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# Evaluation of Cultivars, Experimental Lines and Plant Introduction Collection of Sainfoin for Resistance to *Meloidogyne hapla* Chitwood[1]

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*Abstract:* Stands of several cultivars and experimental lines of sainfoin (*Onobrychis viciifolia*) were severely reduced (92% average loss) in a field naturally infested with *Meloidogyne hapla*. Stands of two alfalfa cultivars included in the test were unaffected. In studies conducted in the greenhouse with plants inoculated at the time of seeding, average mortality was 55% for sainfoin entries and 7% for Ladak alfalfa. Little mortality occurred when plants were inoculated after establishment. Three months after inoculation, all sainfoin entries were heavily galled (range of 3.3–3.7 on a scale of 1–4) while roots of Ladak were only slightly galled (rating of 1.6). Intermating of plants selected in the field plots for resistance to *M. hapla* showed a slight increase in resistance. Of the 147 plant introduction lines tested in the greenhouse, none were resistant to *M. hapla*.

*Key words:* *Onobrychis viciifolia* Scop., northern root-knot nematode, *Meloidogyne hapla*, sainfoin, resistance.

Sainfoin, *Onobrychis viciifolia* Scop., is a potentially valuable forage species in the United States. Sainfoin has two advantages over alfalfa: it does not cause bloating in cattle (20) and it is resistant to the alfalfa weevil, *Hypera postica* Gyll. (2). While sainfoin has a long history as a forage crop in Europe (3), its cultivation in North America has had only limited success, primarily because of rapid stand decline in the field generally attributed to disease (2,6,15,17,21,23). In addition, greenhouse studies have shown that several fungal and nematode pathogens, including *Meloidogyne hapla* Chitwood and *Ditylenchus dipsaci* (Kuhn) Filipjev, cause disease in sainfoin (7–9,11).

Three cultivars of sainfoin were highly susceptible to *M. hapla* under greenhouse conditions (9). Growing inoculated plants at 25 C resulted in the maximum root galling of sainfoin. Top weight of plants of the cultivar 'Eski' inoculated with *M. hapla* was significantly lower than noninoculated plants at all temperature regimes evaluated. Root weights of inoculated and control plants were not different at any temperature level. In Eski sainfoin, 35% of the inoculum matured as females at the most favorable temperature. At inoculum levels of 1,000 and 10,000 juveniles per seedling, mortality in Eski was 60% and 70%, respectively.

Extensive mortality was caused by *M. hapla* in a sainfoin planting in eastern Canada (21). Several lines of sainfoin were evaluated in the greenhouse, and root gall ratings and number of nematodes recovered from plants were similar among lines. Mortality differences, however, were observed among sainfoin entries (21).

The objectives of this study were to determine the reaction of several newly released cultivars and experimental lines of sainfoin to *M. hapla* under field and greenhouse conditions, the reaction of the plant introduction collection of *Onobrychis viciifolia* to *M. hapla*, and the reaction of progenies of plants selected under field conditions for resistance to *M. hapla*.

## Materials and Methods

Five sainfoin cultivars—Eski and 'Remont' (Montana Agricultural Experiment Station), 'Melrose' and 'Nova' (Canada Department of Agriculture), and 'Renumex' (New Mexico Agricultural Experiment Station)—and four experimental lines—Bozeman, Creston, and W-40 (Montana Agricultural Experiment Station) and WY-PX1-84 (Wyoming Agricultural Experi-

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ment Station)—were tested in three field and greenhouse experiments. Alfalfa cultivars used for comparative purposes were 'Ladak' (USDA and Office of Plant Introduction) and 'Perry' (USDA and Nebraska Agricultural Experiment Station).

*Field trial:* The field trial was conducted at the University of Wyoming Research and Extension Center, Torrington, Wyoming. Six sainfoin entries (Bozeman, Creston, Eski, Melrose, Remont, and W-40) and two alfalfa entries (Ladak and Perry) were established on 26 April 1981 to evaluate stand persistence and forage yield. The experimental design was a randomized complete block with four replications. Soil in the test site area, a sandy mixed mesic family of the Torriorthentic Haplustolls, was naturally infested with *M. hapla*. In the fall of 1981 ratings were not taken because stand loss was not evident. Stand depletion was evident in all sainfoin entries, but not in the alfalfa entries, in the spring of 1982; loss percentage was rated on 23 May 1982. Many of the remaining plants were severely stunted and showed severe root galling, typical of root-knot nematode infection. Plants were left in the field for an additional year, and selections for resistance to *M. hapla* were made on 15 May 1983 from the surviving plants in each of the sainfoin entries. Selection criteria were plant vigor and plant height. Plants not selected were rogued, and selected plants were allowed to cross-pollinate in 1983 in order to produce a genetically broad-based germplasm with *M. hapla* resistance. Deer, however, consumed most of the seed in 1983. The test site was fenced in the fall of 1983 and managed for seed production during 1984. Stand counts of the remaining plants were taken on 10 August 1984 prior to seed harvest. Approximately 800 g of seed collected from the remaining plants was bulked and designated WY-PX1-84.

