was used in all experiments. To obtain microsclerotia, the fungus was maintained in 250-ml Erlenmeyer flasks containing 100 ml of Czapek-Dox broth (Difco, Detroit, MI). Cultures were shaken continuously at 160 rpm for 17 days under ambient conditions (21–22 C). To collect microsclerotia, cultures were removed from the shaker and allowed to stand for 30 min so that microsclerotia settled to the bottom of the flask. The top layer, containing hyphae and conidia, was removed by decanting. Microsclerotia were resuspended in 100 ml of sterile distilled water (SDW) and allowed to settle for 30 min, and additional floating hyphae and conidia were decanted. The resuspending and settling procedure was repeated three times. Microsclerotia were resuspended in 100 ml of SDW and macerated twice for 1 min each time in an Omnimixer (Sorvall, Newtown, CN) at full speed. After maceration, microsclerotia were sus-

pended in SDW, allowed to settle for 30 min, and the top layer decanted off and discarded. This procedure was repeated three times. The slurry containing the microsclerotia was spread uniformly in a thin layer in 9-cm-diameter glass petri plates without tops, using approximately one plate for each original Erlenmeyer flask. The slurry was dried in a laminar airflow hood for 1–2 h. Plates were then covered and transferred to a 30 C incubator for 30 h to destroy remaining hyphae and conidia. Microsclerotia from each plate were resuspended in 100 ml of SDW and macerated twice for 1 min each in an Omnimixer at full speed. Approximately 10^5 microsclerotia were suspended in 3 ml of SDW in a 1.6-cm-wide × 15-cm-tall test tube (1 cm depth). Tubes were placed in water baths at temperatures representing those found at 30 cm soil depth during solarization (11,12). Regime 1 consisted of 31 C for 10 h followed by 35 C for 14 h. Regime 2 consisted of 33 C for 10 h followed by 36 C for 14 h. Regime 3 consisted of 35 C for 10 h followed by 38 C for 14 h. Nontreated microsclerotia were held under ambient conditions in water. Microsclerotia were heated for 1, 2, 3, 4, or 5 days in a given regime with two replicate tubes for each regime × day combination. *Talaromyces flavus* isolate Tf-1 was maintained on potato-dextrose agar (Difco) at 27 C. Ascospores of *T. flavus* were collected from 3-wk-old plates for use as drenches or for formulation in prill.

Germination and melanin deposition. Tubes containing treated microsclerotia were mixed for 30 s on a vortex mixer and 2 ml of SDW was used to wash microsclerotia from the walls of the tube into the suspension, resulting in 5 ml total volume for each tube. Contents of the tube were plated onto a soil extract medium (17) by spreading 1 ml over the surface of each of five plates. Plates were incubated in the dark at 21 C and examined daily for 5 days to record percent germination of microsclerotia. Plates with microsclerotia from regimes 2 and 3 were examined daily for 14 days for formation of new, melanized microsclerotia. Differences among final numbers of germinated microsclerotia and of new, melanized microsclerotia were determined using analysis of variance. Differences in the rates of germination and formation of new, melanized microsclerotia were determined by analysis of variance on the slopes of the curves. The experiment was repeated once.

Interaction of sublethal heating with *T. flavus*. Microsclerotia were grown and prepared as described above. Microsclerotia were not heated or were heated for 1 or 2 days with regime 3. Microsclerotia were stored overnight at 5 C. To facilitate recovery of microsclerotia from soil, microsclerotia were placed inside nylon mesh envelopes. Two pieces (approximately 0.5 × 0.5 m each) of nylon mesh (30 μm pore size) (Tetco, Elmsford, NY) were sewn using nylon thread. Sewing was in a grid pattern with stitching lines 2.5 cm apart. A second line of stitching was then sewn approximately 0.5 cm from each original stitching line. The mesh was then cut between the stitching lines to result in pieces 15 cm × 15 cm (5 envelopes × 5 envelopes). Glue (Super Strength Adhesive, 3M, St. Paul, MN) was spread along the stitching lines to prevent loss of microsclerotia from stitching holes. Microsclerotia were suspended in 150 ml of SDW in a 200-ml beaker to result in 10^3 microsclerotia per milliliter. The suspension was continuously stirred with a magnetic stirrer. One milliliter of this suspension was inserted into a corner of each envelope using a syringe with a broad needle (Precision Glide Needle 16G 1½, Becton Dickinson, Franklin Lakes, NJ). The microsclerotia were deposited in the corner opposite the hole made by the entry of the needle and the needle hole was sealed with the same glue. When all envelopes in a block of 25 envelopes contained microsclerotia, the individual envelopes were cut apart.

