

The Effects of Root Knot Nematodes on Bacterial Wilt in Alfalfa

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ABSTRACT

Meloidogyne hapla in association with *Corynebacterium insidiosum* increased the incidence of bacterial wilt disease in alfalfa *Medicago sativa* in studies at Logan, Utah, and Reno, Nevada. The incidence of bacterial wilt symptoms in Moapa and Ranger alfalfa plants infected with *M. hapla* approached the incidence in plants where the rootball was cut before inoculating with *C. insidiosum*. The cultivars DuPuits and Lahontan showed 90 and 42% *C. insidiosum* infection, respectively, where *M. hapla* and *C. insidiosum* were in association in uncut roots at Logan, Utah. *Corynebacterium insidiosum* alone on uncut roots resulted in bacterial wilt symptoms in only 14 and 5% of the plants in the two cultivars.

Studies at Reno, Nevada, in which seedlings of the cultivar DuPuits were inoculated with *M. hapla* and *C. insidiosum* alone and in combination, then

transferred to individual pots at 28, 44, and 65 days after emergence, showed no visible symptoms of bacterial wilt after 4 months with *C. insidiosum* alone and the untreated check. *Meloidogyne hapla* and *C. insidiosum* in combination caused bacterial wilt symptoms in 32, 25, and 56% of treated plants transferred to individual pots 28, 44, and 65 days after inoculation, and 57% in those continuously grown in initial pots. *Corynebacterium insidiosum* was present in plants inoculated with pure cultures of the organism alone.

These studies indicate a close relationship between the incidence of bacterial wilt disease and presence of *M. hapla* in the plants. They also indicate that *M. hapla* may be hastening the colonization of alfalfa plants by *C. insidiosum*. *Phytopathology* 61: 256-259.

Northern root knot nematode (*Meloidogyne hapla* Chitwood) and bacterial wilt (*Corynebacterium insidiosum* [McCull] H. L. Jens) of alfalfa occur under similar environmental conditions in many areas where winter-dormant alfalfa (*Medicago sativa* L.) is grown. *Meloidogyne hapla* larvae enter alfalfa roots, where they usually become sedentary and feed on vascular parenchyma tissue. Rapid physical and chemical changes occur in plant cells in the feeding site. Characteristic galling of the roots usually occurs. The effects of Northern root knot nematode on alfalfa have not been clearly defined, since direct damage is difficult to assess under field conditions. Bacterial wilt causes severe damage to alfalfa in many alfalfa-growing regions of the country.

Interactions between root knot nematodes and other pathogenic organisms may be more important than the effect of the nematode alone. Several studies have shown that root knot nematodes directly affect the incidence and severity of diseases caused by soil-inhabiting fungi. McGuire et al. (5) found that the severity of *Fusarium* wilt caused by *Fusarium oxysporum* Schlecht. f. sp. *vasinfectum* (Ark.) Snyder & Hans. in Buffalo alfalfa increased as root knot infection increased. Interactions between root knot nematodes and other organisms have been described in several recent reviews (12, 13, 19).

The specific role of nematodes in interactions with other pathogens apparently is complex and as yet poorly understood. Root wounding was first considered the most likely relationship. Newhall (8) showed that the burrowing nematode (*Radopholus similis*) feeds on cortex of the banana as deep as the stele, and could

expose the stele to direct invasions by *F. oxysporum* f. sp. *ubense*. Miller (7) indicated that in the case of wilt diseases the organism must reach the vascular system to cause injury; therefore, wounds by endoparasitic nematodes provide an easier, and, in the case of resistant varieties, the only pathway of entry.

Nematodes contribute more to these complexes than avenues of entry (6, 16, 17). Nusbaum (10) showed that the black shank fungus of tobacco (*Phytophthora parasitica* var. *nicotianae*) does not require root wounds to enter a plant; however, a complex involving this disease and nematodes has been described (10, 15). The fungus was able to thrive on hyperplastic and hypertrophic tissue, and extensive colony formation occurred around nematode infection sites (14).

