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Biocontrol of Plant Diseases Laboratory, Plant Sciences Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland, U.S.A.

Evaluation of Oospore Hyperparasites for the Control of Phytophthora Crown Rot of Pepper

E. D. SUTHERLAND and G. C. PAPAVIZAS

Authors' addresses: E. D. SUTHERLAND, Department of Horticulture, Valencia Community College, Orlando, Florida, 32811 and G. C. PAPAVIZAS, Research Leader, Biocontrol of Plant Diseases Laboratory, U.S. Department of Agriculture, Beltsville, Maryland, 20705 (U.S.A.).

With 3 figures

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Abstract

Nine isolates of known oospore mycoparasites comprised of six actinomycetes (*Actinoplanes missouriensis, A. philippinensis, A. utahensis, Amorphosporangium auranticolor, Ampullariella regularis, Spirillospora albida*) and three fungi (*Acremonium* sp., *Humicola fuscoatra, Verticillium chlamydosporium*) were tested in the greenhouse for their ability to suppress or delay the onset of crown rot of pepper caused by *Phytophthora capsici. Verticillium chlamydosporium* applied as a root dip increased the number of healthy plants by more than 100% when peppers were transplanted into soil artificially infested with oospores of *Phytophthora capsici,* but not when peppers were transplanted into soil naturally infested with *P. capsici.* The other mycoparasites were ineffective in the greenhouse. All the mycoparasites tested parasitized oospores of *P. capsici in vitro.*

Zusammenfassung

Die Beurteilung von Oosporenparasiten bei der Bekämpfung der Phytophthora-crown-rot-Krankheit von Paprika

In Gewächshausversuchen wurden neun als Oosporenmycoparasiten bekannte Isolate, und zwar sechs Actinomyceten (*Actinoplanes missouriensis, A. philippinensis, A. utahensis, Amorphosporangium auranticolor, Ampullariella regularis* und *Spirillospora albida*) und drei Pilzarten (*Acremonium* sp., *Humicola fuscoatra* und *Verticillium chlamydosporium*), auf ihre Fähigkeit, das Auftreten der crown-rot-Krankheit von Paprika, verursacht durch *Phytophthora capsici,* zu unter-

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This research was performed while the senior author was a postdoctoral research associate at the Biocontrol of Plant Diseases Laboratory, Beltsville, MD. Address correspondence to second author.

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drücken oder herauszuzögern. Nach einer Wurzeltauchbehandlung mit *V. chlamydosporium* wurde die Anzahl von gesunden Pflanzen um mehr als 100 % erhöht, wenn die behandelten Pflanzen in einen mit *P. capsici* künstlich inokulierten Boden, jedoch nicht in einen natürlich infizierten Boden, umgesetzt wurden. Alle anderen Mycoparasiten waren in den Gewächshausversuchen wirkungslos. *In vitro* waren alle geprüften Mycoparasiten in der Lage, *P. capsici*-Oosporen zu parasitieren.

Species of *Phytophthora* cause some of the most destructive plant diseases worldwide (GREGORY 1983). Among these diseases, Phytophthora blight and crown rot of pepper, caused by *P. capsici* Leonian, results in substantial losses in pepper production (JOHNSTON and SPRINGER 1978). Although pepper lines are now available with considerable resistance of this pathogen (BARKSDALE, PAPAVIZAS and JOHNSTON 1984), none of these lines is commercially available yet. Because of the potential of *Phytophthora* spp. to mutate and form new races or strains, crop resistance and chemical control may offer only a partial solution to disease control. Cultural controls such as site selection, planting on raised beds, and crop rotation have been partially successful (JOHNSTON and SPRINGER 1978). Experimental biological control with hyperparasites has also been investigated (AYERS and LUMSDEN 1975, HUMBLE and LOCKWOOD 1981, HSU and LOCKWOOD 1984, SUTHERLAND and LOCKWOOD 1984). Hyperparasites applied as seed or soil treatments effectively reduced disease on soybean caused by *Phythophthora megasperma* f. sp. *glycinea* under greenhouse conditions (FILONOW and LOCKWOOD 1985, SUTHERLAND and LOCKWOOD 1984).