Two eggplant seeds (*Solanum melongea* L. cv. Black Beauty) were planted in soilless potting mix (Pro-Mix BX, Premier Brands, New Rochelle, NY) in 54 7-cm-diameter plastic pots. Plants were maintained at 21–25 C in a greenhouse under natural light. After emergence, plants were thinned to one plant per pot. After 3 wk, plants were transplanted into 15-cm-diameter plastic pots containing a nonsterile Glaestown sandy loam (GSL) soil (pH 5.8, 77.8% sand, 12.6% silt, 9.6% clay). After an additional 3 wk, plants were transplanted into 27-cm pots containing GSL with or without envelopes of microsclerotia not heated or heated for 1 or 2 days under regime 3. Pots were filled approximately one-fourth full and three envelopes were arranged horizontally in a triangle on the soil surface. The transplant was placed on top of the triangle and the pot was filled with GSL.

Microsclerotia received one of three treatments of *T. flavus*: no *T. flavus*, *T. flavus* in alginate prill, or *T. flavus* applied as a drench. In the drenching treatment, 3 ml of 10^6/ml ascospore suspension of *T. flavus* was placed on each envelope with an additional 1 ml in the center of the triangle formed by the envelopes. One week after transplanting, plants in the drenching treatment received an additional 10 ml of 10^6/ml ascospores poured directly on the soil surface. Alginate prill were prepared with or without ascospores of *T. flavus* as previously described (5) using ground corn cobs as the carrier (Grit-o-Cobs, The Andersons, Cob Division, Maumee, OH). For microsclerotia receiving prill, 0.1 g of prill was divided among the 3 envelopes in each pot. Prill were placed directly on the envelopes before transplanting the eggplant seedlings. Envelopes were destructively sampled at 4 and 8 wk after transplanting. There was a total of 18 treatments (3 heating regimes × 3 biocontrol treatments × 2 sampling times) with three pots/treatment.

Sampling. Mesh envelopes with microsclerotia of *V. dahliae* were recovered 4 or 8 wk after transplanting. Individual envelopes were washed under running tap water for 2 min and rubbed slightly to disperse aggregates of microsclerotia. Microsclerotia were washed into one corner of each envelope, which was then cut diagonally. The inside of the envelope was washed out with 10 ml of distilled water and the contents collected in a test tube. After the suspension settled for 30 min, the top 7 ml was removed by aspiration. Each suspension was adjusted so that it contained 100–200 microsclerotia per milliliter. One milliliter of each suspension was plated on a 15-cm-diameter plastic petri plate containing ethanol-streptomycin agar (ESA), a medium semiselective for *V. dahliae* (1). After 10 days incubation at 21 C, the percentage of microsclerotia giving rise to melanized colonies in each case was recorded. Data were analyzed by analysis of variance. This experiment was repeated with one modification. In the second experiment, rather than plating 1 ml of the suspension containing recovered microsclerotia, microsclerotia were washed into a test tube with approximately 10 ml of distilled water. The contents of each tube were collected on a 0.45-μm pore size filter (Nuclepore, Pleasanton, CA). The filter with microsclerotia was placed in a glass petri plate. Working under a dissecting scope at 30×, a syringe needle (Precision Glide Needle 26G 3/8 Becton Dickinson, Franklin Lakes, NJ) was used to transfer 25 individual microsclerotia to each of two 9-cm-diameter petri plates of ESA (10). Plates were incubated as previously. After germination, some microsclerotia with developing hyphae (0.8-cm-diameter plug) were transferred to ESA and colony diameter was recorded every 2–3 days.