Relationships between pathogenic fungi and nematodes have received much greater attention than relationships between pathogenic bacteria and nematodes. Pitcher (12), in reviewing work on bacterial-nematode relationships, stated that this subject was to some extent "a poor relation" of fungus-nematode and virus-nematode relationships. He illustrated the relationship between *Aphelenchoides ritzemabosi* and *Corynebacterium fascians* in the cauliflower disease of strawberry. The full expression of the disease requires that both organisms be present in the meristems. Hawn (3) showed that *Ditylenchus dipsaci* may act as a carrier of *Corynebacterium insidiosum*, and had a decided influence on development of bacterial wilt in a wilt susceptible variety. Hawn & Hanna (4) showed that stem nematode infestation broke down the bacterial wilt resistance of Beaver alfalfa, making it comparable in susceptibility to Grimm. In greenhouse tests at Iowa,

M. hapla increased the incidence of bacterial wilt caused by *Corynebacterium insidiosum* in both a resistant and susceptible variety (9). Stands of a wilt-resistant variety were reduced significantly when both organisms were tested in combination as compared with either alone.

Since bacterial wilt caused by *C. insidiosum*, and root knot caused by *M. hapla*, are major diseases of alfalfa with similar geographic distribution, we have studied the interactions between these two pathogens and the diseases they incite.

MATERIALS AND METHODS.—Three separate experiments were conducted to study the relationships between Northern root knot nematode and the bacterial wilt disease of alfalfa, Experiments I and II at Logan, Utah, and Experiment III at Reno, Nevada.

Experiment I.—Six replications of eight plants each were made for each of five treatments: (i) *M. hapla* alone; (ii) *M. hapla* plus *C. insidiosum*; (iii) *C. insidiosum* with cut rootball; (iv) *C. insidiosum* with uncut rootball; and (v) untreated control. Three-week-old plants of the alfalfa cultivars Vernal, Ranger, and Moapa were transplanted into 10-cm clay pots at the rate of eight/pot; *M. hapla* was added at this time. *Corynebacterium insidiosum* was added 4 weeks later.

Meloidogyne hapla inoculum was obtained by picking egg masses from infected tomatoes, surface sterilizing, and hatching in distilled water. Two hundred-fifty second-stage larvae/plant were added.

The 7-week-old potted plants were soaked in water for 5 min, rootballs gently removed to minimize root damage, and transplanted to 15-cm clay pots. Cut rootball inoculations were made by cutting through the rootball 5 cm below the plant crown and adding inoculum to severed roots (1).

Corynebacterium insidiosum inoculum was obtained by suspending finely ground infected alfalfa root tissue in distilled water for 8 hr before inoculating. At time of transplanting, 100 ml of the inoculum was poured over the appropriate rootballs.

Plants were grown on a greenhouse bench for 3 months after second transplanting at 22 ± 4 C. Plants were cut 5 cm above ground line at monthly intervals. Plants showing vascular discoloration at 3 months were considered infected with *C. insidiosum*.

Experiment II.—A study, similar in most respects to Experiment I, was conducted at Logan, Utah, by using plants of the cultivars Lahontan and DuPuits. The cut rootball treatment was not included in this experiment; therefore, treatments included (i) *M. hapla*; (ii) *C. insidiosum*; (iii) *M. hapla* + *C. insidiosum*; and (iv) untreated control.

Experiment III.—We studied DuPuits alfalfa at Reno, Nevada, using treatments as in Experiment II and sterilized soil containing 50% loam and 50% washed sand. The soil and inoculum were thoroughly mixed, and a water suspension of *C. insidiosum* cells prepared from pure cultures was sprinkled on the soil as it was mixed. Additional bacterial suspensions were added to the soil surface 1 and 2 weeks after seedlings emerged. *Meloidogyne hapla* inoculum was obtained by chopping infected tomato roots, mixing these thor-

oughly with soil in which they were growing, adding a measured quantity to sterilized soil, and mixing. The mixed soil was transferred to plastic containers 20 cm in depth and 46 cm in diam. Seeds were presoaked before seeding. Approximately 45 swollen seeds were seeded in each container. To minimize the danger of injury to the young radical which might allow entry of the bacterial wilt organisms, we did not use seeds with radicals showing. Each treatment was replicated 4 times.

