Novel Use of a Pyrenomycetous Mycoparasite for Management of Fusarium Wilt of Watermelon

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ABSTRACT

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Fusarium wilt of watermelon, caused by *Fusarium oxysporum* f. sp. *niveum*, is a destructive disease that limits watermelon production in many areas of the world. The discovery of several pyrenomycetous ascomycetes occurring naturally in association with different formae speciales of *F. oxysporum* identified these fungi as potential biological control organisms for watermelon wilt. One such mycoparasitic isolate, identified as *Sphaerodes retispora* var. *retispora*, was chosen for biological control and ecological trials in the greenhouse. Four different methods to inoculate the mycoparasite were evaluated, three of which utilized the parasite encapsulated into sodium alginate pellets. The other method employed root-dipping plants with mycoparasite ascospore suspensions. Ecological factors also were investigated, including the ability of *S. retispora* var. *retispora* to colonize watermelon roots, and its ability to survive in soil over time and reduce propagules of *F. oxysporum* f. sp. *niveum*. In the biological control studies, the use of the mycoparasite significantly reduced plant mortality and increased dry weights of watermelon plants after being challenged with *F. oxysporum* f. sp. *niveum*, compared with pathogen-inoculated controls. It appears that the incorporation of the parasite into alginate pellets in the planting mix at seeding may be the most practical method for future field evaluations of transplant-grown vegetable crops. In the ecological studies, the mycoparasite was recovered from infested soil after 9 months, but was only isolated from watermelon roots when applied in the presence of *F. oxysporum*. *S. retispora* var. *retispora* had no effect on *F. oxysporum* f. sp. *niveum* propagules after being applied to soils in the greenhouse.

Fusarium wilt and root rot diseases caused by formae speciales of *Fusarium oxysporum* include some of the oldest plant diseases described and are among the most severe and difficult to manage in the world. They cause significant economic losses to a wide range of plant species and occur on all plant families except *Graminaceae* (3). Most control methods available are either inefficient or difficult to apply. Fungicides are generally ineffective because of the location of the pathogen within the plant's vascular system. Soil fumigants can be useful, but disease control is erratic and fumigation often is cost-prohibitive. Furthermore, the beneficial effects of fumigation are generally only temporary and must be reapplied each season due to some formae speciales's ability to rapidly recolonize soils (26).

Short-term crop rotation is of little value because *F. oxysporum* colonizes non-host plants and survives in soils for long periods as chlamydospores (28). Therefore, genetic resistance, when available, is often considered the most effective strategy for managing wilt diseases. However, this also can be problematic because the presence of different biological races necessitates the need for germ plasm containing multiple resistance genes.

Biological control of Fusarium wilts, in the form of suppressive soils, has been recognized for over 70 years (4,16). Control in these soils is based on the exploitation of natural communities of microorganisms and generally is recognized to be among the best and most successful examples of biological control with microorganisms (5). Soils suppressive to *F. oxysporum* have been identified from and intensively studied in numerous locations throughout the world (2-4,15,20,21). The majority of these soils tend to have different mechanisms of suppression; therefore, no one suppressive site would be consistent with any other in terms of generating standard suppressive-soil characteristics or control recommendations.

The wilt disease of watermelon, caused by *F. oxysporum* f. sp. *niveum*, is a major factor that limits production in many watermelon-growing areas (27). The disease was first reported in 1894 from Georgia

and South Carolina (31) and, as with other wilt diseases induced by *F. oxysporum*, effective control measures for the watermelon wilt disease are limited. One of the few successful examples using biological control for this disease involved the induction of wilt-suppressive plots within a known wilt-conducive research field in Florida. These suppressive plots were created by monoculturing the moderately wilt-resistant watermelon cv. Crimson Sweet for 4 to 7 years (6,14,15). This is not a practical solution for all watermelon-growing areas; therefore, investigating alternative measures for managing watermelon wilt is warranted.

