

Interaction of *Meloidogyne hapla* and *Fusarium oxysporum* f. sp. *medicaginis* on Alfalfa

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ABSTRACT

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The incidence of fungal infection and vascular discoloration from a single inoculation with *Fusarium oxysporum* f. sp. *medicaginis* was similar in Ranger alfalfa, susceptible to both *Meloidogyne hapla* and *F. o. f. sp. medicaginis*, and Nevada Synthetic XX alfalfa, resistant to *M. hapla* and susceptible to *F. o. f. sp. medicaginis*; less fungal infection and vascular discoloration occurred in Moapa 69 alfalfa, susceptible to *M. hapla* and resistant to *F. o. f. sp. medicaginis*. Fungal infection and root discoloration increased ($P < 0.05$) and plant growth decreased ($P < 0.01$) in Ranger and Moapa 69 at 20–30 C when inoculation with *M. hapla* preceded inoculation with *F. o. f. sp. medicaginis* by 28 days. Plant mortality in Ranger and

Moapa 69 also increased ($P < 0.05$) following sequential inoculations with the two organisms and was greatest at 25 C. In Nevada Synthetic XX, the incidence of fungal infection and root discoloration from combined inoculations (compared to the effects of a single inoculation with the fungus) increased ($P < 0.05$) only at 30 C, where resistance to *M. hapla* is lost. Combined inoculations suppressed the incidence of root-knot galling ($P < 0.05$) and nematode reproduction ($P < 0.05$) on both Ranger and Moapa 69, but not on Nevada Synthetic XX. Single inoculations with *M. hapla* reduced ($P < 0.05$) the growth of Ranger and Moapa 69 at 25 and 30 C and Nevada Synthetic XX at 30 C.

Additional key words: additive, galling indices, *Medicago sativa*, northern root-knot nematode.

One of the most important interactions between plant-parasitic nematodes and economically important plant cultivars is the ability of some nematodes to affect the plant's resistance to other plant pathogens. The literature is replete with instances where nematode species affected the host-parasite relationship between a plant and another pathogen (16). *Meloidogyne* spp. are most commonly associated with nematode-pathogen interactions, and several root-knot nematode species, including *M. hapla* Chitwood, reportedly interact with *Fusarium* spp. in plant disease complexes (2–4, 10–12, 14, 17, 20). Nematode-pathogen relationships (7, 8), including that of *M. hapla* and *F. oxysporum* Schlect., have been reported in alfalfa (13, 18).

We have observed the decline of alfalfa stands in fields infested with both *M. hapla* and *F. o. f. sp. medicaginis* (Weimer) Snyder & Hansen in the western United States. In view of this, a study was made to determine the importance of this nematode-fungus relationship.

MATERIALS AND METHODS

Inoculum of *M. hapla*, obtained originally from a lettuce planting in northern Utah, was cultured in the greenhouse on tomato plants (*Lycopersicon esculentum* Mill. cultivar Bonny Best) for inoculum production. Egg masses were picked from roots with the aid of a stereomicroscope, surface sterilized with a 1% solution of NaOCl for 5 min, and rinsed through four series of sterilized water, and juveniles (J2) were obtained by hatching eggs in oxygenated distilled water at 24 ± 4 C. These juveniles were decanted daily and used as inoculum within 2 days after hatching. Inoculum of *F. o. f. sp. medicaginis* was obtained from alfalfa in Nevada and cultured on potato-dextrose agar as outlined by Morrell and Bloom (14).

Since the greatest damage to alfalfa by *F. o. f. sp. medicaginis* has been observed in sandy soils, sandy loam soil (91% sand, 5% silt, and 4% clay; pH 7.2), fumigated with methyl bromide at 488 kg/ha, was used in all experiments.