In a preliminary study to find the earliest date of infection, *M. hapla* damage was observed 7 days after plant emergence, and *C. insidiosum* was cultured from taproots 28 days after seeding. Based on these findings, 25 seedlings from each treatment of a replication were transferred to individual pots 28, 44, and 65 days after seeding, to determine if initial infection by *C. insidiosum* was sufficient to cause bacterial wilt. One replication was allowed to grow continuously. Potted plants were removed from original containers by soaking in water to minimize root damage. The plants were then washed in tap water and transplanted to sterile soil, one/15-cm pot.

Plants were grown on greenhouse benches for 4 months at 24 ± 5 C. Alfalfa was cut to a 5-cm height at 25% bloom. After 4 months, plants showing vascular discoloration were considered to be infected. A random sample of plants from each treatment was washed, and root-tissue surface sterilized. Root cross sections were planted on beef lactose agar medium to determine the presence or absence of *Corynebacterium insidiosum*.

The cultivar Vernal is highly resistant to bacterial wilt. Ranger and Lahontan are moderately resistant, while Moapa and DuPuits are susceptible. All cultivars are highly susceptible to *M. hapla*.

RESULTS AND DISCUSSION.—The incidence of bacterial wilt infection increased significantly in plants inoculated with a combination of *M. hapla* and *C. insidiosum* over those inoculated with *C. insidiosum* alone. Results from three separate tests showed increases of 7 to 76% in the percentage of plants infected with bacterial wilt where a combination of *M. hapla* and *C. insidiosum* was used (Tables 1, 2, 3).

Combining *M. hapla* with *C. insidiosum* was almost as effective in producing bacterial wilt symptoms in alfalfa as the commonly used cut root technique. The cut root technique gave a higher incidence of wilt only in the highly susceptible cultivar Moapa. Varieties reacted as they would be expected to, based on their relative resistance to bacterial wilt. There is little indication that resistance to wilt is affected by *M. hapla*. The percentage of plants of wilt-resistant Vernal showing wilt symptoms was the same in both the cut root + *C. insidiosum* and *M. hapla* + *C. insidiosum* treatments.

Mortality of plants was almost identical in cut rootball + *C. insidiosum* and *M. hapla* + *C. insidiosum* treatments. Mortality was affected by wilt resistance of the cultivars; *M. hapla* alone resulted in mortality comparable to the cut rootball + *C. insidiosum* and *M. hapla* + *C. insidiosum* treatments; however, there was no cultivar response to the *M. hapla* treatment. Mor-

TABLE 1. Survival and per cent of *Corynebacterium insidiosum*-infected plants of three alfalfa varieties as affected by combinations of *Meloidogyne hapla* and *C. insidiosum* at Logan, Utah

Treatment	Variety					
	% infected plants			% dead plants		
	Moapa	Ranger	Vernal	Moapa	Ranger	Vernal
<i>C. insidiosum</i> + <i>M. hapla</i>	78 b ^a	56 c	7 d	16 c	14 c	6 a
<i>C. insidiosum</i> + roots cut	95 e	64 c	9 d	16 c	13 c	7 ab
<i>C. insidiosum</i>	11 b	9 d	0 a	5 a	8 b	4 a
<i>M. hapla</i>	0 a	0 a	0 a	12 b	13 c	10 b
Untreated	0 a	0 a	0 a	7 a	4 a	4 a

^a Means with the same letter are not different at the .05 level of significance.

tality and vascular discoloration in plants inoculated with *M. hapla* alone may have been partially due to other organisms. In Experiment III, where vascular discoloration occurred in the absence of *C. insidiosum*, we isolated *Fusarium* and *Pythium* from root tissue in the *M. hapla* alone treatment.

The virulence of *C. insidiosum* decreases rapidly when maintained in pure culture. Where we used pure cultures in Experiment III, the incidence of bacterial wilt was much lower than it was in the same cultivar in Experiment II. Inoculum source, therefore, could af-

fect the results of this type of experiment, especially where highly resistant cultivars are used.