The idea for conducting the present study began while surveying Texas sugar beet fields and collecting pathogenic isolates of *F. oxysporum* f. sp. *betae* (12). In approximately 10 to 15% of the field isolations, a pyrenomycete was observed intermingled with the *Fusarium* cultures, and was later identified as *Melanospora zamiae* (8,10). Its presence in the cultures also was observed to seriously affect growth and vigor of the *F. oxysporum* isolates (8,10). Over the next several years, an additional five pyrenomycetous ascomycetes closely resembling *M. zamiae* were found in Florida in constant association with field populations of three distinct formae speciales of *F. oxysporum* (8,10). Four of these isolates were identified as *Sphaerodes retispora* var. *retispora* and the fifth isolate proved to be a new report for *Persiciospora moreau* (9,10).

For several reasons, *S. retispora* var. *retispora* (isolate LE) was selected as the candidate for evaluating wilt disease reduction of watermelon in greenhouse tests. First, it sporulated readily in conjunction with the watermelon wilt isolate that was used in the tests. Second, of the six mycoparasites tested, isolate LE was the most aggressive in dual culture with the wilt pathogen and most effectively inhibited and reduced mycelial growth as measured in laboratory assays (11). Last, LE originally was isolated from diseased watermelon roots in association with *F. oxysporum* f. sp. *niveum* from one of the wilt-suppressive research plots in Leesburg, FL. This study was begun with the purpose of addressing the following objectives under greenhouse conditions: (i) to evaluate the pyrenomycetous mycoparasite *S. retispora* var. *retispora* as a biological control agent for Fusarium wilt of wa-

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termelon, (ii) to evaluate different methods for delivering the parasite into the infection court, and (iii) to evaluate the survival of the mycoparasite in soil and its ability to colonize watermelon roots and to reduce *F. oxysporum* f. sp. *niveum* propagules in artificially infested soils.

MATERIALS AND METHODS

Preparation of sodium alginate pellets. Efforts to develop an optimal method of inoculating *S. retispora* var. *retispora* (isolate LE) began with the encapsulation of the mycoparasite into sodium alginate pellets (sodium salt of alginic acid, Acros Organics, NJ) using several modifications of previously reported studies by Fravel et al. (7) and Lewis and Papavizas (22,23). Our modified recipe consisted of an aqueous suspension of ascospores and hyphal fragments of isolate LE, wheat germ ground to pass through a 0.425-mm screen, and sodium alginate (2.6%). Sodium alginate was dissolved in distilled water (10 g/375 ml) on a stirring hot plate. Ground wheat germ was combined with distilled water (20 g/250 ml) in a separate flask. Both containers were autoclaved for 45 min and cooled to room temperature before adding the mycoparasite. Isolate LE was prepared by grinding 3-week-old

potato dextrose agar (PDA) or mycological agar (MA) cultures in sterile, distilled water for 45 to 60 s in a blender. The entire contents of five petri plates (100 by 15 mm) were used for each 200 ml of water. The finished aqueous suspension was blended once again and consisted of 300 ml of sodium alginate solution, 100 ml of wheat germ slurry, and 100 ml of macerated liquid fungal culture (8). The suspension was placed into an aspiration bottle and continuously stirred on a stir plate while dripping through a Pasteur pipette by gravity into a beaker containing 1 liter of 0.25 M CaCl₂ (8). As each drop of the suspension hit the CaCl₂ solution, it was encapsulated into a spherical pellet. The calcium in the receiving solution displaced the sodium in the alginate suspension, and each pellet should contain equal amounts of sodium alginate, wheat germ, and fungal spores (7). Finished pellets were drained in a colander, washed twice with sterile water to remove excess CaCl₂, and stored in sterile glass bottles at 4°C.

After preparation, pellets were viewed under a compound microscope as squash mounts to observe numbers of incorporated ascospores. Pellets also were plated onto PDA or MA to confirm viability of the LE

isolate and to monitor for the presence of contaminants. This same procedure was performed for all other treatments that were evaluated after encapsulation into sodium alginate pellets. Other pelleted treatments used later in these studies included a nonpathogenic *F. oxysporum* isolate (96-14), isolate 96-14 combined with isolate LE, and blank pellets alone with no microorganisms included in the alginate suspension.