Pathogenicity. Eggplant seeds were planted in soilless potting mix in 7-cm-diameter plastic pots as above. In previous studies with *T. flavus*, prill were added at seeding (4). In this experiment, for treatments with prill, 0.5 g of prill was placed around each root ball at transplanting. Plants receiving drenches were drenched with 10 ml of 10^6/ml ascospores at 2, 3, and 5 wk after seeding. Microsclerotia of *V. dahliae* were grown as above. Microsclerotia were heated by placing 100 ml of 2.2–2.5 × 10^4/ml microsclerotia suspension in a 250-ml Erlenmeyer flask. Microsclerotia were not heated or were heated in a water bath for 1, 2, or 3 days under regime 3. Microsclerotia suspended in 100 ml of SDW were mixed in a bread mixer for 1 min into 5 kg (fresh wt) of GSL soil (Hobart Corporation, Troy, OH) and this soil was mixed with an additional 50 kg (fresh wt) of GSL soil in a cement mixer for 5 min to result in 40–45 microsclerotia per gram of soil. Plants were maintained in a greenhouse under natural light at 21–25 C. Wilt incidence was recorded every 2–3 days for 93 days. Petioles were collected from plants with wilt symptoms and were plated onto ESA to confirm infection by *V. dahliae*. At the termination of the experiment, petiole isolations were made from all remaining

plants. The experiment was repeated once.

Analysis of disease progress curves is usually based on populations of plants and percentage of incidence in each replicate is recorded. In our experiments, data recorded were binary, that is, each plant was either diseased or not diseased. Thus, an alternative data analysis was needed. Data were analyzed using the LIFETEST procedure in SAS (7). Gehan's generalized Wilcoxon test was used to compare survival curves of the various treatment groups (8). This test gives more weight to early wilting than to late wilting and thus is more likely to detect early differences in survival distributions.