The objective of this study was to evaluate the potential of several hyperparasites to reduce the incidence of crown rot of peppers (*Capsicum annuum* L.) caused by *P. capsici.*

Materials and Methods

Hyperparasites and *P. capsici* isolates

The following known parasites of oospores of *Phytophthora* spp. were selected for this study (HUMBLE and LOCKWOOD 1981, SNEH *et al.* 1977): The actonomycetes *Actinoplanes missouriensis* Couch, *A. philippinensis* Couch, *A. utahensis* Couch, *Amorphosporangium auranticolor* Couch, *Ampullariella regularis* Couch, and *Spirillospora albida* Couch; and the fungi *Acremonium* sp., *Humicola fuscoatra* Traaen., and *Verticillium chlamydosporium* Goddard. The actinomycetes were maintained on modified Czapek's agar and transferred biweekly and the fungi were maintained on Difco® potato-dextrose agar (PDA) transferred monthly (SUTHERLAND and LOCKWOOD 1984).

The isolates of *P. capsici* used in this study were S1, compatibility type A1, obtained from infected squash in New Jersey in 1978 by G. C. Papavizas, and isolate ATCC 15399 (A2) from the American Type Culture Collection. These isolates were maintained on Difco® lima bean agar (LBA) and transferred biweekly. For oospore production, a 2-week-old lima bean broth culture of each isolate was comminuted in sterile distilled water (SDW) in an Omni-mixer (Sorvall Omni-mixer, DuPont Instruments, Newtown, CT 06470) and 1 ml of the suspension was used to inoculate each of several plates of V-8 liquid medium containing 30 mg/l cholesterol (AYERS and LUMSDEN 1975). Cultures were incubated at 25 °C in the dark for 30 days.

Oospore infection

Hyperparasites were tested for their parasitic efficacy on oospores of *P. capsici* with the methods of SUTHERLAND and LOCKWOOD (1984). Oospores were separated from mycelia by comminuting liquid cultures in water in an Omni-mixer. Oospores were concentrated by centrifugation,

washed twice with sterile distilled water by centrifugation, and approximately 10^3 oospores were applied with suction to 1-cm^2 pieces (25 μm pore size) on monofilament nylon fabric (Tetko, Inc. Elmsford, NY 10523). The fabric was inverted onto unsolidified water agar and removed after solidification, leaving oospores embedded on the agar surface. Spores and mycelial fragments in sterile distilled water of potential mycoparasites were counted in a hemacytometer and the numbers adjusted to deliver 10^4 propagules with 1 ml per plate. The 1-ml suspension was spread on the surface of the agar containing the oospores with a glass rod. Parasitism was assessed microscopically after 1 week. Oospores were considered infected when mycelia or spores of the hyperparasite replaced the oospore cytoplasm, or when the cytoplasm was granulated and disorganized. A vital stain was also used to assess hyperparasitism (SUTHERLAND and COHEN 1983).

Greenhouse studies

The actinomycetes and fungi were evaluated in the greenhouse for their potential biocontrol ability. Peppers (cv. California Wonder) were planted in a complete peat moss and vermiculite planting mix (New Jersey Ext. Bull., 1983) in 5-cm^2-plastic inserts (J. T. A. Speedling Cutting Tray, Chicago, IL 60608). After 6 weeks, the peppers were transplanted to 10-cm-diameter plastic pots (one plant per pot) containing a Beltsville sandy loam (pH 6.9) that had been artificially infested with 2-week-old cultures of *P. capsici* at a concentration of 500 oospores/g of soil 2 weeks before transplanting.