Methods for inoculating plants with *S. retispora* var. *retispora* isolate LE. Four separate methods were employed in the greenhouse to inoculate watermelon plants with the mycoparasite and evaluate its potential for reducing Fusarium wilt (Tables 1 and 2). Method #1 consisted of combining alginate pellets treated with the mycoparasite into a peat:perlite mix at a rate of 5 g (pellet wet weight) to 1 liter of potting soil (wt:vol). Seedling trays were filled with this mixture and one watermelon seed (cv. Charleston Gray) was sown per cell. Plants were grown for 6 weeks (two to three true leaves), removed from cells, and roots were washed free of the soilless medium. Plants then were challenged with the pathogen by dipping washed roots into a *F. oxysporum* f. sp. *niveum* (CSI-24) microconidial suspension (1×10^6 conidia/ml). Isolate CSI-24 is a moderate-weak race 1 pathogen, was originally isolated from a watermelon wilt-suppressive research plot in Leesburg, FL, and was used throughout the course of the study. Pathogen-inoculated plants were transplanted into 1-gal plastic pots filled with the same peat-based, soilless medium (three plants per replication per pot).

Inoculation method #1 was evaluated using two separate assays. The first assay included four treatments: (i) untreated control, (ii) pathogen-inoculated control, (iii) inoculated with LE only, and (iv) inoculated with LE. The assay was challenged with the pathogen and was repeated once (assays 1a and 1b; Table 1). After promising results were obtained from these assays, the concept was expanded with a second assay consisting of seven treatments, including: (i) untreated control, (ii)

Table 1. Watermelon plant biomass after preliminary inoculation with *Sphaerodes retispora* var. *retispora* (isolate LE) followed by pathogen challenge from a microspore suspension of *Fusarium oxysporum* f. sp. *niveum* (inoculation method #1)

Treatment ^a	Plant dry weight (g) ^b		
	Assay 1a	Assay 1b	Assay 2
Control	4.26 ab	4.67 a	4.05 a
LE control	5.60 a	5.23 a	5.39 a
LE + pathogen	3.50 b	5.10 a	3.65 b
Pathogen control	1.12 c	0.55 b	0.67 e
96-14 + pathogen	...	...	0.77 de
LE/96-14 + pathogen	...	...	1.93 c
Pellets + pathogen	...	...	0.91 de

^a Each value is the average of 18 plants (3 plants per replication). Means followed by the same letter in a column are not significantly different according to Fisher's least significant difference test ($P = 0.05$). Assays 1a and 1b use the same treatments, but are presented separately due to significant differences between repeated assays.

^b Watermelon plants (Charleston Gray) were grown for 6 weeks in a soil mix containing alginate pellets (5 g/liter) of either isolate LE, LE + nonpathogenic *F. oxysporum* isolate 96-14, isolate 96-14, or blank pellets.

Table 2. Survival and biomass of watermelon plants treated with *Sphaerodes retispora* var. *retispora* (isolate LE) and grown in soils artificially-infested with *Fusarium oxysporum* f. sp. *niveum*^a

Treatment ^a	Method #2		Method #3		Method #4	
	Mortality (%)	Dry wt (g)	Mortality (%)	Dry wt (g)	Mortality (%)	Dry wt (g)
Control	0.0 a	1.35 a	0.0 a	2.0 a	0.04 a	1.32 a
Pathogen only	25.0 b	0.18 c	66.6 c	0.40 c	50.0 b	0.26 c
LE + pathogen	0.0 a	0.61 b	25.0 ab	1.70 a	0.04 a	0.86 b
LE/96-14 + pathogen	0.0 a	0.39 c	25.0 ab	0.84 b	58.3 b	0.47 c
96-14 + pathogen	50.0 d	0.19 c	41.6 bc	0.37 c	58.3 b	0.22 c
Blank pellets + pathogen	33.3 c	0.21 c	41.6 bc	0.54 bc	58.3 b	0.28 c

^a Average of 24 plants (2 plants per replication). Means followed by the same letter in a column are not significantly different according to Fisher's least significant difference test ($P = 0.05$). Method 2 = root dip plants with treatment suspensions (250 to 300 ascospores/ml or 1×10^6 conidia/ml for LE and 96-14, respectively) before transplantation; method 3 = mix pellet treatments in potting soil (5 g per 1 liter of mix), and grow plants for 6 weeks before transplantation; method 4 = mix pellet treatments into pathogen-infested soils (5 g per 300 g of soil) and transplant with healthy plants. All treatments involved transplantation of watermelon plants into pathogen-infested soils (250 propagules/g of soil), except the control treatment.

