Failure of Ditylenchus dipsaci to Promote Infection by an Antagonistic Strain of Corynebacterium insidiosum

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ABSTRACT

Corynebacterium insidiosum and an antagonistic variant of the species that was inhibitory to the growth of *C. insidiosum* in culture induced wilt symptoms in wounded roots. Only *C. insidiosum* infected alfalfa when transmitted to crown buds by the alfalfa stem nematode, *Ditylenchus dipsaci*. Neither strain affected the action of the nematode. Phytopathology 61:1097-1098.

A virulent isolate of *Corynebacterium insidiosum* (McCulloch) Jensen, the alfalfa wilt bacterium, was shown by Nelson & Semeniuk (5) to be antagonistic to several isolates of *C. insidiosum* on agar platings. Both *C. insidiosum* and the antagonistic variant were kept in agar culture for almost 8 years without passage through the host. We presumed, from past experience, that these stock cultures had lost their virulence during this time. Hawn (2) found that *C. insidiosum* was transmitted to alfalfa by *Ditylenchus dipsaci* (Kuehn), the stem nematode. Hawn & Hanna (3) reported that the stem nematode broke down the inherent resistance of Beaver alfalfa to bacterial wilt. This prompted us to determine whether the stem nematode might render alfalfa plants susceptible to strains of *C. insidiosum* which are normally avirulent. Cultures of *C. insidiosum* and the antagonistic variant were used to test this hypothesis and the results are reported here.

MATERIALS AND METHODS.—Alfalfa (*Medicago sativa* L. 'Rhizoma') was seeded in 15-cm pots in the greenhouse, thinned to 10 plants/pot after emergence, and grown for 3 months or longer before inoculation.

Stock cultures of *C. insidiosum* and the antagonistic variant were used to inoculate alfalfa in a preliminary experiment with the stem nematode. Virulent cultures of *C. insidiosum* were obtained for subsequent experiments by isolation from naturally infected field alfalfa or by reisolation from alfalfa artificially inoculated in the greenhouse. A culture of the antagonistic variant proved virulent in the preliminary experiment. To ensure a virulent culture of this strain in subsequent experiments, it was inoculated into alfalfa and reisolated after wilt symptoms had developed. Both isolates of *C. insidiosum* and the antagonistic variant were spot-planted on agar seeded with a known sensitive strain to check their sensitive or antagonistic properties (5).

Before each experiment, cultures of *C. insidiosum* were transferred twice on 6-ml slants of modified Burkholder's agar (5) to assure physiologically active cells. A final transfer was made to 300-ml slants. All cultures were grown at 22 C. Cells were scraped from the larger slants, suspended in water, and used for plant inoculations. Nematodes were obtained from crown buds of alfalfa (2).

Bacteria were introduced into wounded roots of alfalfa by the root-ball soak method (1), by stem nematode transmission (2), and (in one experiment) by wetting the soil surface and plant crowns with bacterial suspensions without wounding the plants. Noninoculated plants with wounded roots served as controls.

Pots of alfalfa were arranged in simple randomized blocks with four replications/treatment. The tops of the plants were trimmed back at monthly intervals. All plants were examined and rated for bacterial wilt about 3 months after inoculation. Disease data were subjected to analysis of variance, and differences among individual means were compared by Duncan's multiple range test.

RESULTS.—In the preliminary experiment (G. A. Nelson, *unpublished data*), two cultures of *C. insidiosum* and four cultures of the antagonistic variant grown on agar since 1960 were inoculated into alfalfa. Only a single culture of the antagonistic variant incited severe wilt symptoms after root inoculation, whereas none of the six cultures caused infection following transmission by *D. dipsaci*. An isolate of *C. insidiosum* from field alfalfa caused good wilt development after both root inoculation and transmission by *D. dipsaci*.

Two experiments were performed to determine what effect *D. dipsaci* had on the antagonistic variant. Experiment 1 was carried out during the short daylight hours of winter; Experiment 2 was performed during the long daylight hours of spring and summer.

The data in both experiments (Table 1) suggested that wilt developed when the nonantagonistic variant of *C. insidiosum* was transmitted by *D. dipsaci*, but did not develop after similar transmission of the antagonistic variant.