Mycelia and spores were scraped from the surface of 2-week-old cultures of the actinomycetes, or 4-week-old cultures of the fungi, and comminuted in an Omni-mixer with sterile distilled water (one culture per 40 ml of water). Actinomycetes and fungi were added to the mix at 10^2—10^3 and 10^4—10^5 colony-forming units per ml, respectively. Each of the antagonists was applied onto the soil or mix surface in one of the following time sequences: at seeding, at seeding and transplanting, 2 weeks after seeding, and at 2-week intervals until transplanting. In addition, the hyperparasites *A. missouriensis* (10^3 sporangia/ml), *H. fuscoatra* (10^5 conidia/ml) and *V. chlamydosporium* (10^5 chlamydospores/ml) were tested as root dips at transplanting, as seed treatments, or by mixing them into the soil artificially infested with *P. capsici* and incubating the soil for 8 weeks before transplanting. *Humicola fuscoatra* and *V. chlamydosporium* were also tested by incorporating them into the pepper planting mix at 10^5 and 10^4 colony forming units per g of mix, respectively. The microorganisms were grown for 4 weeks in sand-bran medium (600 g quartz sand + 200 g corn meal + 100 g bran + 425 ml water) and the air-dried preparation was used as inoculum and carrier. The preparation was added to the planting medium at the rate of 20% (v/v).

Ten replicate pots were prepared per biocontrol treatment in a completely randomized design. Controls consisted of water treatments or, where applicable, non-inoculated sand-bran carriers. The pots were placed in a temperature controlled chamber maintained at 27 ± 2°C. All pepper plants were maintained on a watering regime (flooded, then watered normally) conducive to the development of Phytophthora crown rot (TSAO and GARBER 1960). The percentage of pepper plants with stem cankers was assessed weekly. Success of the biocontrol agents was determined by the ability of the organisms to reduce stem canker incidence by 50% or more, or to delay disease onset by at least 30% (JOHNSTON and SPRINGER 1978).

Verticillium chlamydosporium was also tested in naturally infested soil as a root dip. The sandy loam soil had been collected from a New Jersey field naturally infested with *Phytophthora* and had been planted to four generations of peppers in greenhouse beds to obtain 100% disease incidence. Six-week-old pepper plants were dipped into water suspension (2 l) of chlamydospores of *V. chlamydosporium* (10^5 chlamydospores per ml) and planted in the naturally infested soil. Ten replicate pots were arranged in a randomized complete design with five blocks. All experiments were repeated one or more times.

Results

Oospores of *P. capsici* on water agar were infected at percentages of 90 or more after 1 week by all the potential biocontrol agents tested. However, biological control of the phytophthora crown rot in the greenhouse was obtained

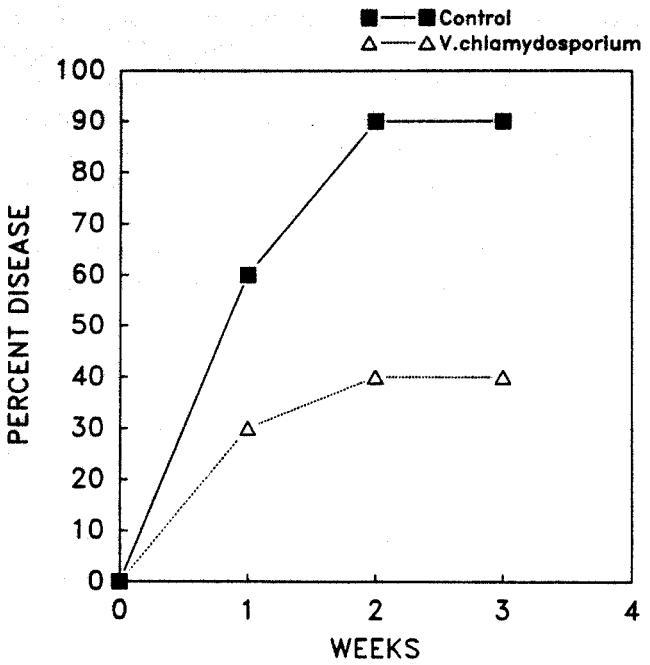

Fig. 1. Percentage of pepper plants that developed crown rot in soil artificially infested with *Phytophthora capsici* over a 3-week period after root dip treatment with *Verticillium chlamydosporium*

