Biological Control of Pythium Root Rot of Table Beet with *Corticium* sp.

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ABSTRACT

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An isolate of *Corticium* sp. was highly effective in controlling preemergence and postemergence damping-off of table beets caused mainly by *Pythium ultimum* in naturally infested soil. The antagonist was grown first on corn leaf meal (CLM) and then incorporated in soil from a beet field with a history of severe root rot. Damping-off was reduced significantly when CLM colonized with *Corticium* sp. was incorporated at a rate as low as 0.1% (v/v). However, *Corticium* sp. was most effective in natural soil at a rate of 5 or 10% (v/v). Effectiveness of *Corticium* sp. remained high in natural beet soil for as long as 21 days after soil incorporation. This antagonist was equally effective in controlling damping-off disease of table beets at 15, 20, and 25 C in both wet and dry soils.

New York produces about one-fourth of the total crop of table beets (*Beta vulgaris* L.) in the United States. In recent years, root rot has often diminished yield and quality and threatens the future of the processing industry in New York. *Pythium ultimum* Trow is considered the primary causal agent of this disease (1) and causes both preemergence and postemergence damping-off. Seedlings that survive the damping-off stage and are infected or subsequently become infected develop various stem and root rots that can ultimately affect the quality of the processed product.

Varieties of table beet with resistance to *P. ultimum* are not commercially available. Registered fungicides for use on beets and effective against Pythium root rot are likewise not available. Alternative strategies using biological control were thus investigated. The beet-*P. ultimum* relationship appeared particularly amenable to such strategies because the beet plant is susceptible to attack by *P. ultimum* only during the first 2–3 wk after planting. Thus, control measures only need be effective for a short period. Of several control strategies, incorporation of antagonistic microorganisms into the soil was particularly attractive.

This article demonstrates the effectiveness of an isolate of *Corticium* sp. for control of preemergence and postemergence damping-off of table beets under greenhouse conditions.

MATERIALS AND METHODS

Potential biological control organisms were grown under aseptic conditions for 2–4 wk at 25 C in 500-ml flasks containing 6 g of corn leaf meal (CLM) and 35-ml of distilled water. The CLM was prepared from senesced field-dried sweet corn leaves ground with a Wiley mill to pass through a 2-mm mesh screen. The resulting inoculum was incorporated uniformly into either autoclaved (1 hr at 120 C and 15 psi) or untreated beet field soil at a 10% ratio (v/v), unless indicated otherwise. The autoclaved soil was left to aerate at least 2 wk before use. The soil was obtained from a commercial beet field in central New York with a previous history of severe beet root rot. The amended soil was placed in 2-oz untreated paper cups (Solo Cup Company, Urbana, IL 61801) and immediately planted with five untreated, size 11, beet seed-balls (cv. Ruby Queen). In some tests, seed-balls treated with Thiram 75WP [bis (dimethylthiocarbamoyl)disulfide] or Dexon 35WP (p-dimethylaminobenezenediazo sodium sulfonate) were used as checks and compared with the test organisms. Eight cups (replicates) were buried in washed sand in 8 × 9 × 55 cm wooden flats. Total emergence and preemergence and postemergence damping-off were recorded at weekly intervals up to 6 wk. Only data from the 4th wk are reported in the tables. The tests were conducted in a greenhouse at approximately 21 C day and 16 C night. In all greenhouse tests, the soil was kept moist at all times by daily watering to favor Pythium root rot.

The fungi tested were: *Corticium* sp. from M. Boosalis, University of Nebraska; *Gliocladium roseum* (Link) Bainer from H. L. Barnett, West Virginia University; *Penicillium vermiculatum* Dang. from M. Boosalis; *Trichoderma harzianum* Rifai (isolates 14 and 117) from sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary. The soil population of *Pythium* spp. was determined using a modification of Tsao and Ocana's medium (5).

The effect of soil temperature and moisture on the ability of *Corticium* sp. to control damping-off of table beet was studied in a growth chamber at an air temperature of 21 ±2 C, 60–70% relative humidity, and 14 hr of cool-white fluorescent light (11,000 lux) per day. Soil temperatures were maintained at 15, 20, or 25 C using controlled temperature tanks (2). Two soil moisture (wet and dry) regimes were maintained at each temperature. In the wet treatment the soil moisture was kept at or above field capacity at all times by watering two to three times daily. In the dry treatment the soil was watered once every 2 days; thus the soil moisture fluctuated considerably between waterings.

