Synergistic Effect of Pythium ultimum and the Additive Effect of P. aphanidermatum with Heterodera schachtii on Sugarbeet

E. D. Whitney

Research Plant Pathologist, ARS, USDA, U.S. Agricultural Research Station, Salinas, California 93901.
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ABSTRACT

The effect of *Pythium ultimum* and *Heterodera schachtii* in combination was synergistic on the preemergence and postemergence damping-off of sugarbeet, but a synergistic effect occurred only rarely on the root rot of sugarbeet. The increased damping-off appeared to be associated with the increased growth of the fungus around the infection centers because the number of centers remained constant whether or not the nematode was present. This observation is consistent with the fact that the period of susceptibility to the fungus was not lengthened by nematode infection. The effect of *P. aphanidermatum* and the nematode in combination was additive for both damping-off and root rot of sugarbeet.

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Synergistic effects between *Rhizoctonia solani* Kühn and *Heterodera schachtii* A. Schm. on damping-off of sugarbeet have been reported (4). Several other important pathogens of seedling sugarbeets, *Pythium* spp., *Fusarium* spp., *Aphanomyces cochlioides* Drechs., and *Phoma betae* Frank, could interact with *H. schachtii* similarly. Tests were conducted to determine whether *Pythium ultimum* Trow and *P. aphanidermatum* (Edson) Fitzp., interacts in a synergistic way with *H. schachtii*. A preliminary report has been published (5). Additional studies were made to determine whether the synergistic effect on damping-off noted between *P. ultimum* and *H. schachtii* extended to the postdamping-off root rot of sugarbeet.

MATERIALS AND METHODS.—The *P. ultimum* and *P. aphanidermatum* cultures used were isolated from field-grown sugarbeets that were also infected with *H. schachtii*. The fungi were maintained on cornmeal agar, and inocula were produced in sand-cornmeal culture (1:1, v/v) for 72 h at 28 C. Inocula for the sand-cornmeal cultures (145 g/culture bottle) were 25 ml of washed and blended *P. ultimum* mycelial mats or *P. aphanidermatum* zoospores (200,000/bottle). The mats were grown in 100 ml soytone broth (3%) for 9 days at 24 C and blended in 50 ml of water. The sand-cornmeal cultures were sieved through a 10-mesh screen just before use. The nematode larvae were hatched and partly surface-disinfested as previously described (6).

Damping-off tests.—To about 10 kg of steam-treated soil, 0.0, 0.5, or 5.0 g of *P. ultimum* inoculum or 0.0, 0.33, or 3.3 g of *P. aphanidermatum* inoculum was added by mixing in a cement mixer. Superimposed upon these fungal treatments was 0.0, 1.0, or 10 *H. schachtii* larvae/g of soil to produce a 3 × 3 factorial experiment. One-hundred-fifty, surface-disinfested hybrid sugarbeet seed were planted 1 cm deep and 1 cm apart in six rows of seed

Fig. 1-6. The effects of *Pythium ultimum* (Fig. 1-4) or *P. aphanidermatum* (Fig. 5-6) in combination with *Heterodera schachtii* on damping-off of sugarbeet (●, 0.0; ▲, 1.0; and ■, 10.0 larvae/g soil). **1, 3)** Synergistic effect on postemergence damping-off by *P. ultimum* (0.5 g/10 kg of soil) and *H. schachtii*. **2, 4)** Synergistic effect on preemergence damping-off by *P. ultimum* (0.5 and 5.0 g/10 kg of soil, respectively) and *H. schachtii*. **5,6)** Additive effects on damping-off of sugarbeet by *P. aphanidermatum* (0.33 and 3.3 g/10 kg, respectively) and *H. schachtii*. Damping-off did not occur in the controls or nematode treatments; therefore, the x-axis represents these treatments. Inset data are number of plants which emerged at each nematode inoculum level.