only with the hyperparasite *V. chlamydosporium* applied as a root dip or when the planting mix was supplemented with a *V. chlamydosporium*-bran preparation. The plants treated with *V. chlamydosporium* as a root dip developed about 50% less Phytophthora crown rot than plants dipped in water alone (Fig. 1). Two weeks after transplanting, 40% of the peppers developed crown rot with the *V. chlamydosporium* treatment, while 90% of the water treated plants had crown rot. No further disease developed in plants treated with the hyperparasite or with the water controls. Results were similar in a second experiment.

A preparation of *V. chlamydosporium* on sand-bran mix added to the potting mix delayed the onset but did not prevent the development of Phytophthora

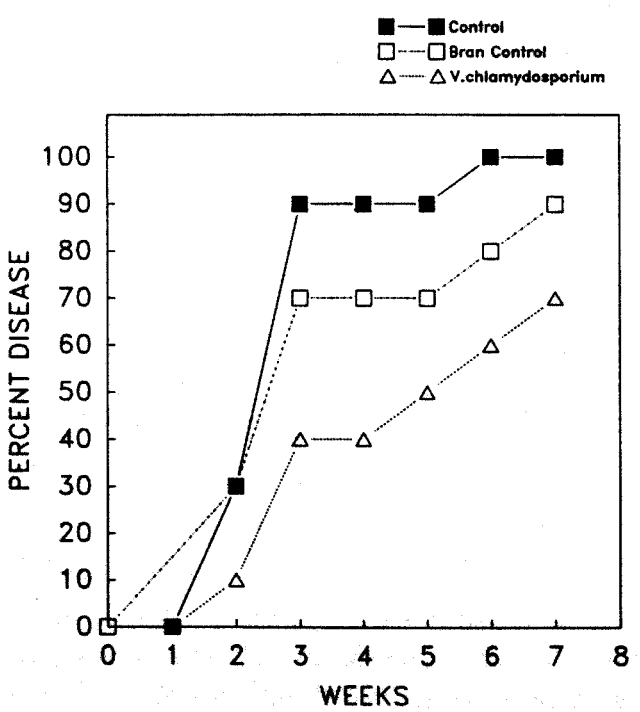

Fig. 2. Percentage of pepper plants that developed crown rot in soil artificially infested with *Phytophthora capsici* and amended with sand-bran mix colonized by *Verticillium chlamydosporium* over a period of 7 weeks

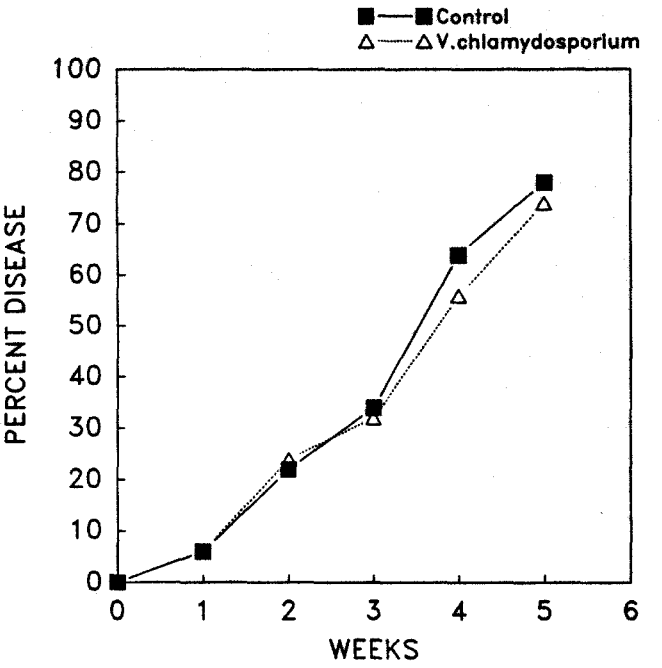

Fig. 3. Percentage of pepper plants that developed crown rot in soil naturally infested with *Phytophthora capsici* over a 5-week period after root dip treatment with *Verticillium chlamydosporium*