Greenhouse bench test 1. A greenhouse bench test determined if there is an interaction between *M. hapla* and *F. o. f. sp. medicaginis* on alfalfa. Pregerminated seeds of Ranger alfalfa, susceptible to both organisms, were treated with captan, washed in sterilized water to remove the captan, and planted in 15-cm-diameter containers of soil (one seed per container). After 28 days of growth, plant roots were inoculated by adding equal amounts of inocula through four soil openings made around each plant. Each plant was treated with either 1,000 J2 of *M. hapla*; a 10-ml suspension of *F. o. f. sp. medicaginis* (12×10^6 microconidia per milliliter); simultaneous inoculation with 1,000 J2 of *M. hapla* and a 10-ml suspension of *F. o. f. sp. medicaginis*; or sequential inoculation with 1,000 J2 of *M. hapla* followed 28 days later with a 10-ml suspension of *F. o. f. sp. medicaginis*; uninoculated plants were used as controls. Since a preliminary study showed no significant differences ($P < 0.01$) in infection, discoloration, and growth of Ranger, Moapa 69, and Nevada Synthetic XX alfalfa inoculated with *F. o. f. sp. medicaginis* at 28 and 56 days of age, a fungal inoculation treatment of 56-day-old plants was eliminated from the experiment. All treatments were replicated 20 times. The plants were lightly watered after inoculation and grown on a greenhouse bench at 22 ± 4 C. To partially simulate field conditions, shoots were harvested when the plants were 72 days old and every 30 days thereafter. The experiment was terminated 280 days after planting, and wet root and total wet shoot weights from all harvests were determined. Root-knot indices (0 = no galling; 6 = very severe galling) and nematode egg reproduction per plant (final population divided by initial population) were determined for *M. hapla* (1, 9). Alfalfa root and stem tissues were sectioned and stained in 1% cotton blue or plated out on potato-dextrose agar, to determine infection by *F. o. f. sp. medicaginis* and vascular discoloration (14). The data were analyzed by a split-plot analysis of variance, using cultivars as whole plots.

Greenhouse bench test 2. A second greenhouse bench test investigated the differential effects of combined inoculations with *M. hapla* and *F. o. f. sp. medicaginis* on the growth of Nevada Synthetic XX (resistant to *M. hapla* and susceptible to *F. o. f. sp. medicaginis*), Moapa 69 (resistant to *F. o. f. sp. medicaginis* and susceptible to *M. hapla*), and Ranger. The experimental design was identical to that described for greenhouse bench test 1. Alfalfa

plants were grown in the greenhouse for 280 days at 22 ± 4 C, and shoot and root weights and the incidence of wilt were determined as in greenhouse bench test 1.

Growth chamber temperature test. A final experiment studied the effect of soil temperature on the interaction between *M. hapla* and *F. o. f. sp. medicaginis* on alfalfa. The experimental design and alfalfa cultivars duplicated those of the second greenhouse bench study, except the simultaneous inoculation treatment was not included. Pregerminated seeds were planted in 10-cm-diameter containers of soil, and each treatment was replicated 20 times. The plants were grown in temperature-controlled growth chambers at 15, 20, 25, and 30 C. In each of the treatments, 10 replicates were harvested when the plants were 100 days old, and the other 10 replicates were harvested when they were 280 days old. Total plant weights, nematode galling and reproduction, and the incidence of disease were determined as previously outlined.

Data from all experiments were analyzed using a split-plot analysis of variance.

RESULTS

Greenhouse bench test 1. *M. hapla* decreased ($P < 0.05$) both shoot and root growth of Ranger alfalfa (Table 1). There were no

TABLE 1. Interaction of *Meloidogyne hapla* and *Fusarium oxysporum* f. sp. *medicaginis* on shoot and root weights and nematode galling indices of susceptible Ranger alfalfa

Treatment	Plant weights (g) ^a		Nematode indices ^b	
	Shoots	Roots	Reproduction	Root galling
<i>M. hapla</i> ^c	20.8*	42.6*	16.3	5.0
<i>F. o. f. sp. medicaginis</i> ^c	30.4	51.0	0.0	0.0
<i>M. hapla</i> + <i>F. o. f. sp. medicaginis</i> ^c	18.6*	40.1*	12.8†	4.2†
<i>M. hapla</i> + <i>F. o. f. sp. medicaginis</i> ^d	8.2**	25.2**	8.4††	3.1†
Uninoculated control	27.9	53.9	0.0	0.0

^a Cumulative shoot weights from harvest starting 72 days after seeding and every 30 days thereafter for 280 days. Root weights after 280 days. Means with asterisks (* and **) are significantly different from those of untreated controls at $P < 0.05$ and $P < 0.01$, respectively.