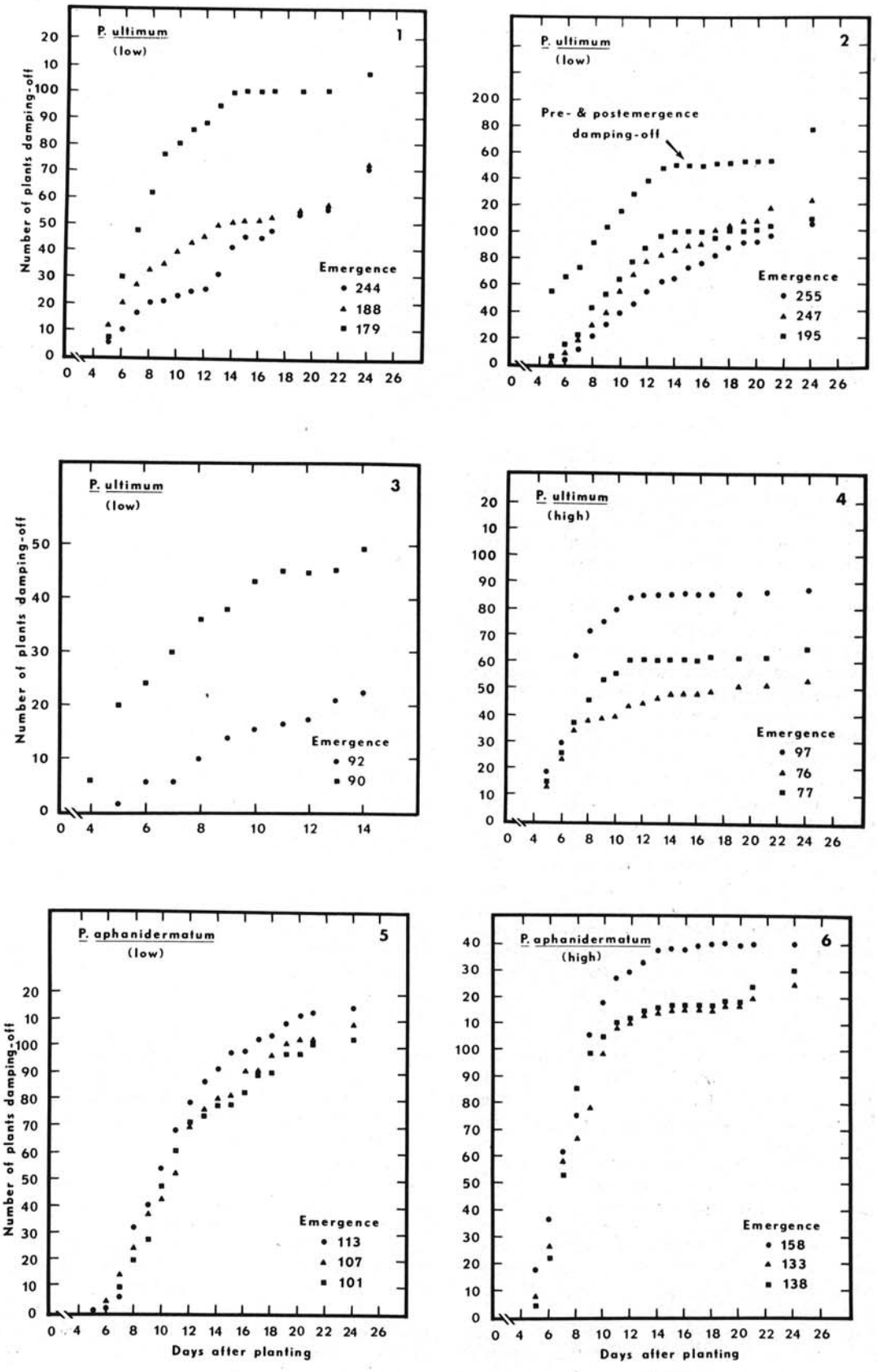
P. ultimum
(low)
1
Number of plants damping-off
Emergence
244
188
179
P. ultimum
(low)
2
Pre- & postemergence
damping-off
Emergence
255
247
195
P. ultimum
(low)
3
Number of plants damping-off
Emergence
92
90
P. ultimum
(high)
4
Emergence
97
76
77
P. aphanidermatum
(low)
5
Number of plants damping-off
Emergence
113
107
101
Days after planting
P. aphanidermatum
(high)
6
Emergence
158
133
138
Days after planting