crown rot in peppers transplanted to soil artificially infested with *P. capsici* (Fig. 2). Fewer than half as many plants were infected by *P. capsisi* in the *V. chlamydosporium* treatment (40%) than in the controls (90%) during the third and fourth week following transplanting, but disease continued to develop thereafter. At 7 weeks disease was 100% in the control and 70% with the biological control agent. Similar results were obtained in a second experiment.

Since application of *V. chlamydosporium* inoculum to pepper plants as a root dip was the most efficient delivery method in soil artificially infested with the pathogen, root dipping was further used to test the biocontrol efficacy of *V. chlamydosporium* in soil naturally infested with *P. capsici.* Biocontrol was not obtained when pepper plants were dipped in either water or *V. chlamydosporium* prior to transplanting into naturally infested soil (Fig. 3). Crown rot of peppers was observed in 6% of the plants in both the untreated control and *V. chlamydosporium* treatment the first week after transplanting. Symptom expression increased steadily over a 5-week period to 78% in the water control and to 74% in the *V. chlamydosporium* treatment. No differences were found in either disease development or in the amount of disease observed.

Discussion

The present study extended the host range of nine known oospore mycoparasites to include *P. capsici.* These results also support those of SUTHERLAND and LOCKWOOD (1984) who described parasitism of oospores of some Peronosporales by the same actinomycetes used in this study and by the fungi *H. fuscoatra* and *V. chlamydosporium.* Chlamydospores of *P. parasitica* var. *nicotianae* were also infected by the actinomycetes and by the two mycoparasitic fungi (E. D. SUTHERLAND, unpubl.).

The mycoparasite *V. chlamydosporium* used as a root dip suppressed disease incidence in natural soil artificially infested with *P. capsici*, but not in naturally infested soil (Figs 1, 3). The discrepancy in biocontrol results in artificially infested soil vs. naturally infested soil cannot be readily explained. Oospores in naturally infested soil may be embedded in organic debris that protects them from the immediate action of the mycoparasites. Also, naturally produced, weathered oospores may be more resistant to the mycoparasitic action. For several reasons, including possible differences in chemical composition, oospores produced on agar and added to soil may be more sensitive to the debilitating action of various microbial and physiochemical factors that make them more susceptible to the mycoparasitic action of the actinomycetes and fungi than naturally produced oospores. Similar views on mycoparasitism were expressed elsewhere (AYERS and ADAMS 1981).

In all of the screening tests performed to test the biological control potential of *V. chlamydosporium*, disease was delayed and/or suppressed in tests only where the biocontrol agent was present at transplanting. Apparently, *V. chlamydosporium* was not in an activated state because even when it had been incorporated in the planting mix on sand-bran, disease was delayed but not prevented by *V. chlamydosporium*. The application of the antagonist as a root dip apparently allowed the introduction of activated chlamydospores into the disease management system. In contrast, experiments by MAROIS *et al.* (1982) have shown that the establishment of the antagonist *Talaromyces flavus* in the planting mix afforded protection from Verticillium wilt of eggplant.

Although *V. chlamydosporium* was ineffective in reducing disease in naturally infested soil under the conditions tested, this oospore parasite should not be eliminated from consideration as a biological control agent. The tenacity of this organism in soil may allow it to be particularly effective in controlling disease over a long period of time. In a field test where *V. chlamydosporium* inoculum was added to the pepper transplanting mix before transplanting, the number of healthy plants was higher by seven-fold in blocks where the disease was severe than when the antagonist was not added to the mix (SUTHERLAND, unpubl.). The difficulty in obtaining consistent disease makes it very difficult to perform reliable field tests, but only experiments of this type can validate the reliability of biological control agents.

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