^b Pathogen = *Fusarium oxysporum* f. sp. *niveum*, LE = *Sphaerodes retispora* var. *retispora*, 96-14 = nonpathogenic isolate of *F. oxysporum*.

LE-only control, (iii) pathogen-only control, (iv) inoculated with LE, (v) inoculated with LE and 96-14, (vi) inoculated with 96-14, and (vii) blank pellets with no microorganisms. All treatments except numbers i and ii were challenged with the pathogen afterward by dipping treated roots into an *F. oxysporum* microconidial suspension and transplanted as described for assays 1a and 1b. After growing 8 weeks in the greenhouse, the inoculated plants from both assays were removed from media, roots were washed, and samples were air dried in paper bags and weighed for total biomass. The nonpathogenic *F. oxysporum* 96-14 with isolate LE was included because of the inability of isolate LE to produce vigorous growth in the absence of *F. oxysporum* in initial lab isolations and assays (8,11).

The remaining three inoculation methods (#2, #3, and #4) included similar experimental practices. The same six treatments were used in all three as those described above from the second assay of method #1, minus the LE-only control (Table 2). Method #2 used plants grown in soilless mix for 6 weeks (two- to three-leaf stage). Roots were washed free of potting mix, then dipped into the various biological control suspensions. Suspensions used for inoculations included 250 to 300 ascospores/ml for isolate LE and 1×10^6 microconidia/ml for 96-14. Inoculated plants were transplanted into 10-cm clay pots (two plants/pot) containing noncultivated, unsterilized field soils artificially infested with *F. oxysporum* f. sp. *niveum* at approximately 250 propagules per gram of soil. Infestation levels of the pathogen were estimated from soil dilution plating of stock soil on Komada's medium (19). After 8 weeks, survival was recorded and the remaining watermelon plants were removed, dried, and weighed.

Inoculation method #3 consisted of the same six treatments except that alginate pellets were used to deliver the mycoparasite and nonpathogenic *F. oxysporum* treatments. Watermelon plants were inoculated with isolate LE as described before by planting seed in soilless media amended with alginate pellets. After 6 weeks, plants were removed and transplanted into clay pots with infested field soil as described

for method #2, and surviving plants were recorded, harvested, dried, and weighed after 8 weeks.

Inoculation method #4 consisted of mixing pellets from same six treatments as previously described into pathogen-infested field soil in clay pots. The pellets were applied at a rate of 5 g of pellets to 300 g of soil, which was the approximate volume of soil used for all clay pot experiments. Six-week-old healthy watermelon plants then were transplanted into infested soil. After 8 weeks, survival again was recorded and remaining plants were removed, dried, and weighed as described above.

Evaluation of *S. retispora* var. *retispora* isolate LE in naturally infested wilt soils. Soil was obtained from research plots in Leesburg, FL in which watermelon cultivars susceptible to Fusarium wilt (race 1) had been grown in a monoculture (14). This soil, naturally infested with *F. oxysporum* f. sp. *niveum*, was collected and mixed with alginate pellets containing isolate LE, *F. oxysporum* isolate 96-14, or both at 5 g per 300 g soil. Six-week-old watermelon plants were transplanted into treated soils as described for inoculation method #4. Treatments for this portion of the study included (i) naturally-infested soils alone as a pathogen control, and pellets containing (ii) isolate LE alone, (iii) isolate LE plus the nonpathogenic 96-14 isolate, and (iv) the 96-14 isolate alone (Table 3). After 8 weeks, survival was recorded and dry weights were again determined from remaining plants.

Ecological studies with *S. retispora* var. *retispora* isolate LE. Watermelon root colonization by *S. retispora* var. *retispora* isolate LE was evaluated in unsterilized, noncultivated field soil contained in clay pots in the greenhouse. Four treatments were used and included: (i) 6-week-old watermelon seedlings root-dipped into a suspension (250 to 300 ascospores/ml) of isolate LE and transplanted into pots, (ii) seedlings root-dipped into a mixture of isolate LE and nonpathogenic *F. oxysporum* f. sp. *niveum* isolate 96-14 (1×10^6 microconidia/ml), (iii) seeds planted into the same nonsterile field soils mixed with alginate pellets prepared with isolate LE, and (iv) seeds planted into soil amended

with alginate pellets containing isolates LE and 96-14. Treatments were watered as necessary to maintain plant turgor. After 2 months, plants were removed and roots were washed and plated on PDA or MA amended with a liquid culture filtrate of *F. oxysporum* f. sp. *niveum* (9,10,12) to determine if isolate LE could be recovered from watermelon roots. The entire root system (both tap and feeder roots) was cut into 1-cm-length pieces and plated (five pieces per plate) onto five replicate plates for each plant/pot.

