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Pathogenicity of Two Populations of *Meloidogyne hapla* Chitwood on Alfalfa and Sainfoin

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Abstract: The pathogenicity of two populations of the northern root-knot nematode, *Meloidogyne hapla* Chitwood, population 1 (P1) from alfalfa and population 2 (P2) from sainfoin, was studied on both alfalfa and sainfoin for 25 weeks. Alfalfa and sainfoin plants inoculated with P2 had significantly ($P \leq 0.05$) higher mortality than plants inoculated with P1. Plant stands over all weeks for the uninoculated control, P1, and P2 were 90.5, 78.5, and 64.0% for alfalfa and 84.5, 51.0, and 41.0% for sainfoin, respectively. The increased virulence of P2 was again shown when means of plant species were combined (inoculation × week of count interaction). Plants inoculated with P2 had significantly higher mortality than either those inoculated with P1 or the uninoculated control beginning at week 7 and continuing through week 25. Plant stands over species at 25 weeks for the uninoculated control, P1, and P2 were 82.5, 29.0, and 18.0%, respectively. Sainfoin was significantly more susceptible to either population than alfalfa (plant species × week of count interaction). Separation between species first occurred after week 7 and continued until week 25. Percentages of plants remaining for alfalfa and sainfoin were 61.5 and 25.0 after 25 weeks. Significantly higher reproduction occurred in the alfalfa plants remaining after 25 weeks in P2 than in P1. Mean number of eggs per root system were 60,371 for P1 and 104,438 for P2, a difference of 42%. The results of this study indicate a need for breeders to adequately sample nematode populations present in the intended area of cultivar use and to design screening procedures to account for population pathogenicity variability.

Key words: alfalfa, *Medicago sativa, Meloidogyne hapla,* northern root-knot nematode, *Onobrychis viciifolia,* pathogenic race, sainfoin.

The northern root-knot nematode, *Meloidogyne hapla* Chitwood, is a serious pest on many perennial forage legume species including alfalfa, *Medicago sativa* L., (1,7–9) and sainfoin, *Onobrychis viciifolia* Scop. (5,6,14) in the northern United States. In the development of disease-resistant cultivars, physiological races or biotypes of the pathogen to which a cultivar may be subjected under field conditions should be considered. This type of information is vital in designing a plant breeding program to develop cultivars that perform more proficiently under field situations.

There is disagreement among researchers relating to physiological races of *M. hapla.* Goplen et al. (4) compared the response of alfalfa genotypes to two populations of northern root-knot nematode collected from alfalfa fields in California. Based on the reaction of selfed progeny of a selection from 'Vernal' alfalfa, they concluded that these populations were different physiological races of *M. hapla.* Ogbuji and Jensen (10) identified five races of *M. hapla* based on the reaction of 12 test plant species. Races 4 and 5 were collected from alfalfa fields in Prineville and Redmond, Oregon, respectively. Inoculation with race 4 resulted in no infection with the *M. hapla*-resistant alfalfa line 65-298, whereas a disease rating of 3 (on a scale of 0 = none to 4 = severe) was observed when the same line was inoculated with race 5. Eisenback (3) noted differences among 11 *M. hapla* populations for galling and reproduction on marigold (*Tagetes erecta* L. cv. Carnation). He observed a difference between *M. hapla* cytological race A and all other populations for infection and reproduction.

Triantaphyllou (12,13) characterized cytological races A and B. Race A consists of hypotetraploid populations which reproduce by meiotic parthenogenesis, aphimixis, or both and have haploid chromosome numbers ranging from 13 to 17. Race B populations reproduce by mitotic parthenogenesis with chromosome number ranging from 30 to 48. Triantaphyllou hypothesized that race B had evolved from race A, suggesting that genetic changes had

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occurred. Recently Curran et al. (2) were able to biochemically differentiate between cytological race A and race B populations based on DNA restriction fragment lengths.