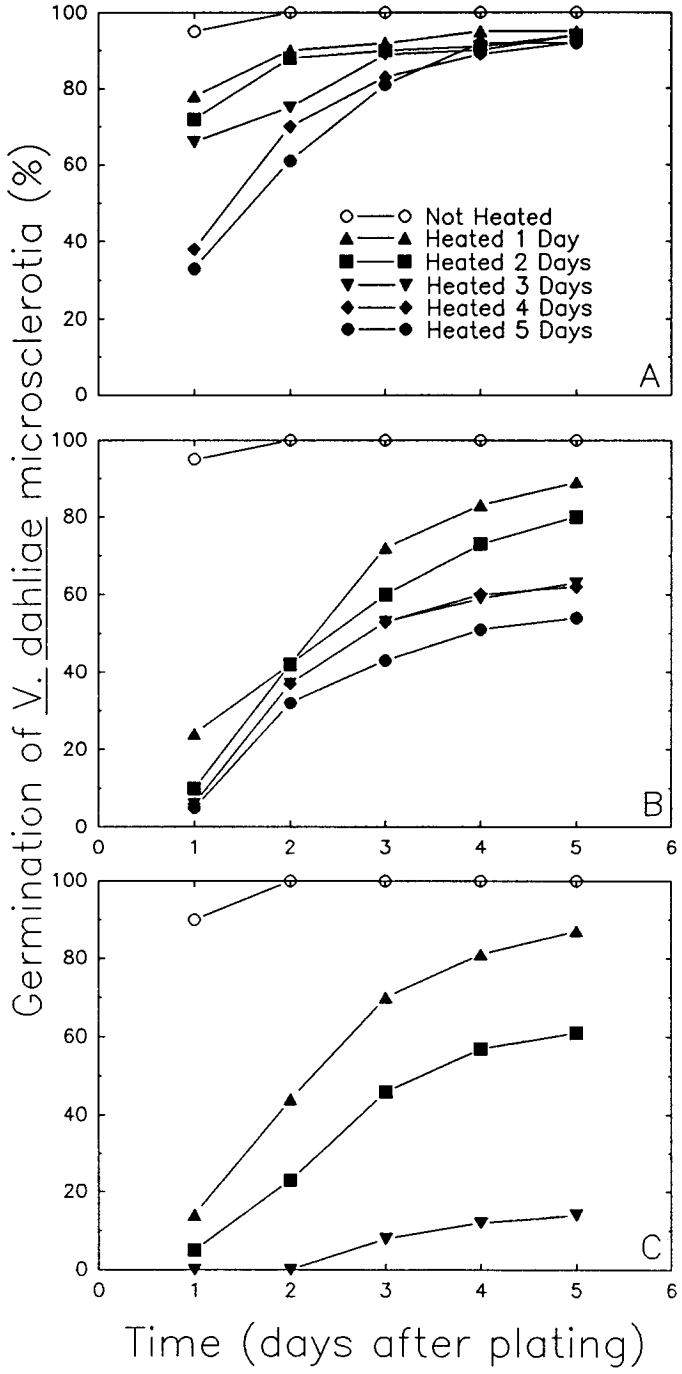

Fig. 1. Relationship of germination of microsclerotia of *Verticillium dahliae* to heating. Microsclerotia were not heated or heated 1–5 days with regime 1 **(A)** (31 C for 10 h then 35 C for 14 h); regime 2 **(B)** (33 C for 10 h then 36 C for 14 h); or regime 3 **(C)** (35 C for 10 h then 38 C for 14 h). Microsclerotia were plated onto an agar medium and germination was recorded for 5 days.

RESULTS

Effect of sublethal heating on microsclerotial germination. All microsclerotia that were not heated germinated 1–2 days after plating. Ninety percent of the nonheated microsclerotia germinated within 24 h and 100% within 48 h after plating (Fig. 1A). Regardless of the duration of heating, 90% of the microsclerotia heated under regime 1 (31–35 C) germinated 5 days after plating. Although additional days of heating with regime 1 did not affect the number of microsclerotia that had germinated after 5 days, it did affect the rate at which they germinated ($P \leq 0.0001$) (Fig. 1A). Additional days of heating with regime 2 or 3 affected both the final number of germinated microsclerotia ($P \leq 0.01$) and the rate at which they germinated ($P \leq 0.0001$) (Fig. 1B, C). Under regime 2 (33–36 C), 90% of microsclerotia heated for 1 day germinated in 5 days while 82, 62, 62, and 50% of those heated for 2, 3, 4, and 5 days, respectively, had germinated in 5 days (Fig. 1B). Five days after plating, 87, 61, 14, 0.9, and 0.2% of microsclerotia treated for 1 to 5 days with regime 3 (35–38 C), respectively, had germinated (Fig. 1C).

Effect of sublethal heating on the formation of melanized microsclerotia. Of the microsclerotia that germinated (above), nearly 40% of the colonies arising from microsclerotia in the nonheated control formed numerous round and melanized microsclerotia by 3 days (Fig. 2). By 7 days after plating, 75% of the germinated microsclerotia had formed melanized microsclerotia. In contrast, only 4% of the colonies arising from microsclerotia that were heated for 1 day under regime 3 formed melanized microsclerotia 6 days after plating and 11% at 9 days after plating. Compared with the control, there was at least a 3-day delay in melanin deposition in colonies arising from microsclerotia heated with regime 3. When treated with regime 2 for 1 day, 17 and 34% of germinated microsclerotia produced new, melanized microsclerotia in 6 and 10 days after plating, respectively.

Heating regime (nonheated, regime 2, or regime 3) significantly affected both the final number of melanized microsclerotia ($P \leq 0.003$) and the rate at which they were produced ($P \leq 0.001$). Additional days of heating with regime 3 also significantly affected both the final number of melanized microsclerotia ($P \leq 0.01$) and the rate at which melanized microsclerotia were produced ($P \leq 0.001$).