The same nonsterile field soils were infested with alginate pellets containing isolate LE to determine its survival over time. This mycoparasite-infested field soil was then put into clay pots, and half of the infested soil was sown to watermelon while the other was left fallow. To mimic the normal time period between watermelon plantings in central Florida, pots were maintained in a walk-in growth chamber for a period of 9 months (June to February). Fallow pots were watered each time the pots planted with watermelons were watered. After 10 weeks, watermelon plants were removed and pots from both treatments were allowed to sit fallow for the remainder of the 9-month period. Temperature in the chamber was maintained at 25 to 30°C, with an interval of light and dark cycles set at 12 h for the entire 9-month period.

To determine survival of isolate LE in soil after 9 months, soil dilutions from each pot were performed and plated to either PDA or MA (10 replications/pot). Because of the faster growth of various soil

Table 3. Survival and biomass of watermelon plants treated with *Sphaerodes retispora* var. *retispora* (isolate LE) and grown in soils naturally-infested with *Fusarium oxysporum* f. sp. *niveum*^y

Treatment ^z	Mortality (%)	Plant dry weight (g)
Naturally infested pathogen control	95.8 d	0.19 c
Isolate LE alone + pathogen	29.2 a	1.29 a
Isolate LE + 96-14 + pathogen	58.3 bc	0.86 ab
96-14 alone + pathogen	70.8 cd	0.79 b

^y Average of 24 plants (2 plants per replication). Means followed by the same letter are not statistically different according to Fisher's least significant difference test ($P = 0.05$).

^z Treatments were sodium alginate pellets containing isolate LE, 9614 (nonpathogenic isolate of *F. oxysporum* f. sp. *niveum*), or both and mixed into soils naturally infested with the Fusarium wilt pathogen (5 g of pellets per 300 g of soil) except the control. Infested pots were watered daily for 1 week prior to transplantation with healthy 6-week-old watermelon plants.

Table 4. Evaluation of *Sphaerodes retispora* var. *retispora* (isolate LE) to affect soil populations of *Fusarium oxysporum* f. sp. *niveum* with and without watermelon plants grown in soils

Treatment ^y	Mean propagule counts ^z
Pathogen + isolate LE (with)	293.20 ab
Pathogen + isolate LE (without)	279.96 ab
Pathogen alone (with)	248.00 b
Pathogen alone (without)	236.80 b

^y Treatments with and without plants. Pathogen = *F. oxysporum* f. sp. *niveum* infested in soils at 250 propagules per gram of soil. Isolate LE = *S. retispora* var. *retispora* mixed into soils at 5 g of pellets per 300 g soil. Those treatments with plants were sown with watermelon seed, cv. Crimson Sweet (1 per pot), and maintained for the duration of the experiment.

^z Average number of *F. oxysporum* f. sp. *niveum* colonies formed on Komada's medium after dilution plating of infested soils and 3 months of incubation in the greenhouse. Each value is the mean of 100 plates per treatment (10 plates per replication). Means followed by the same letter are not significantly different according to Fisher's least significant difference test ($P = 0.05$).

saprophytes, a special dilution-plating technique was attempted to more readily detect *S. retispora* var. *retispora* (24). Soil samples were suspended in 50% ETOH and stirred for 5 min. The suspension (1 ml) was pipetted into plates before the addition of molten media. After solidification, plates were incubated at 40°C for 24 h before being transferred to an incubator maintained at 18°C (24).