^b Nematode egg reproduction indices: $(Pf/Pi) \times 10^{-2}$, where Pf is the final population and Pi is the initial population. Root-galling indices: 0 = no galling; 6 = very severe galling. Means with daggers († and ††) are significantly different from those of plants inoculated with *M. hapla* alone at $P < 0.05$ and $P < 0.01$, respectively.

^c Twenty-eight-day-old plants inoculated with *M. hapla* or *F. o. f. sp. medicaginis* or with both organisms.

^d Twenty-eight-day-old plants inoculated with *M. hapla* and 28 days later with *F. o. f. sp. medicaginis*.

differences in the shoot and root weights of plants inoculated with *F. o. f. sp. medicaginis*, and the growth of Ranger simultaneously inoculated with both organisms was not lower than that of plants inoculated with *M. hapla* alone. There was a reduction in shoot and root growth ($P < 0.01$) and in root galling and nematode reproduction ($P < 0.05$) from sequential inoculations, when nematode inoculum preceded fungal inoculum by 28 days (Table 1).

Simultaneous and sequential inoculations with both organisms significantly increased ($P < 0.05$) the incidence of fungal infection and vascular discoloration in Ranger over that produced by inoculation with *F. o. f. sp. medicaginis* alone. Infection or discoloration was found in 45, 75 and 100% of Ranger roots following inoculation with *F. o. f. sp. medicaginis* alone, simultaneous inoculations, and sequential inoculations, respectively.

Greenhouse bench test 2. *M. hapla* significantly reduced ($P < 0.05$) shoot and root growth of Ranger and Moapa at 22 ± 4 C (Table 2). Reductions of shoot and root growth of Ranger were significantly greater ($P < 0.01$) from simultaneous and sequential inoculations with *M. hapla* and *F. o. f. sp. medicaginis*, and reductions in shoot and root growth from sequential inoculations were significantly greater ($P < 0.05$) than those from simultaneous inoculations. Simultaneous inoculations of Moapa 69 did not reduce shoot and root growth below that of plants inoculated with *M. hapla* alone, but sequential inoculations significantly reduced ($P < 0.05$) growth below that of plants receiving single or combined inoculations. There was no suppression in shoot or root growth of Nevada Synthetic XX from single, combined, or sequential inoculations, and *F. o. f. sp. medicaginis* did not affect the resistance of Nevada Synthetic XX to *M. hapla*; all Nevada Synthetic XX plants were free of galling.

The incidence of fungal infection and vascular discoloration from single inoculations with *F. o. f. sp. medicaginis* were similar in Ranger and Nevada Synthetic XX and significantly less ($P < 0.05$) in Moapa 69. However, in the presence of *M. hapla*, there was significantly more ($P < 0.05$) fungal infection and vascular discoloration in Ranger and Moapa 69, but not in Nevada Synthetic XX. The percentage of plants that were infected or showed vascular bundle discoloration from inoculation with *F. o. f. sp. medicaginis* alone, simultaneous inoculations with both organisms, and sequential inoculations were 35, 65, and 90% in Ranger; 40, 35, and 45% in Nevada Synthetic XX; and 10, 35, and 65% in Moapa 69.

Growth chamber temperature test. Single inoculations with *F. o. f. sp. medicaginis* significantly reduced ($P < 0.05$) plant growth of Ranger and Nevada Synthetic XX at 15 C after 100 and 280 days, and single inoculations with *M. hapla* significantly reduced ($P < 0.05$) plant growth of Ranger and Moapa 69 at 25 and 30 C after

TABLE 2. Effect of combined inoculation with *Meloidogyne hapla* and *Fusarium oxysporum* f. sp. *medicaginis* on root and shoot weights of resistant and susceptible alfalfa cultivars at 22 ± 4 C

Treatment	Alfalfa cultivars ^a					
	Ranger		Nev Syn XX		Moapa 69	
	Shoot weight (g)	Root weight (g)	Shoot weight (g)	Root weight (g)	Shoot weight (g)	Root weight (g)
<i>M. hapla</i> ^b	20.8*	50.6*	26.4	67.3	24.6*	61.5
<i>F. o. f. sp. medicaginis</i> ^b	28.4	58.3	26.8	71.7	31.3	65.9
<i>M. hapla</i> + <i>F. o. f. sp. medicaginis</i> ^b	15.4**	37.5**	27.1	66.7	21.3*	45.8*
<i>M. hapla</i> + <i>F. o. f. sp. medicaginis</i> ^c	10.5**†	28.1**††	28.1	64.7	16.9**†	39.9**†
Uninoculated control	27.9	56.9	27.5	69.2	29.8	70.0