Taylor and Sasser (11) reported differential reproduction for *M. hapla* populations on tobacco (*Nicotiana tabacum* L. cv. NC95) and on pepper (*Capsicum frutescens* L. cv. California Wonder). They did not, however, consider this to be evidence for different races of *M. hapla.* They also disagreed with other manuscripts reporting the existence of races of *M. hapla.* Their opinion was based on problems they perceived with sample size of the nematode populations. Ogbuji and Jensen (10) established populations by selecting three egg masses for increasing nematode populations. Using this type of sample from a heterogeneous population of nematodes could possibly result in differential reactions on test species. Goplen et al. (4) did not describe their sampling technique.

A sainfoin breeding program is being developed at the University of Wyoming. Two populations of *M. hapla* collected in Wyoming, P1 from alfalfa and P2 from sainfoin, are presently being maintained in a greenhouse. In our previous study (14) we described the extreme susceptibility of sainfoin to P2, compared with alfalfa in terms of root galling and reproduction. The objective of this study was to compare the pathogenicity of these two *M. hapla* populations on alfalfa and sainfoin. Specifically, we wanted to measure the comparative plant death rate from the two populations over time.

Materials and Methods

The two populations of *Meloidogyne hapla* Chitwood used in this study were collected from alfalfa (P1) and sainfoin (P2) in Goshen County, Wyoming. Both fields had sandy loam soils. At each location, the roots of several plants dispersed throughout the field were dug and nematode eggs were extracted from the composite samples. The extraction procedure was described previously (14). Nematode populations were increased and maintained in the greenhouse on tomato (*Lycopersicon esculentum* L. cv. Rutgers). Both P1 and P2 populations were initially identified as *M. hapla* by G. D. Griffin and confirmed by A. Morgan Golden.

To study the comparative pathogenicity of the populations, a greenhouse experiment was initiated on 28 April 1986. Three-day-old seedlings of Ladak alfalfa, or Eski sainfoin, were planted in steam-sterilized river bottom sand in 24 clay pots (25.4 cm d, 20 seedlings/pot). Each plant species was inoculated as follows: 1) P1, 1,500 eggs/pot; 2) P2, 1,500 eggs/pot; and 3) uninoculated control. Nematodes were applied at the time of planting. Plants were maintained in a greenhouse at 27 ± 2 C with a 14-hour photoperiod. Plants were watered daily and fertilized as needed throughout the study. The experimental design was a 3 × 2 factorial arrangement of treatments in a randomized complete block with four replications.

Plant stand counts were made 1, 2, 4, 7, 11, 14, 17, and 25 weeks after planting to compare mortality in alfalfa and sainfoin between the two populations. Plants were harvested at 10% bloom on 17 July, 12 September, and 21 October 1986, and forage yield was determined. At week 25, surviving plants were removed from the pots and root systems were rated for nematode galling. The rating scale was as follows: 1 = no galls, 2 = 1–10 galls, 3 = 11–100 galls, and 4 = > 100 galls. Nematode eggs were extracted from roots to estimate reproduction rates, and root dry weights were determined. Nematode egg extraction procedure was described previously (14). Data were subjected to standard analysis of variance techniques and means separated with the Student-Newman-Keuls range test.

Results and Discussion

Plant mortality: Plant stand count data revealed the following significant sources of variation: inoculation treatment, plant species, week of count, week × inoculation interaction, week × plant species interaction, and plant species × inoculation in-

TABLE 1. Plant stand count means of alfalfa and sainfoin inoculated with two populations of *Meloidogyne hapla*.

Treatment	Week 1	Week 2	Week 4	Week 7	Week 11	Week 14	Week 17	Week 25
				Alfalfa				
Uninoculated	18.5 a	18.3 a	17.8 a	17.5 a	18.0 a	16.3 a	16.3 a	16.3 a
P1	19.5 a	19.3 a	19.0 a	17.8 a	14.3 ab	11.3 b	11.3 b	10.3 b
P2	19.0 a	18.5 a	18.3 a	14.3 a	9.0 c	8.3 b	8.3 b	7.0 c
				Sainfoin				
Uninoculated	19.0 a	18.0 a	18.0 a	16.5 a	16.5 a	15.8 a	15.8 a	15.0 a
P1	18.0 a	17.5 a	16.5 ab	13.8 ab	13.8 ab	1.8 b	1.8 b	0.0 b
P2	17.5 a	17.0 a	13.0 b	8.3 c	5.5 c	0.3 b	0.3 b	0.0 b