To evaluate the potential influence of isolate LE on *F. oxysporum* f. sp. *niveum* soil populations, clay pots were filled with *F. oxysporum* f. sp. *niveum*-infested soil (250 propagules/g) and mixed with isolate LE incorporated into alginate pellets (5 g per 300 g of soil), and maintained in the greenhouse. Control treatments consisted of pathogen-infested soils without isolate LE-treated pellets. Two other treatments were included which involved using the same two soil amendments, but were sown with watermelons. Changes in *F. oxysporum* f. sp. *niveum* population levels in treated soils were determined by dilution plating on Komada's medium after 3 months of incubation in the greenhouse (19).

All experiments (biological control inoculation methods and ecological studies) utilized treatments arranged in a completely randomized design with six replications (pots) per treatment, and all experiments were repeated once, with the exception of assay two of inoculation method #1. All collected data were subjected to the general linear model procedure. Treatment means were separated by Fisher's least significant difference test, and variance between repeated experiments was additionally analyzed for homogeneity.

RESULTS

Sodium alginate pellets. The sodium alginate pellets were an efficient technique for inoculation with *S. retispora* var. *retispora*. Fungal germination and growth of the mycoparasite occurred whenever pellets were plated onto media. Although viability of the isolate within the pellet was determined to be at least 2 months under refrigeration, biological activity was not tested after this period, and new batches of pellets were produced. Examination of pellets in squash mounts averaged 10 to 15 ascospores per field of view using the $\times 10$ power objective. A similar number of ascospores consistently was observed with each batch of pellets, provided that the LE cultures used were the same age (2 to 3 weeks).

Inoculation methods for *S. retispora* var. *retispora* isolate LE. None of the plants died after using inoculation method #1, but there were significant differences in total dry biomass (Table 1). Watermelon plants inoculated with isolate LE alone and not challenged with the pathogen were not adversely affected by the mycoparasite,

because those treatments produced dry weights equal to those of the untreated controls from both assays (Table 1). Since no deleterious effects were observed on plants after inoculation with isolate LE singly, this treatment was omitted from subsequent inoculation methods. The LE + pathogen treatment had significantly higher dry weights than the pathogen-inoculated control treatment in all experiments employing this method. The second assay using this inoculation method included three new treatments that were added to preliminarily evaluate techniques for future experiments. The addition of the nonpathogenic isolate 96-14 and blank pellets in assay 2 had no beneficial effect on disease reduction. Plant dry weights using these treatments were no different than the pathogen-inoculated control (Table 1).

Three additional methods for inoculation with isolate LE were developed that evaluated these additional treatments, but employed *F. oxysporum* f. sp. *niveum*-infested soils as the challenge inoculum rather than microconidial suspension. These additional methods were designed to more realistically measure mycoparasite performance in a field situation (Table 2). Unlike inoculation method #1, methods #2, #3, and #4 resulted in plant mortality ranging from 0% in untreated controls to 66% in pathogen-inoculated controls (Table 2). However, the total biomass produced from these methods was similar to that from method #1. Treatments from method #3 produced dry weights from the LE-inoculated treatment that were no different than those from the untreated control. Methods #2 and #4 resulted in dry weights from the LE treatments that were intermediate between the untreated and pathogen-inoculated controls.

The three treatments involving blank pellets or isolate 96-14 alone again did not reduce disease or increase plant biomass when compared with the pathogen-only treatment (Table 2). The combination treatment (LE and 96-14) had plant mortality similar to the LE-inoculated treatment in inoculation methods #2 and #3, but did not improve plant dry weights as consistently as did the LE-inoculated treatments. Regardless of the inoculation method employed, the combination treatment of LE and 96-14 did not improve plant response to disease over that of isolate LE alone (Table 2).

Evaluation of *S. retispora* var. *retispora* isolate LE in naturally infested soil. The results from this experiment were very similar to those using the artificially infested soils. The mycoparasite LE significantly reduced watermelon mortality and increased plant dry weights grown in soil naturally infested with *F. oxysporum* f. sp. *niveum* (Table 3). Treatments including *F. oxysporum* isolate 96-14 provided no additional disease reduction compared with isolate LE alone. Isolate 96-14 did not

reduce plant mortality compared with the naturally infested control, but did significantly improve plant dry weights (Table 3).