^a Cultivar Ranger is susceptible to *M. hapla* and *F. o. f. sp. medicaginis*; cultivar Nevada Synthetic XX (Nev Syn XX) is resistant to *M. hapla* and susceptible to *F. o. f. sp. medicaginis*; and cultivar Moapa 69 is susceptible to *M. hapla* and resistant to *F. o. f. sp. medicaginis*. Cumulative shoot weights from harvests starting 72 days after seeding and every 30 days thereafter for 280 days. Root weight after 280 days. Means with asterisks (* and **) are significantly different from those of untreated controls at $P < 0.05$ and $P < 0.01$, respectively. Means with daggers († and ††) are significantly different from those of plants simultaneously inoculated with *M. hapla* and *F. o. f. sp. medicaginis* at $P < 0.05$ and $P < 0.01$, respectively.

^b Twenty-eight-day-old plants inoculated with *M. hapla* or *F. o. f. sp. medicaginis* or with both organisms.

^c Twenty-eight-day-old plants inoculated with *M. hapla* and 28 days later with *F. o. f. sp. medicaginis*.

100 and 280 days (Fig. 1). Sequential inoculations with both organisms significantly reduced ($P < 0.01$) plant growth of Ranger at 20–30 C and 15–30 C after 100 and 280 days, respectively. Reductions ($P < 0.05$) in plant growth of Moapa 69 from sequential inoculations occurred at 20–30 C after 100 and 280 days. The growth of Nevada Synthetic XX was not affected by single or combined inoculations of *M. hapla* and *F. o. f. sp. medicaginis* except following sequential inoculation with the two organisms at 30 C.

The incidence of fungal infection and vascular discoloration, which resulted in growth suppression, was enhanced by the longer period of exposure to the two organisms (Fig. 2). Fungal infection and vascular discoloration following single inoculations with *F. o. f. sp. medicaginis* were greatest at 25 C. *M. hapla* increased ($P < 0.05$) the incidence of root infection and discoloration on Ranger and Moapa 69; the increase was positively correlated with an increase in soil temperature from 15 to 25 C. The incidence of infection and discoloration in Nevada Synthetic XX increased only at 30 C and was related to a loss of nematode resistance. Vascular discoloration increased as plant exposure increased from 100 to 280 days.

The most intensive root galling resulted from single inoculations of Ranger and Moapa 69 with *M. hapla* at 25 C after 280 days (Fig. 3). Although combined inoculations with *M. hapla* and *F. o. f. sp. medicaginis* did not affect galling after 100 days, galling was significantly reduced ($P < 0.05$) after 280 days following combined inoculations. Nematode reproduction closely paralleled root galling and was reduced by combined inoculations on Ranger and Moapa 69 at 15–25 C after 280 days (Fig. 4).

Following sequential inoculations, plant mortality of Ranger was 10 and 20% at 20 and 25 C, respectively, after 280 days; mortality of Moapa 69 was 10 and 15%, respectively, under comparable conditions. No mortality resulted in Nevada Synthetic XX from any of the treatments.

DISCUSSION

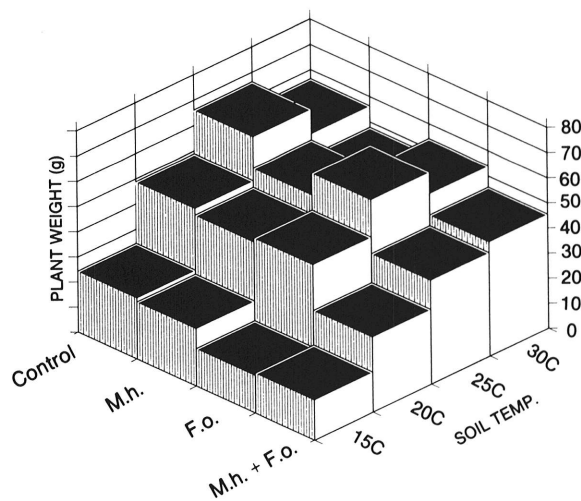
The possible use of a mildly virulent strain of *F. o. f. sp. medicaginis* may explain why single inoculations failed to suppress the growth of susceptible Ranger and Nevada Synthetic XX. However, our data agree with the findings of Walters and Slack (18), who showed reduction in the growth of alfalfa with *F. o. f. sp. medicaginis* only in the presence of *M. hapla*. Since fungal infection and root discolorations resulted from single inoculations with *F. o. f. sp. medicaginis*, this treatment may have resulted in reduced plant growth if the experiment had been continued for a longer period of time.