Effectiveness of *Corticium* sp. for control of Pythium root rot as affected by incubation time after soil infestation also was investigated. Inoculum of *Corticium* sp. was prepared as described and thoroughly mixed into sterilized or natural field soil at 1, 5, and 10% (v/v). Treated soils and the checks were planted with beet seed-balls 0, 4, 7, 14, and 21 days after soil infestation. Before planting, the soils were stored at 5 or 22 C in polyethylene bags. The tests were conducted in the greenhouse, and data collection was the same as described above. All tests were repeated at least twice and the data analyzed by the Waller and Duncan's Bayesian K-ratio (LSD) rule (6).

RESULTS

Of the organisms tested, only *Corticium* sp. exhibited a pronounced ability to prevent both preemergence and postemergence damping-off of table beet seedlings caused by *P. ultimum* (Table 1). Generally, treating field soil with *Corticium* inoculum at 10% (v/v) was as good as the Thiram and Dexon treatments and at times approached the steam treatment in its effectiveness against damping-off diseases. The isolates of *Gliocladium*, *Penicillium*,

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and *Trichoderma* were ineffective.

Occasionally, the incidence of damping-off was higher than expected in the autoclaved and *Corticium*-amended treatments. This was undoubtedly due to contamination of these soil treatments accompanied by a rapid buildup of disease-causing agents, especially in the sterilized soil treatments. Generally, addition of sodium nitrate to the CLM at a rate of 1% before inoculation with *Corticium* sp. did not statistically change the effectiveness of *Corticium* sp. as a control for damping-off (Table 1). Addition of the nitrate increased the amount of mycelial growth of *Corticium* sp., however, compared with CLM medium without added nitrate.

Corticium sp. incorporated in soil in amounts as low as 0.1% (v/v, CLM-*Corticium*/soil) significantly reduced the total incidence of damping-off; it was most effective, however, at 5 and 10% (Table 2). The antagonist was especially effective in controlling postemergence damping-off, in contrast to Thiram, which was most effective against the preemergence damping-off stage (Tables 1 and 2).

In all tests, beet seedlings growing in field soil amended with sterilized CLM developed the highest percentage of damping-off (Tables 1 and 2). The CLM probably provided an exploitable substrate for damping-off organisms to rapidly invade and increase in numbers, thus increasing inoculum potential. The *Corticium* sp. was equally effective in controlling damping-off of table beets at 15, 20, and 25 C (Table 3). Emergence and stand counts were lower in the dry than in the wet soil treatments (Table 3), but soil moisture did not significantly alter the effectiveness of *Corticium* sp. in reducing the incidence of damping-off. Factorial analysis of the disease data confirmed that the amount of soil amendment with *Corticium* sp. significantly influenced disease incidence and that there was no interaction with temperature and moisture.

Activity of *Corticium* sp. remained high in field soil even after incubation for 21 days, the longest period tested (Table 4). Total emergence and stand counts were significantly higher ($P = 0.05$) in sterilized soil or soil amended with 1, 5, or 10% (v/v) *Corticium* sp. than in untreated field soil. Furthermore, storing *Corticium*-amended soil at 22 or 5 C for 21 days did not significantly alter its effectiveness for control of Pythium damping-off of table beets.

No effects or macroscopic symptoms and signs were observed on table beet seedlings, either in sterilized or untreated field soil, that could be attributed to the isolate of *Corticium* sp. In addition, no effects were noted on field or sweet corn, alfalfa, wheat, oats, soybeans, snap beans, black turtle soup beans, or garden peas sown in *Corticium*-amended sterilized soil. Untreated seeds and seeds treated with Captan or Thiram were used. Cabbage plant height however, was decreased by about 50% in the *Corticium*-amended soil. No roots of any test plants exhibited symptoms of disease caused by *Corticium*. A slight reduction in height of beet seedlings was noted in CLM-amended sterilized soils.

Similar results were obtained when all these experiments were repeated. In addition, in trials where data were taken at 6 wk instead of 4 wk after planting, the incidence of disease generally held constant in the *Corticium*-amended soils.