Survival in soil. Recovery of microsclerotia of *V. dahliae* from envelopes buried in soil with eggplants was difficult due to contaminants that grew around the plated microsclerotia and germination and subsequent melanization were determined microscopically. Melanization of newly formed microsclerotia could be seen 6–7 days after plating. By 10 days after plating, melanized colonies could be easily observed and recorded. With respect to melaniza-

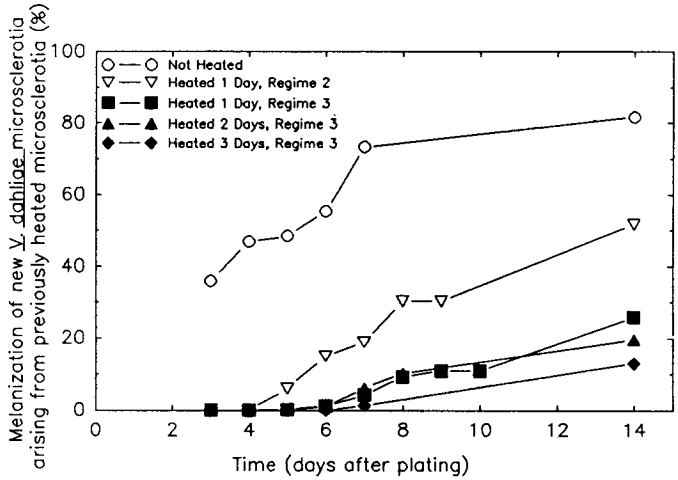

Fig. 2. Production of new melanized microsclerotia from germinated microsclerotia of *Verticillium dahliae* receiving heat treatments. Microsclerotia were not heated or heated 1–5 days with regime 1 (31 C for 10 h then 35 C for 14 h); regime 2 (33 C for 10 h then 36 C for 14 h); or regime 3 (35 C for 10 h then 38 C for 14 h). Data reported are for microsclerotia that germinated.

tion of newly formed microsclerotia, there was a highly significant ($P \leq 0.0075$) and a synergistic interaction between heating and the presence of *T. flavus* (Fig. 3). Results from the first and second samplings were similar and only data from the first sampling are shown.

Pathogenicity tests. At 49 days after planting (median wilt incidence for many treatments), all but three treatments were similar to the healthy control (Fig. 4). At the same time, the healthy controls and microsclerotia heated for 3 days were different from the disease control ($P \leq 0.02$). All other treatments were similar to the nonheated microsclerotia (disease control) although there was a trend ($P \leq 0.07$) for all treatments with *T. flavus* prill suppressing wilt as well as the combination of microsclerotia heated for 3 days and plants drenched with *T. flavus*. There was no overall interaction between heating and *T. flavus* ($P \leq 0.32$).

DISCUSSION

Sublethal heating of microsclerotia of *V. dahliae* had detrimental effects on germination of microsclerotia as well as on the rate of melanization of newly formed microsclerotia and the final percentage of new microsclerotia that became melanized. A key enzyme(s) involved in the production of melanin in *V. dahliae* is inactivated by heating at 40 C for 35 min (30). Possibly, sublethal heating inactivates this enzyme or disrupts its activity. Melanin functions in the defense of microsclerotia from microbial attack (3,10) as well as in protection of microsclerotia from ultraviolet irradiation (10). Thus, reductions in the percentage of microsclerotia that become melanized and reduced rates of melanin deposition would be expected to result in increased susceptibility to attack by biocontrol agents.

Freeman and Katan (7) demonstrated that *Fusarium oxysporum* f. sp. *vasinfectum* propagules are weakened by sublethal heating. Lifshitz et al (19) also showed the effect of sublethal heating on sclerotia of *Sclerotium rolfsii*. The levels of heat used in our experiments were sufficient to cause some loss of viability. It is plausible to envisage that the remaining, viable microsclerotia were weakened and that the weakening was expressed as reduced melanin deposition and pathogenicity, and increased susceptibility to *T. flavus*.