Root discoloration was increased and plant growth was significantly suppressed by sequential inoculation with both organisms, compared to inoculation with *F. o. f. sp. medicaginis* only. This agrees with the findings of Porter and Powell (15), who reported maximum wilt symptoms of tobacco only when plants were exposed to *M. incognita* 3 to 4 wk prior to *F. oxysporum*.

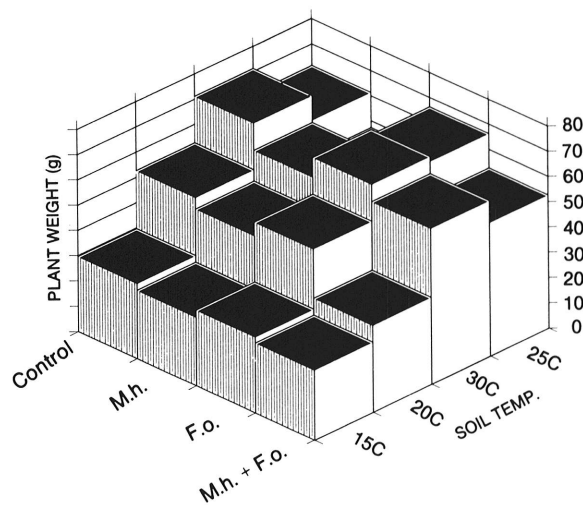
Single inoculation with *F. o. f. sp. medicaginis* did not affect plant growth of the susceptible Ranger alfalfa, and it did not affect the resistance of Nevada Synthetic XX to *M. hapla*. Only when Nevada Synthetic XX lost this resistance, at 30 C (5,6), were fungal infection and discoloration greater after combined inoculations than after a single inoculation with *F. o. f. sp. medicaginis*; vascular discoloration following a single inoculation with *F. o. f. sp. medicaginis* was greatest at 25 C, in agreement with the findings of Weimer (19).

The reduction in nematode reproduction and root galling from combined inoculations may be due to competition between *M. hapla* and *F. o. f. sp. medicaginis* for infection loci or feeding sites and to a reduction in root growth; both organisms are vascular parasites, and the optimum temperatures for reproduction or expression of both organisms are similar (5,6,19).

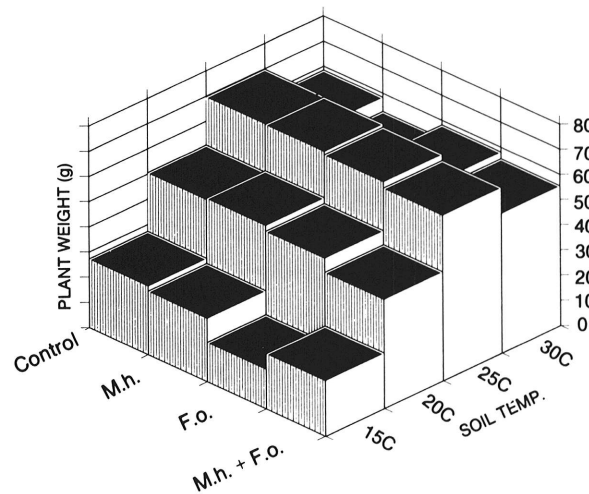
The ability of *M. hapla* to increase the virulence of *F. o. f. sp. medicaginis* to alfalfa agrees with previous data involving nematodes and fungi (16). Since *M. hapla* adversely affects the resistance of alfalfa to *F. o. f. sp. medicaginis*, it is important that



RANGER, 280 DAYS



MOAPA 69, 280 DAYS



NEV SYN XX, 280 DAYS

Fig. 1. Effect of the interaction of *Meloidogyne hapla* (M.h.) and *Fusarium oxysporum* f. sp. *medicaginis* (F.o.) on the growth of Ranger, Moapa 69, and Nevada Synthetic XX (Nev Syn XX) alfalfa at different soil temperatures after 280 days. All inoculations were made when the plants were 28 days old, except the combined inoculations, in which plants were inoculated with *F. o. f. sp. medicaginis* 28 days after inoculation with *M. hapla*.