Soil population of low-temperature *Pythium* spp. was

TABLE 1. Effect of antagonistic fungi on the incidence of Pythium damping-off of table beets evaluated 4 wk after planting

	% Damping-off[w]				% Total disease	
	Preemergence		Postemergence			
Treatment	I[x]	II[x]	I	II	I	II
Sterilized soil	0 a	...[y]	4 ab	...	3 ab	...
Sterilized soil + CLM[z]	0 a	11 ab	0 a	18 b	0 ab	28 bc
Field soil	41 bc	...	79 e	...	87 de	...
Field soil + CLM	...	59 c	...	90 e	...	93 e
Field soil + Thiram	8 a	...	29 bc	...	37 c	...
Field soil + Dexon	14 ab	...	4 ab	...	18 b	...
Field soil + *Corticium* sp.	8 a	1 a	7 ab	38 c	14 b	37 c
Field soil + *Gliocladium*	51 c	44 c	54 cd	71 de	79 d	82 de
Field soil + *Penicillium*	28 bc	29 bc	82 e	71 de	85 de	75 d
Field soil + *Trichoderma* (No. 14)	42 bc	49 c	74 e	57 de	85 de	77 d
Field soil + *Trichoderma* (No. 117)	28 bc	26 b	64 de	81 e	74 d	84 de

[w] Means in a column followed by the same letter do not differ significantly ($P = 0.05$) by Waller and Duncan's Bayesian K-ratio (LSD) rule.
[x] I and II = With and without the addition, respectively, of 1% $NaNO_3$ to the CLM on which test fungi were grown. Figures of both columns were analyzed statistically together.
[y] Experimental conditions not tested.
[z] CLM = Corn leaf meal.

TABLE 2. Effect of different levels of incorporation of *Corticium* sp. in field soil on the control of Pythium damping-off of table beets evaluated 4 wk after planting

	% Damping-off[y]		% Total disease
Treatment	Preemergence	Postemergence	
Sterilized soil	0 a	0 a	0 a
Sterilized soil + CLM[z]	2 a	0 a	2 a
Sterilized soil + 10% *Corticium* sp.	11 ab	0 a	11 a
Field soil	35 c	80 d	86 e
Field soil + CLM	56 d	67 cd	93 e
Field soil + Thiram	9 ab	54 c	59 cd
Field soil + 10% *Corticium* sp.	17 bc	23 b	37 bc
Field soil + 5% *Corticium* sp.	21 bc	16 ab	34 bc
Field soil + 2.5% *Corticium* sp.	18 bc	41 bc	49 cd
Field soil + 1% *Corticium* sp.	26 bc	18 ab	38 bc
Field soil + 0.5% *Corticium* sp.	16 b	39 bc	54 cd
Field soil + 0.25% *Corticium* sp.	30 c	53 c	68 d
Field soil + 0.1% *Corticium* sp.	27 bc	51 c	65 d

[y] Means in a column followed by the same letter do not differ significantly ($P = 0.05$) by Waller and Duncan's Bayesian K-ratio (LSD) rule.
[z] CLM = Corn leaf meal.

TABLE 3. Influence of soil temperature and moisture on the ability of *Corticium* sp. to control Pythium damping-off diseases of table beets when evaluated 4 wk after planting

	Soil temperature/moisture[x,y]					
	15 C		20 C		25 C	
Treatment	Wet	Dry	Wet	Dry	Wet	Dry
Total emergence						
Sterilized soil + *Corticium* sp.	7.1	5.3	7.9	6.1	6.2	6.2
Field soil + CLM[z]	2.4	3.7	4.4	2.9	4.8	3.0
Field soil + *Corticium* sp.	6.7	6.2	7.1	5.9	6.1	5.3
Final stand count						
Sterilized soil + *Corticium* sp.	6.9	5.2	7.8	5.9	7.2	5.9
Field soil + CLM	1.0	1.1	2.0	2.0	2.9	1.1
Field soil + *Corticium* sp.	6.3	5.6	6.1	4.4	5.2	4.1

[x] Factorial analysis of the emergence and stand count data showed that soil treatment with *Corticium* sp. and moisture but not temperature influenced disease development significantly ($P \leq 0.001$); however, there was no significant interaction between temperature, moisture, soil treatment, and *Corticium* sp. Waller and Duncan's multiple range test showed that the sterilized and field soil + *Corticium* sp. treatments were significantly different ($P = 0.05$) from the untreated field soil + CLM for each column.
[y] Data refer to average number of seedlings per replicate from a total of five seed-balls.
[z] CLM = Corn leaf meal.