The additive effects on disease incidence and the synergistic interaction of sublethal heating with the biocontrol fungus *T. flavus* offer new opportunities for integrated disease control. Because of the levels of heat generated, plants cannot be grown during solarization (11) and the land is out of production for 1–2 mo. By combining sublethal solarization with biocontrol, it may be possible to keep the land in production by using a plastic that generates lower temperatures than those usually used in solarization. Applying preparations of *T. flavus* to soil prior to plastic mulching for solarization may facilitate establishment of the antagonist in the solarized soil. This may have particular importance for perennial crops such as olive. Alternatively, it may be possible to reduce the length of time that the soil is covered by conventional solarization plastics if the solarization is combined with thermotolerant biocontrol agents. There is convincing evidence that *T. flavus* and other potential thermotolerant natural populations of bioantagonists are able to survive solarization and increase after solarization (25,27,28) and *T. flavus* readily colonizes solarized soils (29). Work is in progress to determine if sublethal metham sodium and other stressing agents cause similar responses in *V. dahliae* and thus offer additional opportunities for integrated control of Verticillium wilt diseases.

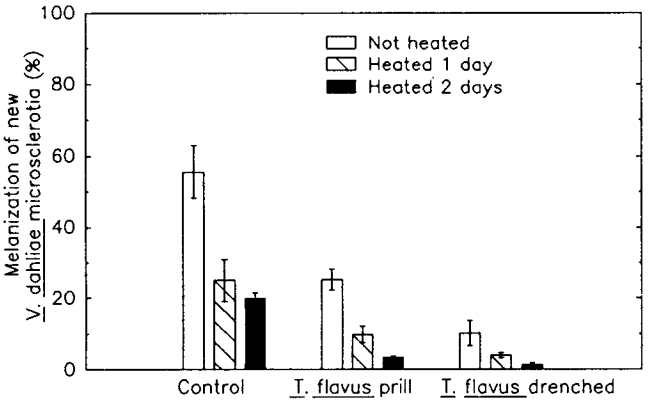

Fig. 3. Effect of heating and the biocontrol fungus *Talaromyces flavus* on production of melanized microsclerotia. Microsclerotia were not heated or heated 1–2 days at 35 C for 10 h then 38 C for 14 h. Microsclerotia were then placed in nylon mesh envelopes and placed on soil. *Talaromyces flavus* was applied as a drench or alginate prill to the envelopes. Envelopes were covered with additional soil and a 6-wk-old eggplant was transplanted into the pot. Microsclerotia were recovered and plated on an agar medium. Germinated microsclerotia were observed for production of new, melanized microsclerotia.