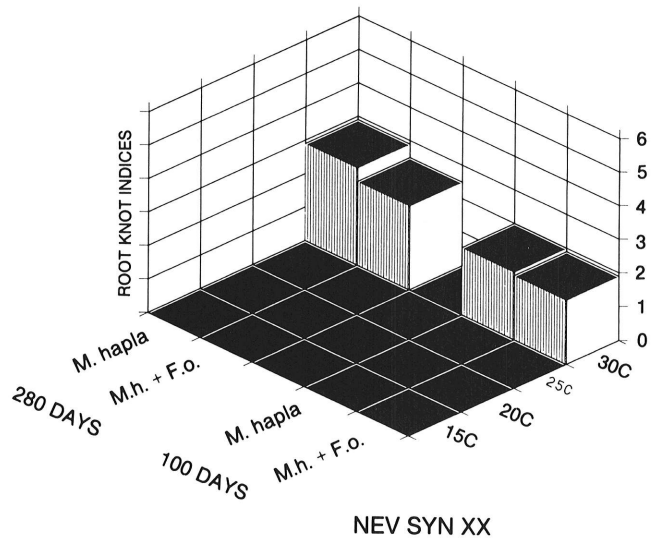
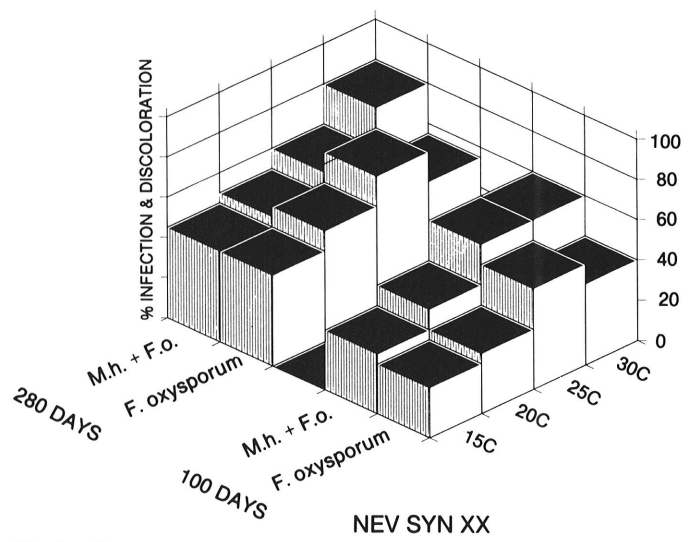
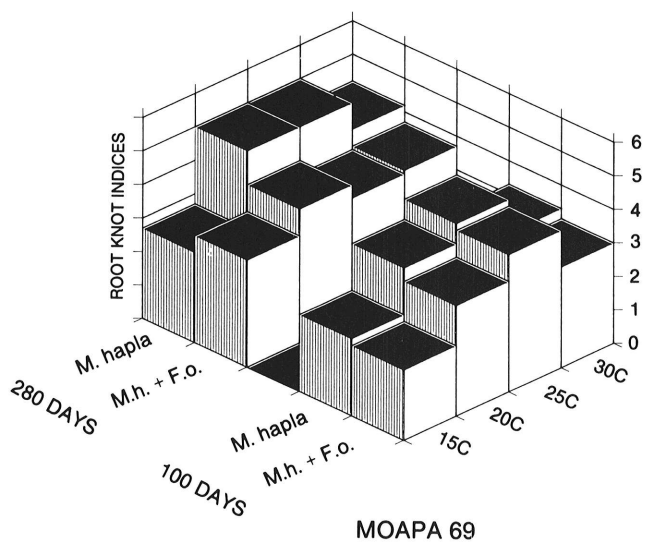