Ecological studies with *S. retispora* var. *retispora* isolate LE. In root colonization tests, isolate LE never was recovered in culture from watermelon roots that were grown in LE-treatments alone. It was, however, isolated in association with all treatments including *F. oxysporum* isolate 96-14. Isolate 96-14 rapidly grew from plated roots within several days. The mycoparasite was observed growing in the *Fusarium* cultures 10 to 14 days later. These results suggest that isolate LE did not colonize watermelon roots, but did colonize isolate 96-14 as it grew on the roots.

In soil survival assays, isolate LE was recovered from soil dilutions after 9 months (*data not shown*). A selective or semiselective medium was not available for *S. retispora* var. *retispora*; therefore, a modified technique was attempted to more easily identify the mycoparasite from soils (24). This effort did help to reduce the incidence of rapidly growing soil saprophytes. However, it was not sufficient for obtaining quantitative values from mycoparasite propagules (ascospores or bulbils and hyphal fragments). Nevertheless, the parasite always was identified after dilution plating from soils in association with other microorganisms found inhabiting the unsterilized field soils, primarily *F. oxysporum*. This demonstrated that the ascospores of isolate LE remained viable for at least 9 months in the growth chamber after being artificially infested into field soils.

Colony counts for *F. oxysporum* f. sp. *niveum* estimated from dilution plating without isolate LE were similar to those originally applied to the soils (250 propagules/g; Table 4). The pathogen propagule counts estimated after being treated with isolate LE were not lower than the original controls, suggesting that LE was not reducing *Fusarium* propagules in soils. Furthermore, the presence or absence of watermelon plants grown in the infested soils had no effect on any of the colony counts from dilution plating (Table 4).

With the exception of assay 1 from inoculation method #1, variance between repeated experiments was homogenous, and no treatment-experiment interactions were observed. Therefore, results from assay 1 (1a and 1b) of method #1 are presented separately (Table 1). The results of all other repeated experiments have been combined (Tables 2-4).

DISCUSSION

The idea of using mycoparasites as biological control organisms for plant pathogens was first suggested by Weindling in 1932 after he observed that *Trichoderma lignorum* was capable of parasitizing several diverse soilborne fungi in culture, including *Rhizoctonia solani*, *Phytoph-*

thora parasitica, *Pythium* spp., *Rhizopus* spp., and *Sclerotium rolfsii* (33). Since that time, many mycoparasites have been tested in this capacity, but few have provided economical control of soilborne plant diseases. This is due primarily to the high rate of mycoparasite application required, and low levels of control achieved (1).

The most successful examples of biological control of *Fusarium* wilt diseases utilizing mycoparasites have consistently been demonstrated with the use of nonpathogenic *F. oxysporum* isolates, often found initially occurring in suppressive soils (2–4,16,20,21). The mechanism of suppression and disease reduction is mainly attributed to an effect similar to cross protection, or induced resistance in host plants prior to infection by the pathogen (4,20,21). The watermelon wilt-suppressive research plots in Florida have been documented to contain higher levels of various microorganisms, including nonpathogenic isolates of *F. oxysporum*, bacteria, and actinomycetes than the corresponding wilt-conductive plots (21). It was further shown that the mechanism of suppression in these plots was due to a protective resistance induced by saprophytic isolates of *F. oxysporum* that infected plants more rapidly than pathogenic isolates (20,21).

The attempt to use *Sphaerodes retispora* var. *retispora* (isolate LE) as a biological control agent is unprecedented. It is a unique organism for this purpose because it does not possess many of the ideal biological traits characteristic of the more successful mycoparasites such as *Trichoderma* or *Gliocladium* spp. (1,4,25,30). Isolate LE does not compete well in culture with other organisms, and exhibits a nutritional dependency upon isolates of *F. oxysporum* for rapid growth and sporulation (8). Furthermore, it does not produce massive numbers of functional conidia, but does form bulbils and ascospores homothallically (8,10). Its potential for survival in soil is apparently adequate, but the fate of individual propagules added to soil or plant root systems is unknown.

Sodium alginate pellets have been successfully deployed on a number of occasions for the introduction of biocontrol agents in the attempt to protect crops from soilborne pathogens (8,22,23). The alginate pellet method also effectively introduced the mycoparasite *S. retispora* var. *retispora* into the experimental system used in this study. The same relative number of ascospores was consistently observed after pellet formation, and viability and survival of the fungus also was consistently demonstrated after isolation from infested soils.