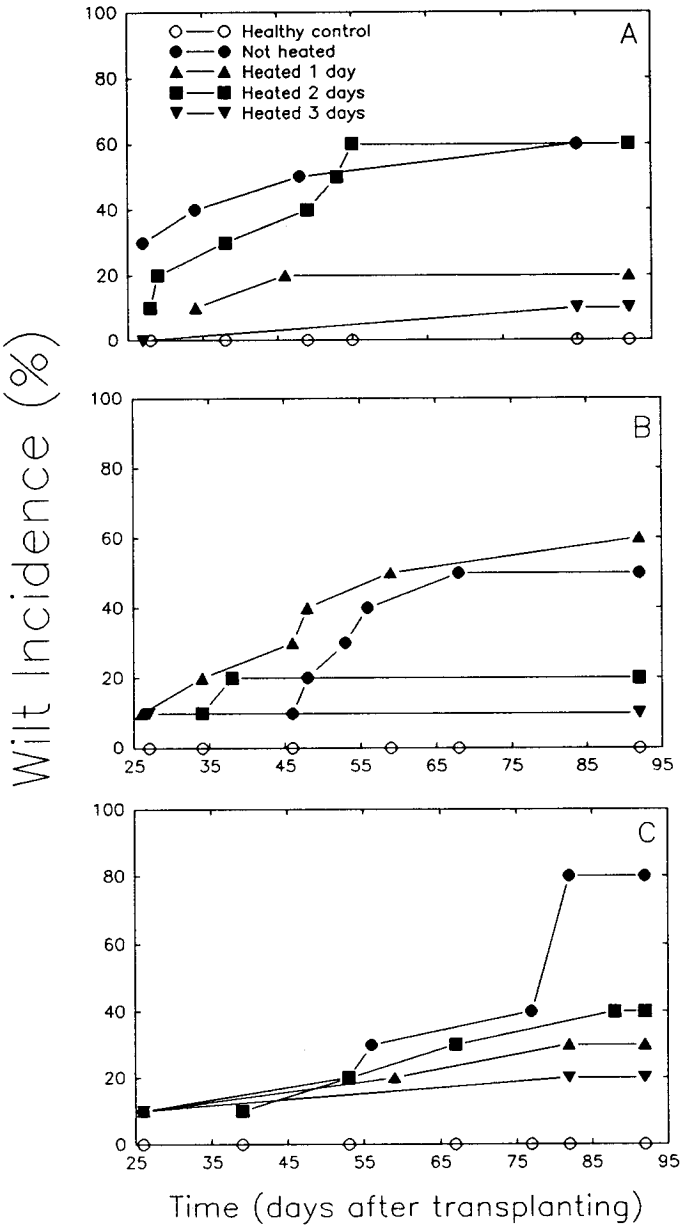

Fig. 4. Effect of sublethal heating of microsclerotia of *Verticillium dahliae* and the biocontrol fungus *Talaromyces flavus* on incidence of Verticillium wilt of eggplant. Microsclerotia were not heated or heated 1–3 days with regime 3 (35 C for 10 h then 38 C for 14 h). **(A)** Wilt incidence over time for plants not receiving the biocontrol fungus *T. flavus*. **(B)** Disease progress for plants receiving drenches with *T. flavus* prior to transplant into Verticillium-infested soil. **(C)** Disease progress for plants receiving prill of *T. flavus* at transplant.