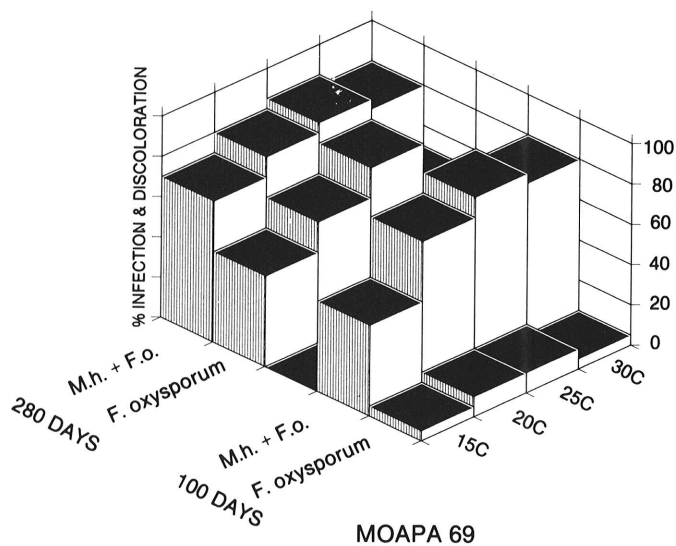
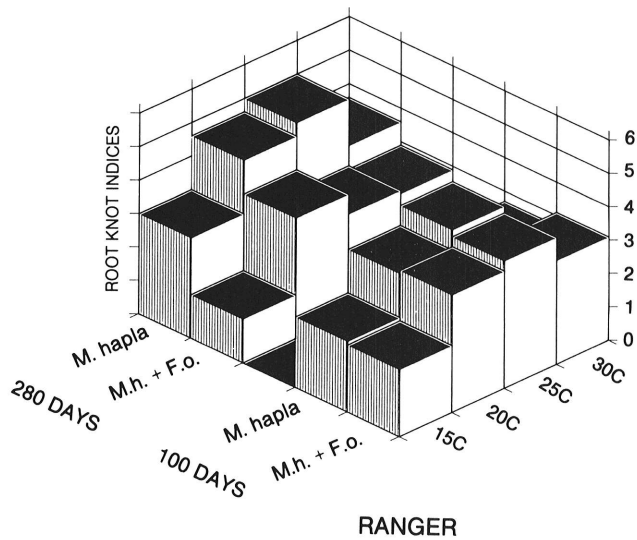
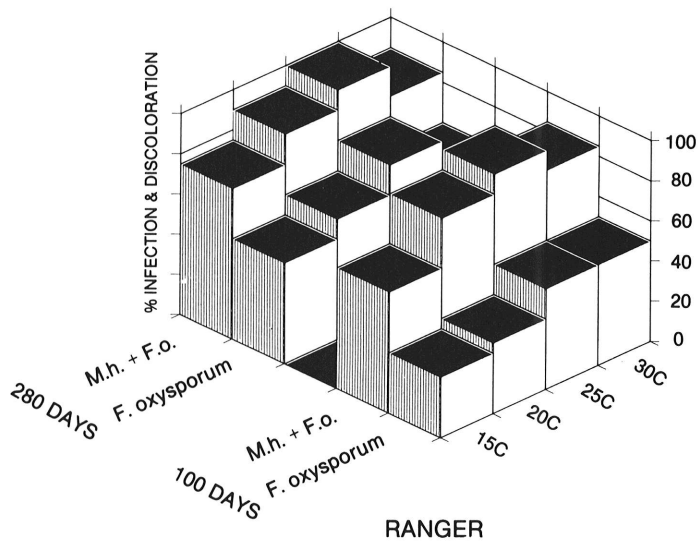