Regardless of the inoculation method evaluated, this study has provided evidence that the *S. retispora* var. *retispora* isolate used has the potential to reduce watermelon mortality and increase plant biomass

compared with *Fusarium* wilt pathogen-inoculated controls at the greenhouse level. The use of isolate LE by itself was significantly better than the pathogen-inoculated control for decreasing numbers of dead plants and increasing plant biomass in all experiments (Tables 1 and 2). The use of LE also resulted in disease reduction that was either equal to untreated controls or intermediate between pathogen and untreated controls (Tables 1 and 2). However, the mechanism for reducing wilt disease development by isolate LE is unknown. The combination of isolates LE and the nonpathogenic *F. oxysporum* isolate 96-14 was not as consistent in reducing numbers of dead plants or increasing dry weights as with isolate LE alone. This is somewhat surprising because this treatment was expected to help improve distribution of the parasite by first colonizing a fungus having the ability to better compete with the pathogen in the rhizosphere. It also demonstrates that isolate 96-14 provided no induced resistance for protecting plants against the wilt pathogen.

Inoculation method #3, which exposed plants to the parasite via the potting mix at seeding followed by transplanting into pathogen-infested soils, would likely be the most efficient and practical technique for applying biocontrol agents into this system. Root-dipping all plants with parasite treatments prior to field transplantation is too labor intensive, and applying treated pellets directly into transplant beds in the field would likely require impractically high volumes of alginate pellets. The technique of combining parasites into planting mixes in the greenhouse is an efficient, inexpensive method of providing protection for developing plants, and is easily incorporated into vegetable production systems (including watermelon) in Florida. This technique has been successfully implemented with tomato, pepper, celery, and citrus using *T. harzianum* (29). Combining different antagonists or the integration of mycoparasitism with other technologies will also likely increase the efficiency or consistency of biological control as a viable disease management technique (2,30).

It is still unknown as to whether isolate LE can effectively colonize watermelon roots without the additional presence of *F. oxysporum*. It was never isolated from inoculated roots by itself, but was successfully recovered in association with the nonpathogenic *F. oxysporum* 96-14 in greenhouse studies. It was also originally isolated in association with a pathogenic isolate of *F. oxysporum* f. sp. *niveum* (8,9,10). Isolate LE was recovered from soils after 9 months, but only as part of a complex with other fungal organisms, not singly. These results indicated that the parasite could survive and regenerate after at least 9 months in soils. They also suggested that *S. retispora* var. *retispora* exhibits a dependency upon other organisms

for germination and growth and is not itself an aggressive soil competitor.

Other ascomycetes closely related to *Sphaerodes*, such as *Melanospora* and *Persiciospora*, have been demonstrated to be parasitic on other fungi, including several *Fusarium* spp. (9–11,18,32). Species of *Melanospora* and their anamorphs are considered to be fusion biotrophs (17). These types of parasites prey upon their hosts without the production of cell wall-degrading enzymes, and have been shown ultrastructurally to establish physical contact without penetration (13,17). Membrane-lined channels similar to plasmodesmata are formed between fungal contact points, thus providing avenues for cytoplasmic exchange (13). Although not investigated here, it is presumed that the *S. retispora* var. *retispora* (isolate LE) used in this study functions in a manner similar to that of some of the more studied fusion biotrophic parasites, such as *Melanospora damnosa* (anamorph *Gonatobotrys simplex*) or *M. zamiae* (13,17,18,32). The isolate of *Sphaerodes* tested in this study does form contact branches and hook cells with *Fusarium* hyphal filaments (8,11) identical to those described for *M. zamiae* (18).

Successful biological control demonstrated at the laboratory or greenhouse level often is not achieved in the field due to a number of interrelated environmental factors. Key among these factors is a thorough understanding of the ecological interactions and relationship between host and parasite. In order to obtain this type of information, the design and conduction of preliminary studies (8–11) are often necessary to more effectively eliminate or accelerate the selection of potential candidates for future biocontrol evaluations. This study has provided some practical evidence of disease control for *Fusarium* wilt of watermelon in the greenhouse that encourages expanded studies of *S. retispora* var. *retispora* and other related mycoparasites into field studies.

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