TABLE 4. Influence of incubation time after incorporation of *Corticium* sp. in field soil on the control of Pythium damping-off diseases of table beets evaluated 4 wk after planting

Treatment	Planting time (days after incorporation)[y]				
	0	4	7	14	21
Total emergence					
Sterilized soil + *Corticium* sp.	6.4 ab	7.8 a	7.4 a	6.8 abc	7.4 a
Field soil + CLM[z]	5.8 b	6.8 ab	4.3 b	5.3 bce	4.8 b
Field soil + 1% *Corticium* sp.	7.4 a	5.4 c	6.9 a	6.5 abc	7.4 a
Field soil + 5% *Corticium* sp.	5.9 b	4.9 c	6.0 a	4.5 e	7.4 a
Field soil + 10% *Corticium* sp.	6.0 b	5.0 c	6.3 a	6.1 abc	7.4 a
Final stand count					
Sterilized soil + *Corticium* sp.	7.5 a	7.6 a	7.1 a	6.6 a	7.3 a
Field soil + CLM	2.0 e	1.6 c	2.9 c	2.8 c	3.5 b
Field soil + 1% *Corticium* sp.	3.0 ce	2.8 bc	3.8 bc	4.6 b	6.0 a
Field soil + 5% *Corticium* sp.	4.4 bc	2.1 bc	4.8 b	4.5 b	7.1 a
Field soil + 10% *Corticium* sp.	5.3 b	3.5 b	4.9 b	4.4 b	6.4 a

[y]Means in a column followed by the same letter do not differ significantly ($P = 0.05$) by Waller and Duncan's Bayesian K-ratio (LSD) rule.
[z]CLM = Corn leaf meal.

determined in soil amended with 10% (v/v) of CLM containing the *Corticium* sp. Two *Pythium* spp. (a fast-growing type, pathogenic and probably *P. ultimum*; and a slow-growing, nonpathogenic *Pythium* sp.) were observed on the isolation plates. Five weeks after planting, the populations of *P. ultimum* were 1,000 and 1,600 propagules per gram of untreated and sterilized CLM-amended field soil, respectively. In contrast, the population of *P. ultimum* was only 120 and 33 per gram of field soils amended with 10% and 1% *Corticium*, respectively. Other levels of incorporation of *Corticium* sp. also resulted in decreased numbers of *Pythium* propagules. Interestingly, the population of the slow-growing *Pythium* sp. increased from 67 propagules per gram of untreated field soil to 1,650 propagules per gram in the *Corticium*-amended (10%, v/v) field soil.

DISCUSSION

The isolate of *Corticium* sp. used in this study is highly effective in controlling damping-off diseases of table beet incited by *Pythium* spp. (principally, *P. ultimum*) under greenhouse conditions. Although an efficient method for applying this biological control agent to beet fields has not yet been devised, commercial use of this organism warrants serious consideration. The practical use of *Corticium* sp. as a possible biological control organism for *Pythium*-induced diseases has several promising characteristics: (i) It appears to be effective over the temperature and moisture conditions that favor the high incidence and severe damage characteristic of Pythium diseases, (ii) results indicate that the antagonist is not pathogenic to a range of host plants commonly grown in rotation with table beet in New York, and (iii) it readily produces a large number of small sclerotia that might be used as a source of inoculum to add to soil or to coat seeds for protection against plant pathogens. Extensive research is required to determine the survival of *Corticium* sp. in the field and its effect on other soil microorganisms. Data also are needed on the factors that promote and maintain an effective *Corticium* population under natural field conditions. Furthermore, the best method of distribution of the antagonist, as a meal or as a seed coating, needs to be explored.

This same isolate of *Corticium* sp. was previously reported to control Rhizoctonia damping-off diseases of bean, soybeans, and sugar beets (4). The mode of antagonism toward *Rhizoctonia solani* is mycoparasitic with the hyphae of *Corticium* sp. coiling around and invading *R. solani* hyphae (Hoch, *unpublished*); however, preliminary results involving the *Corticium* sp. and eight species of *Pythium*, including *P. ultimum*, suggest that antibiosis rather than mycoparasitism is involved in the control of Pythium damping-off (Hoch, *unpublished*).

Liu and Vaughan (3) previously reported on the control of table beet damping-off diseases caused by *P. ultimum* with antagonistic microorganisms. Species of *Trichoderma* and *Penicillium* afforded some protection to the germinating beet seed from preemergence damping-off, but unlike our results with *Corticium*-amended soil, control of postemergence damping-off was not achieved. In addition, the degree of control of Pythium damping-off diseases of table beets obtained in this study in field soil was surprisingly good, especially since the major overwintering propagules of *Pythium* are mostly oospores resistant to many treatments and organisms.

The data from this study were based on experiments conducted with soils containing natural microflora. In the past, biological control studies have been conducted in sterilized soils to which a single pathogen, produced under laboratory conditions in vitro, had been added. Unfortunately, promising biological control strategies formulated under the latter conditions were often ineffective when tested under natural field conditions.

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