TABLE 1. Differences in the effect of the *Pythium ultimum*/*Heterodera schachtii* combination on yield of sugarbeet due to age of plants at the time of inoculation and soil temp

Age of Plant	Temp (C)	Treatments[x]			
		Control	Nematode	Fungus	Combination
2 wk[y]	18	202.4 a	174.6 b	211.2 a	178.0 b
	23	229.0 a	95.4 b	199.1 a	111.0 b
	28	251.0 a	101.3 c	251.6 a	136.4 b
	x[z]	227.4 a	123.8 b	211.8 a	129.8 b
3 wk[y]	18	202.3 ab	179.6 bc	224.2 a	159.1 c
	23	228.5 a	111.4 c	185.0 b	106.6 c
	28	218.6 a	114.4 b	227.5 a	70.1 c
	x[z]	216.4 a	135.1 b	212.2 a	111.9 c

[x]Means for each treatment followed by the same letter are not significantly different at the 5% level (LSD).
[y]Each datum is the mean of eight replications.
[z]Each datum is the mean of 24 replications.

5 cm apart in flats of soil. Each treatment had two replications in a randomized block design. The test was repeated once (Test 1 and 2). Two similar tests with *P. ultimum* were made in growth chambers at the low fungus and high nematode levels in a 2 × 2 factorial experiment (Tests 3 and 4). Each treatment had four rows of 15 seeds each per test. Temperatures were 27 C for 14 h with a fluorescent light intensity of 8,600 lx (800 ft-c); and 22 C for 10 h without light. As plants damped-off, a dated marker was placed between the rows perpendicular to its position in the row. At the conclusion of each test, these data were plotted on graph paper to estimate the number of infection centers per flat. A test similar to the damping-off test with *P. aphanidermatum* and *H. schachtii* was conducted under field conditions in soil-filled crocks (10 kg) to study the effect of this combination on damping-off. Each treatment had 20 replications of 20 seeds each.

Root rot tests.—Four-wk-old plants in 15-cm diam pots of soil placed in temp control beds (24 C) were infested with 0 or 0.2 g of sand-cornmeal inoculum of *P. ultimum* or *P. aphanidermatum*. Superimposed upon these treatments were 0.0, 1.0, or 10 *H. schachtii* larvae/g of soil in a 2 × 3 factorial experiment. The sand-cornmeal inoculum was added to the top of the soil and covered with about 0.5 cm of steam-treated soil. The nematode larvae were added as previously described (3) over a 5-wk period. Each treatment group included 16 single-plant replications. The *P. ultimum* test was repeated.

For yield estimates, plants already established in the crocks in the *P. aphanidermatum*/*H. schachtii* combination damping-off test were thinned to two plants 4 wk after planting; yield was measured when the plants were about 5 mo old.

Two similar tests with *P. ultimum* were made by inoculating 2- and 3-wk-old plants. In one test, the plants (eight per treatment) were placed in temp control beds at 18, 23, and 28 ± 2 C, and the low fungus/high nematode inoculum levels used. In the second test, under greenhouse conditions, the high fungus/high nematode level with 10 replications/test was used. Yield data were about 5 mo old.

RESULTS.—*Damping-off tests.*—Synergistic effects between *P. ultimum* and *H. schachtii* on damping-off of sugarbeet were observed at all levels tested and for both preemergence and postemergence damping-off (Fig. 1-4). The maximum affect was observed 10 to 12 days after planting. The effect of the combination was proportional to the nematode concn, with only a small effect detectable at the 1 larva/g of soil. At the low *P. ultimum* soil treatment, 12 days after planting, damping-off was increased 244% by the nematode (10 larvae/g soil) in test 1 and 180% in test 2 and test 3. The data for tests 3 and 4 are reported as data for test 3.

The effect of the *P. aphanidermatum*/*H. schachtii* combination on damping-off of sugarbeet was the same as the fungus alone (Fig. 5-6). In no test was the number of plants which emerged affected by only the nematode at the levels tested.