Fig. 2. Effect of *Meloidogyne hapla* (M.h.) on infection and discoloration of Ranger, Moapa 69, and Nevada Synthetic XX (Nev Syn XX) alfalfa by *Fusarium oxysporum* f. sp. *medicaginis* (F.o.) at different soil temperatures. All inoculations were made when the plants were 28 days old, except the combined inoculations, in which plants were inoculated with *F. o. f. sp. medicaginis* 28 days after inoculation with *M. hapla*.

Fig. 3. Effect of *Fusarium oxysporum* f. sp. *medicaginis* (F.o.) on galling indices for *Meloidogyne hapla* (M.h.) on Ranger, Moapa 69, and Nevada Synthetic XX (Nev Syn XX) alfalfa at different soil temperatures. All inoculations were made when the plants were 28 days old, except the combined inoculations, in which plants were inoculated with *F. o. f. sp. medicaginis* 28 days after inoculation with *M. hapla*. 0 = No galling; 6 = very severe galling (galling on 91–100% of the root system).

nematode resistance be considered in a program breeding for resistance to this fungal pathogen.

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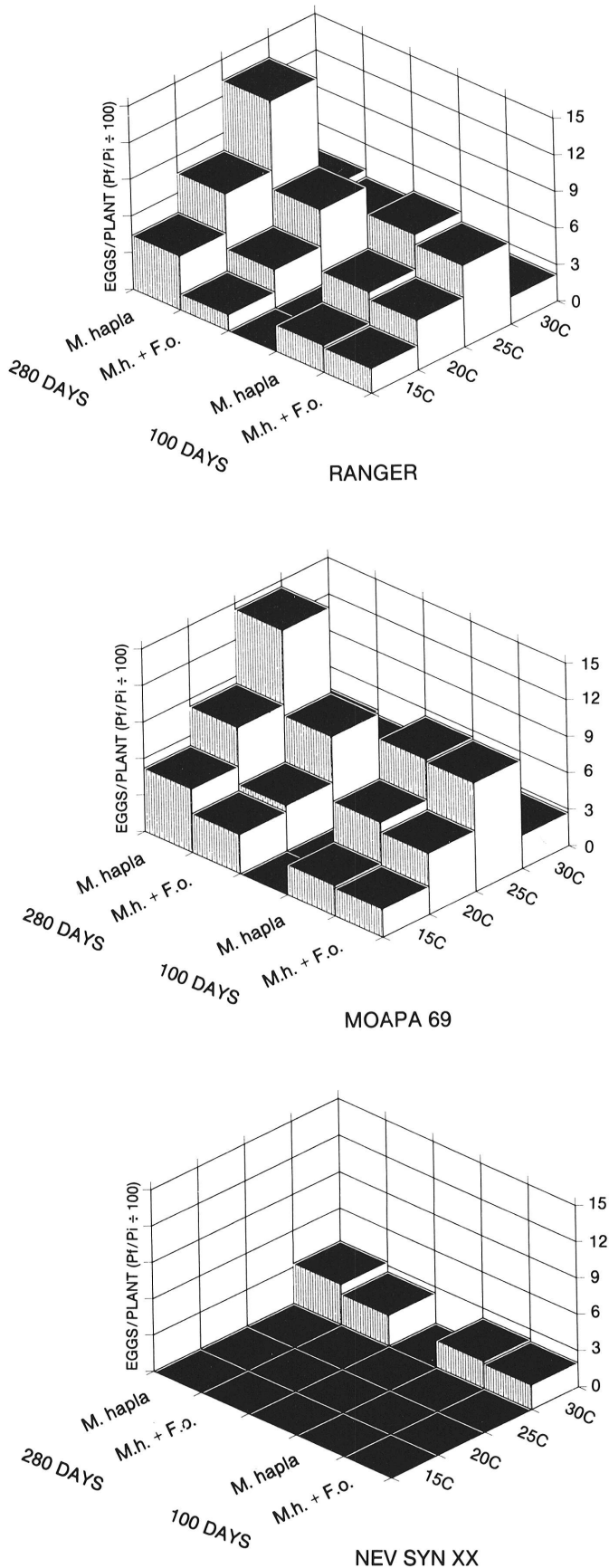


Fig. 4. Effect of *Fusarium oxysporum* f. sp. *medicaginis* (F.o.) on the reproduction of *Meloidogyne hapla* (M.h.) on Ranger, Moapa 69, and Nevada Synthetic XX (Nev Syn XX) alfalfa at different soil temperatures. All inoculations were made when the plants were 28 days old, except for the combined inoculations, in which plants were inoculated with *F. o.* f. sp. *medicaginis* 28 days after inoculation with *M. hapla*. Pf is the final population and Pi is the initial population of *M. hapla*.