LITERATURE CITED

1. Ausher, R., Katan, J., and Ovadia, S. 1975. An improved selective medium for the isolation of *Verticillium dahliae*. Phytoparasitica 3:133-137.
2. Beauchat, L. R. 1988. Thermal tolerance of *Talaromyces flavus* ascospores as affected by growth medium and temperature, age and sugar content in the inactivation medium. Trans. Br. Mycol. Soc. 90:359-364.
3. Bell, A. A., and Wheeler, M. H. 1986. Biosynthesis and function of fungal melanins. Annu. Rev. Phytopathol. 24:411-451.
4. Fravel, D. R. 1989. Biocontrol of Verticillium wilt of eggplant and potato. Pages 487-492 in: Vascular Wilt Diseases of Plants. E. C. Tjamos and C. H. Beckman, eds. Springer-Verlag, Berlin.
5. 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.
6. Freeman, S., Ginzburg, C., and Katan, J. 1989. Heat shock protein synthesis in propagules of *Fusarium oxysporum* f. sp. *niveum*. Phytopathology 79:1054-1058.
7. Freeman, S., and Katan, J. 1988. Weakening effect on propagules of *Fusarium* by sublethal heating. Phytopathology 78:1656-1661.
8. Gehan, E. A. 1965. A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika 52:203-223.
9. Harrison, J. A. C., and Isaac, I. 1969. Survival of the causal agents of "early-dying disease" (Verticillium wilt) of potatoes. Ann. Appl. Biol. 63:277-288.
10. Hawke, M. A., and Lazarovits, G. 1994. Production and manipulation of individual microsclerotia of *Verticillium dahliae* for use in studies of survival. Phytopathology 84:883-890.
11. Katan, J. 1987. Soil Solarization. Pages 77-105 in: Innovative Approaches to Plant Disease Control. I. Chet, ed. John Wiley & Sons, NY.
12. Katan, J., and DeVay, J. E, eds. 1991. Soil Solarization. CRC Press, Boca Raton, FL.
13. Katan, J., DeVay, J. E., and Greenberger, A. 1989. The biological control induced by soil solarization. Pages 493-499 in: Vascular Wilt Diseases of Plants. E. C. Tjamos and C. H. Beckman, eds., Springer-Verlag, Berlin.
14. Katan, J., Greenberger, A., Alon, H. and Grinstein, A. 1976. Solar heating by polyethylene mulching for the control of diseases caused by soil-borne plant pathogens. Phytopathology 66:683-688.
15. Katan, J., Grinstein, A., Greenberger, A., Yarden, O., and DeVay, J. E. 1987. The first decade (1976-1986) of soil solarization (solar heating): A chronological bibliography. Phytoparasitica 15:229-255.
16. Katan, T. 1985. Heat activation of dormant ascospores of *Talaromyces flavus*. Trans. Br. Mycol. Soc. 84:748-750.
17. Keinath, A. P., Fravel, D. R., and Papavizas, G. C. 1991. Potential of *Gliocladium roseum* for biocontrol of *Verticillium dahliae*. Phytopathology 81:644-648.
18. Krikun, J., and Bernier, C. C. 1987. Infection of several crop species by two isolates of *Verticillium dahliae*. Can. J. Plant Pathol. 9:241-245.
19. Lifshitz, R., Tabachnik, M., Katan, J., and Chet, I. 1983. The effect of sub-lethal heating on sclerotia of *Sclerotium rolfsii*. Can. J. Microbiol. 29:1607-1610.
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. 16:381-390.
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. Pullman, G. S., DeVay, J. E., and Garber, R. H. 1981. Soil solarization and thermal death: A logarithmic relationship between time and temperature for four soilborne plant pathogens. Phytopathology 71:959-964.
23. Pullman, G. S., DeVay, J. E., Garber, R. H., and Weinhold, A. R. 1981. Soil solarization: Effects on Verticillium wilt of cotton and soilborne populations of *Verticillium dahliae, Pythium* spp., *Rhizoctonia solani,* and *Thielaviopsis basicola*. Phytopathology 71:954-959.
24. Stapleton, J. J., and DeVay, J. E. 1982. Effect of soil solarization on populations of selected soilborne microorganisms and growth of deciduous fruit tree seedlings. Phytopathology 72:323-326.
25. Tjamos, E. C., Biris, D. A., and Paplomatas, E. J. 1991. Recovery of olive trees with Verticillium wilt after individual application of soil solarization in established olive orchards. Plant Dis. 75:557-562.
26. Tjamos, E. C., Karapapas, V., and Bardas, D. 1989. Low cost application of soil solarization in covered plastic houses for control of Verticillium wilt of tomatoes in Greece. Acta Hortic. 255:139-150.
27. Tjamos, E. C., and Paplomatas, E. J. 1987. Effect of solarization on the survival of fungal antagonists of *Verticillium dahliae*. EPPO Bull. 17:645-653.
28. Tjamos, E. C., and Paplomatas, E. J. 1988. Long-term effect of soil solarization in controlling Verticillium wilt of globe artichokes in Greece. Plant Pathol. 37:507-515.
29. Tjamos, E. C., and Skretis, I. 1990. Establishment of applied or increase of natural *Verticillium dahliae* antagonists in solarized or untreated soils. (Abstr.) Intern. Verticillium Symp., 5th.
30. Wheeler, M. H. 1982. Melanin biosynthesis in *Verticillium dahliae*: Dehydration and reduction reactions in cell-free homogenates. Exp. Mycol. 6:171-179.
31. Wilhelm, S. 1955. Longevity of the Verticillium wilt fungus in the laboratory and field. Phytopathology 45:180-182.