In Experiment 1 (Table 1), more plants had symptoms of nematode infection when inoculated with *C. insidiosum* and *D. dipsaci* than when inoculated with the antagonistic variant and *D. dipsaci*. However, neither of these two treatments differed significantly from plants inoculated with the nematode alone. These three treatments were not significantly different in Experiment 2.

Wetting the soil surface and unwounded plant crown with bacterial suspensions did not cause significant wilt development. Although *C. insidiosum* applied in this manner caused slightly higher percentages of wilted plants than did the antagonistic variant, symptom severity did not differ significantly between the two treatments.

Isolates of *C. insidiosum* and the antagonistic variant did not differ ($P > .01$) in virulence in either experiment.

TABLE 1. Development of bacterial wilt in Rhizoma alfalfa after inoculation with sensitive or antagonistic strains of *Corynobacterium insidiosum* by root inoculation, soil wetting, or by transmission by *Ditylenchus dipsaci*

Treatments	Avg wilt-disease severity[a]		Avg % plants showing wilt		Avg % plants with nematode symptoms	
	Exp. 1[c]	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Sensitive strain—roots[b]	1.9 a[d]	2.5 a	93.8 a	84.3 a	0 c	0 b
Sensitive strain—soil surface		0.4 b		21.3 b		0 b
Sensitive strain + nematode	0.5 b	2.8 a	43.6 b	87.8 a	89.7 a	73.4 a
Antagonistic strain—roots	1.7 a	1.5 a	92.0 a	87.7 a	0 c	0 b
Antagonistic strain—soil surface		0.1 b		8.1 b		0 b
Antagonistic strain + nematode	0.1 c	0.1 b	5.5 c	9.1 b	67.5 b	71.3 a
Nematode alone	0.1 c	0.1 b	3.9 c	12.2 b	76.4 ab	91.3 a
Noninoculated control	0 c	0.1 b	0 c	6.4 b	0 c	0 b

[a] Bacterial wilt severity rated on top and root symptoms of all plants: 0 = no symptoms; 5 = plant dead or dying.
[b] Root-ball soak method (1).
[c] Values given are for two separate experiments designated Exp. 1 and Exp. 2.
[d] Means in the same column not followed by the same letter differ at the 1% level of probability for all ratings.

In Experiment 1, alfalfa plants inoculated with wilt bacteria by nematode transmission had lower wilt severity ratings than root-inoculated plants. Plants inoculated with *C. insidiosum* by both methods in Experiment 2 had essentially the same severity ratings. Experiment 2 was extended for 5 months after inoculation to compensate for an extremely warm period which delayed both wilt and nematode development. This extension and monthly top trimming allowed for improved wilt expression in plants inoculated with *C. insidiosum* and the nematode.

DISCUSSION.—Stem nematode transmission of bacterial cells resulted in wilt development in Rhizoma alfalfa only when a nonantagonistic culture of *C. insidiosum* was used. Cells of the antagonistic strain possibly gained entrance to the alfalfa plant on penetration by the nematode, but were unable to establish themselves there. This may explain why the antagonistic variant has not become widely disseminated in susceptible alfalfa stands. Evidence indicates that nonantagonistic variants were involved in the original work on nematode transmission (2), because all agar isolates made from infected field alfalfa in southern Alberta were nonantagonistic (G. A. Nelson, *unpublished data*).

Ditylenchus dipsaci did not render alfalfa plants susceptible to avirulent strains of the wilt bacterium. Generally, the virulence of *C. insidiosum* declines steadily in agar culture. However, one of the antagonistic cultures was still virulent after 8 years under such conditions.

Neither *C. insidiosum* nor the antagonistic strain appears to affect the action of *D. dipsaci.*

Various mechanisms may account for the failure of wilt symptoms to appear in plants inoculated with the antagonistic variant and *D. dipsaci.* The antagonistic bacteria may have entered the crown buds along with the nematodes, but were unable to establish themselves because of the presence of preformed chemicals or of a chemical induced by nematode infection. An induced chemical, such as a phytoalexin (4) could be strongly inhibitory to the antagonistic variant and could prevent its development in the plant. Further experiments would have to be conducted to clarify these possibilities.

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