Values are the number of live plants of 20 original plants per pot and are the mean of four replicates. Means in a given week within a forage crop followed by the same letter do not differ ($P \leq 0.05$) according to the Student-Newman-Keuls range test.

teraction. Plant stand count means varied by week and treatment (Table 1). Significant ($P \leq 0.05$) differences among inoculation treatments were first detected in sainfoin 4 weeks after inoculation. Significantly more mortality occurred in P2 than in the uninoculated control, whereas neither was different from P1. This same trend continued until week 14 when mortality was similar in both P1 and P2 because few sainfoin plants remained. All P1 and P2 treated plants were dead by week 25. Significant differences among inoculation treatments in alfalfa were not detected until week 11. Here again, P2 caused more mortality than P1 or the uninoculated control. Both P1 and P2 were significantly different from the uninoculated check but not from each other at weeks 14 and 17. By week 25, however, statistical separation between P1 and P2 was observed.

There was a significant ($P \leq 0.05$) difference between P1 and P2 regardless of crop species (inoculation × week interaction) (Table 2). Plant stand count differences among inoculation treatments were not detected until week 7. There was a significant increase ($P \leq 0.05$) in plant mortality in P1 and P2 over uninoculated control plants from weeks 11 to 25 with significantly greater mortality caused by P2. By week 11 all three treatments were significantly different. These significant differences continued throughout the remainder of the experiment, indicating differential mortality rates dependent upon the nematode populations used.

Differences between alfalfa and sainfoin over both *M. hapla* populations (plant species × week of count interaction) were also significant ($P \leq 0.05$) (Table 3). When means were compared over all inoculations (P1, P2, and uninoculated control), significant losses in alfalfa were not detected until week 11. No further change occurred in alfalfa during the remainder of the test. Mortality in sainfoin was observed earlier, with a significant reduction occurring at week 7. Losses continued to occur in sainfoin treatments until week 14. Stand count differences between alfalfa and sainfoin were first detected in week 7 and continued

TABLE 2. Plant stand count means for the inoculation × week of count interaction.

Treatment	Week 1	Week 2	Week 4	Week 7	Week 11	Week 14	Week 17	Week 25
Uninoculated	18.8 a A	18.1 a A	18.0 a A	17.4 a A	17.3 a A	17.0 a A	16.9 a A	16.5 a A
P1	18.9 a A	18.4 ab A	18.4 ab A	16.0 c AB	12.6 d B	6.9 e B	6.8 e B	5.8 e B
P2	18.4 a A	17.8 ab A	17.8 ab A	11.3 c C	7.1 d C	4.3 e C	4.0 e C	3.6 e C

Values are the number of live plants of 20 original plants per pot and are the mean of four replications over plant species. Means followed by the same letter (lower case for rows and upper case for columns) do not differ ($P \leq 0.05$) according to the Student-Newman-Keuls range test.

TABLE 3. Stand count means for the plant species × week of count interaction.

	Week 1	Week 2	Week 4	Week 7	Week 11	Week 14	Week 17	Week 25
Alfalfa	19.1 a A	18.7 ab A	18.6 a–c A	16.8 a–d A	13.7 e A	12.8 e A	12.5 e A	12.3 e A
Sainfoin	18.3 a A	17.5 ab A	17.5 ab A	13.0 c B	11.0 cd B	6.0 e B	5.9 e B	5.0 e B

Values are the number of live plants of 20 original plants per pot and are the mean of four replications over inoculations. Means followed by the same letter (lower case for rows and upper case for columns) do not differ ($P \leq 0.05$) according to the Student-Newman-Keuls range test.

throughout the remainder of the study. This agrees with our earlier report (14) which showed sainfoin to be more susceptible than alfalfa to *M. hapla.*

There were differences between inoculation treatment and plant species regardless of the week counts were taken (plant species × inoculation interaction) (Table 4). Alfalfa and sainfoin plants inoculated with P2 had significantly more mortality than plants inoculated with P1, indicating the aggressive nature of the P2 population. Differences between the nematode populations within each plant species are indicative of different host plant responses to the nematode population. Within either population, sainfoin suffered higher mortality than alfalfa.