Several studies on nematode-pathogen relationships show a reduction in plant growth which is additive. Van Gundy & Tsao (18) and O'Bannon et al. (11) showed an additive relationship between nematodes and *Fusarium* in citrus. It was indicated in both studies that environmental conditions favorable to the nematode could have masked some of the additive effect. Faulkner & Skotland (2) showed a dry wt reduction of 22% in peppermint with either *Verticillium dahliae* or *Pratylenchus minyus* alone, but as much as 68% by a combination of the two organisms. In the present studies, we did not take dry wt of the plants, but observed very striking differences in total growth of the treatments (Fig. 1). Plants inoculated with *M. hapla* and a combination of *M. hapla* and *C. insidiosum* were reduced

TABLE 2. Per cent of *Corynebacterium insidiosum*-infected plants of two alfalfa varieties and per cent of dead plants after subjection to combinations of *Meloidogyne hapla* and *Corynebacterium insidiosum* at Logan, Utah

Treatment	Variety			
	Du-Puits	Lahontan	Du-Puits	Lahontan
	% infected plants		% dead plants	
<i>C. insidiosum</i> + <i>M. hapla</i>	90 e ^a	42 d	22 c	12 b
<i>C. insidiosum</i>	14 c	5 d	8 ab	6 b
<i>M. hapla</i>	0 a	0 a	18 c	12 b
Untreated controls	0 a	0 a	8 ab	6 b

^a Means with the same letter or letters are not different at the .05 level of significance.

TABLE 3. Per cent alfalfa plants showing vascular discoloration after inoculation with *Meloidogyne hapla* and *Corynebacterium insidiosum* alone and in combination on DuPuits, Experiment III, Reno, Nev.

Treatment	% Plants showing vascular discoloration			
	Contin-uous	Time of transfer to individual pots (days after emergence)		
		28	44	65
<i>M. hapla</i> + <i>C. insidiosum</i>	57 c ^a	32 c	25 c	56 c
<i>M. hapla</i>	18 b	13 b	16 b	17 b
<i>C. insidiosum</i>	0 a	0 a	0 a	0 a
Untreated control	0 a	0 a	0 a	4 a

^a Means with the same letter or letters are not different at the .05 level of significance.

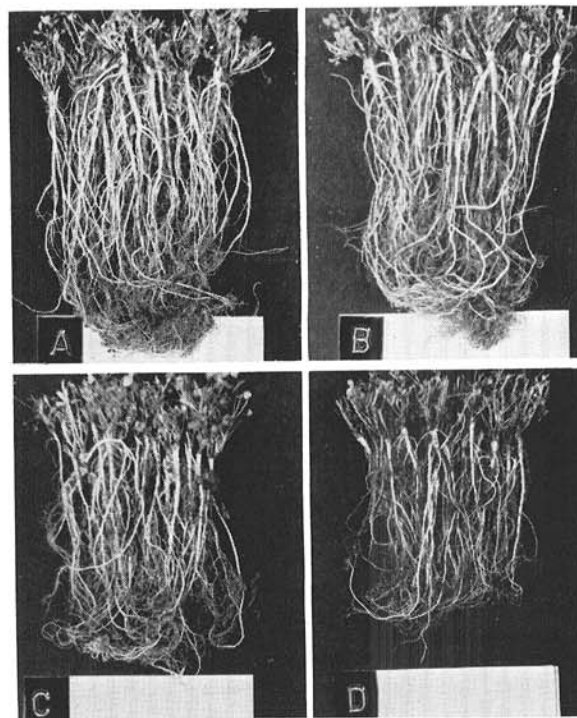


Fig. 1. Effect of *Meloidogyne hapla* and *Corynebacterium insidiosum* on alfalfa. A) Untreated check; B) *C. insidiosum* alone; C) *M. hapla*; D) *C. insidiosum* + *M. hapla*.

compared to the controls. The effect of *M. hapla* and *C. insidiosum* appeared to be additive. *Meloidogyne hapla* alone reduced growth more than did *C. insidiosum*.

Meloidogyne hapla either aided entry of bacteria into roots of alfalfa or hastened the colonization of *C. insidiosum*. Only a small percentage of these plants with intact roots inoculated with *C. insidiosum* alone showed bacterial wilt symptoms. All plants thus treated in Experiment III were found to contain *C. insidiosum* bacteria. A quantitative analysis of the bacteria present in tissue in the various treatments was not made; therefore, we do not know if *M. hapla* in association with *C. insidiosum* increased the progress of the disease or multiplication of the bacteria.

There was no indication in these studies that wilt-resistant plants could be predisposed to the disease in the presence of *M. hapla*. These studies, however, were not designed to determine this relationship. Such an important relationship should be worthy of additional study.

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