The number of infection centers/flat of soil was not affected by the presence of the nematode. The mean number of infection centers for the low level for *P. ultimum* was 9.7, and 18.8 for the high. For *P. aphanidermatum*, the mean number was 8.7 for the low level, and 18.6 for the high.

Root rot yield tests.—In only one of the four yield tests was a significant synergistic effect between *P. ultimum* and *H. schachtii* observed on yield of sugarbeet. The effect was observed for 3-wk-old plants in the soil temp-controlled test but was due to the synergistic effect at 28 C. A fungus effect was observed at 23 C (Table 1), thus, the temp × age × treatment interaction was statistically significant. The effect of the nematode on yield was significantly reduced at the low temp: 54.8% reduction in yield at the high temp, 54.1% at the medium temp, but only 12.5% at the low temp. Total mean yields of 171.4 g at the high temp, 156.5 at the medium, and 185.5 at the low, were significantly different. The effect of the *P. aphanidermatum*/*H. schachtii* combination on yield in all tests was additive.

DISCUSSION.—Two *Pythium* spp. which incite damping-off of sugarbeet are *P. ultimum* and *P. aphanidermatum*. The data presented here indicate an increased importance of *P. ultimum* and *H. schachtii* as pathogens of sugarbeet due to the synergistic effects of the combination. This importance is enhanced by the wide distribution of both pathogens in areas of sugarbeet culture (1, 2). The synergistic effect appears to be associated with increased spread of the fungus around the infection centers, because the number of centers was the same with or without the nematode. Increased growth of the fungus could be attributed to changes in the plant due to nematode invasion that promote fungal growth [as suggested for *R. solani* (4)], or to leakage of nutrients from plants due to nematode invasion. Whether damping-off occurs before or after emergence appears to be dependent upon inoculum concn. However, both are increased by the presence of the nematode. Previously (4) a synergistic effect between *R. solani* and *H. schachtii* was observed and that fungus has a similar growth habit to *P. ultimum* in that spread in the soil is by fungal growth. Spread of *P. aphanidermatum* is by motile zoospores, which may explain the lack of a synergistic effect with this fungus. Resistance to *P. ultimum* alone and to the fungus plus the nematode appeared simultaneously 12 to 14 days after planting; thus, the plants showed no enhanced susceptibility to the fungus with or without the nematode. Therefore, the effect is likely due to the increased spread of the fungus in the soil.

The synergistic effect extended to the root rot of sugarbeets but it was observed in these tests only on 3-wk-old plants inoculated at 28 C. The observation that it does not occur under all conditions is supported by earlier work (7).

LITERATURE CITED

1. ALTMAN, J., and I. THOMASON. 1971. Nematodes and their control. Pages 335-370. *in* R. T. Johnson et al., ed. Advances in sugarbeet production: principles and practices. Iowa State Univ. Press, Ames.
2. BENNETT, C. W., and L. D. LEACH. 1971. Diseases and their control. Pages 223-285. *in* R. T. Johnson et al., ed. Advances in sugarbeet production: principles and practices. Iowa State Univ. Press, Ames.
3. DONEY, D. L., E. D. WHITNEY, and A. E. STEELE. 1971. Effect of Heterodera schachtii infection on sugarbeet leaf growth. Phytopathology 61:40-41.
4. POLYCHRONOPOULOS, A. G., B. R. HOUSTON, and B. F. LOWNSBERY. 1969. Penetration and development of Rhizoctonia solani in sugar beet seedlings infected with Heterodera schachtii. Phytopathology 59:482-485.
5. WHITNEY, E. D. 1971. Synergistic effect between Heterodera schachtii and Pythium ultimum on damping-off of sugarbeet vs. additive effect of H. schachtii and P. aphanidermatum. Phytopathology 61:917 (Abstr.).
6. WHITNEY, E. D., and D. L. DONEY. 1970. Large scale hatching, disinfestation, and storage of Heterodera schachtii larvae. Phytopathology 60:1191-1194.
7. WHITNEY, E. D., and D. L. DONEY. 1970. Effects of Aphanomyces cochlioides and Pythium ultimum, alone and as complexes, with Heterodera schachtii on sugarbeet. J. Am. Soc. Sugar Beet Technol. 16:214-218.