Forage yield: Yield means were affected by treatment at all harvests (Table 5). Yield at the first harvest in alfalfa was significantly lower with P2 than with P1 or the uninoculated control. There was no difference between P1 and the control. At the second harvest, yield with P2 was significantly lower than the uninoculated control but no different from yield with P1. Again, there was no difference between P1 and the control. No differences were detected at the third harvest, possibly because of compensation in growth by the remaining plants within pots. In sainfoin, at the first harvest yield means for all treatments were significantly different from each other. Yield with P2 was the lowest of all treatments. By the second harvest, all plants with P2 and most with P1 were dead and separation between populations could not be made. Both were significantly lower than the uninoculated control. Similar results occurred at the third harvest, at which time all inoculated sainfoin plants were dead.

Both alfalfa and sainfoin plants inoculated with either nematode population yielded significantly less than uninoculated control plants, and both yielded significantly less when inoculated with P2 than when inoculated with P1. Overall, these data indicate differences in pathogenicity between the two *M. hapla* populations and differences in the susceptibility of sainfoin and alfalfa to root-knot nematode as measured by plant mortality and yield.

Reproduction: Significant differences ($P \geq 0.05$) were detected among the inoculation treatments in the number of nema-

TABLE 4. Plant stand count means over weeks for the plant species × inoculation interaction.

	Control	Population 1	Population 2
Alfalfa	18.1 a A	15.7 b A	12.8 c A
Sainfoin	16.9 a A	10.2 b B	8.2 c B

Values are the number of live plants of 20 original plants per pot and are the mean of four replications over weeks. Means followed by the same letter (lower case for rows and upper case for columns) do not differ ($P \leq 0.05$) according to the Student-Newman-Keuls range test.

TABLE 5. Yield of alfalfa and sainfoin inoculated with two populations of *Meloidogyne hapla.*

Treatment	Yield (g dry wt) 1st harvest (17/7/86)	2nd harvest (12/9/86)	3rd harvest (2/10/87)
	Alfalfa		
Uninoculated	14.3 A	14.2 A	9.2 A
P1	12.9 A	12.2 AB	8.2 A
P2	5.5 B	11.0 B	7.2 A
	Sainfoin		
Uninoculated	14.2 A	12.3 A	4.7 A
P1	9.3 B	1.2 B	0.0 B
P2	4.2 C	0.0 B	0.0 B

Values are the average dry weight per pot and are the mean of four replications. Means in a given harvest within a forage crop followed by the same letter do not differ ($P \leq 0.05$) according to the Student-Newman-Keuls range test.

tode eggs extracted per alfalfa plant root system. Number of eggs per root system were 0 for the uninoculated control, 60,371 for P1, and 104,438 for P2. All were significantly different from each other. P2 reproduction was 42% greater than P1 on alfalfa. Since all inoculated sainfoin plants were dead when the test was terminated, reproduction could not be determined; however, reproduction of the P2 population of *M. hapla* on sainfoin was reported in our previous study (14). There was no difference between nematode populations in root galling and root weight of alfalfa plants remaining when the experiment was terminated. Respective treatment means for the control, P1, and P2 were 0, 3.6, and 3.6 for root galling, and 46.2, 16.5, and 12.7 g for root weight. The experiment was repeated with similar results.

In conclusion, a Wyoming population of *M. hapla* from sainfoin was significantly more virulent on both alfalfa and sainfoin than a population from alfalfa, also from Wyoming. Plant mortality resulting from the sainfoin population occurred earlier and increased at a faster rate in both plant species. The sainfoin population also had a significantly higher level of reproduction in alfalfa than did the population collected from alfalfa. Difference in virulence between the two populations is important to a plant breeding program, since such differences in pathogenicity could result in serious problems when resistant lines, selected against a less virulent population, are tested under field conditions when the more virulent population is present. The more rapid rate of mortality due to the sainfoin population must also be considered in a breeding program. Unless the time of evaluation in screening plant material was extended past 11 weeks, the difference observed between nematode populations could result in less efficient selection, particularly if selection was primarily based on survival. The results of this study indicate a need for breeders to adequately sample nematode populations present in the intended area of cultivar use and to design a screening procedure to account for population pathogenicity variability.

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