*Greenhouse experiments:* Three experiments were conducted at the College of Agriculture Greenhouse Complex in Laramie, Wyoming. Cultures of *M. hapla* were maintained and increased in the greenhouse on 'Rutgers' tomato, *Lycopersicon esculentum*. To establish the cultures, sainfoin roots with severe galling were removed from the field test site and washed free of soil. Nematode eggs were extracted by the sodium hypochlorite method (14). Eggs were injected into the soil near the roots of 6-week-old tomato plants, and populations were allowed to reproduce for 6–12 months prior to inoculum extraction. Eggs were extracted using the previously described method modified by the use of three sieves (250-, 45-, and 25-$\mu$m-pore screens) rather than two.

The first experiment included eight sainfoin entries (Bozeman, Creston, Eski, Melrose, Nova, Remont, Renumex, and W-40) and one alfalfa entry (Ladak) arranged in a randomized complete block design. Each of the three replications consisted of two 25-cm-d clay pots filled with river bottom sand for each entry with eight plants per pot. An all-purpose fertilizer was applied weekly starting the third week after seedling emergence. The greenhouse was maintained at day and night temperatures of 25 and 22 C ($\pm$ 2 C), respectively. Supplemental fluorescent lights were used to extend the photoperiod to 16 hours. Three weeks after seeding, 600 eggs per plant (4,800 eggs per pot) (4) were injected with a syringe (no needle) into the soil around the roots, and 1,200 eggs per plant (9,600 eggs per pot) were added three weeks later to ensure adequate infection. Twelve weeks after seeding, plants were removed and roots were washed free of sand and rated for root galling on an individual plant basis using a 1–4 scale as follows: 1 = no galls, 2 = 1–10 galls, 3 = 11–100 galls and 4 = >100 galls (19). To determine the relative rate of nematode reproduction, nematode eggs were extracted from the roots of plants from each entry using the sodium hypochlorite method with one additional modification. Rather than manually shaking each root sample for 4 minutes to dissolve egg matrices, a Model 5400 Red Devil paint shaker (Western Repair, Denver, Colorado) was employed in order to standardize the vigorous shaking portion of extraction and reduce

TABLE 1. Stand loss in sainfoin caused by the northern root-knot nematode, *Meloidogyne hapla*, and survival of plants selected for resistance.

| Entry | Stand loss* (%) | Survival of plants selected for resistance to *M. hapla* | | | | Loss (%) |
|---|---|---|---|---|---|---|
| | | Selected 15 May 1983 | | Remaining 10 August 1984 | | |
| | | Total | $\bar{x}$† | Total | $\bar{x}$ | |
| Sainfoin | | | | | | |
| Bozeman | 93.2 a | 28 | 7.0 a | 17 | 4.3 e | 39.3 |
| Creston | 85.0 a | 40 | 10.0 a | 36 | 9.0 a | 10.0 |
| Eski | 93.2 a | 40 | 10.0 a | 33 | 8.3 ab | 17.5 |
| Melrose | 95.0 a | 35 | 8.8 a | 28 | 7.0 a–e | 20.0 |
| Remont | 94.5 a | 30 | 7.5 a | 30 | 7.5 a–d | 0 |
| W-40 | 88.3 a | 34 | 8.5 a | 32 | 8.0 a–c | 5.9 |
| Alfalfa | | | | | | |
| Ladak | 0.0 b | | | | | |
| Perry | 0.0 b | | | | | |
| Total | | 207 | | 176 | | 15.0 |

Values in each column followed by the same letter are not different at $P \leq 0.05$ as determined by Duncan's multiple-range test.

* Plots established 26 April 1981; values are means of four replications.

† Values are the mean number of plants selected for resistance from four replications.

sample variability. After eggs were extracted, roots were oven dried at 40 C for 24 hours and weighed.

The second experiment involved nine sainfoin entries (Bozeman, Creston, Eski, Melrose, Nova, Remont, Renumex, W-40, and WY-PX1-84) and Ladak alfalfa. Seeds were germinated and seedlings transplanted in furrows in river bottom sand in galvanized aluminum flats (38 × 54 cm). There were 10 rows (one row per entry) in each flat; rows were 32 cm long spaced 5 cm apart with 30 seedlings per row. Inoculum was prepared in the same manner as previously described and placed in the furrows (30 ml containing 2,000 eggs per seedling) at the time of transplanting. After furrows were closed, flats were watered lightly and thereafter daily until harvest. Plants were maintained as in the first experiment. The split-plot experimental design was superimposed on a randomized complete block with four replications. Live plant counts were made 2 weeks after transplanting. Shoots of live plants were harvested at 16 weeks, dried at 40 C for 24 hours, and weighed. Roots were removed, rated for galling, oven dried, and weighed.

In the third greenhouse experiment, the plant introduction collection of *Onobrychis viciifolia*, 147 lines from 18 countries, was evaluated for *M. hapla* resistance. Eski was included in the test as a check. A randomized complete block design with two replications was used in this experiment. Within a replication, seeds of each accession were planted in rows in galvanized aluminum flats containing river bottom sand. Seedlings were inoculated with 2,000 eggs per plant 5 weeks after planting. Row spacings and management procedures were the same as in the second experiment. Twelve weeks after inoculation, plants were removed and counted and roots were rated for galling using the same scale as in the other greenhouse experiments.

## RESULTS

*Field trial:* No statistical differences were detected for percentage of stand loss among sainfoin entries; however, all sainfoin lines had significantly greater loss than either Ladak or Perry alfalfa (Table 1). All sainfoin entries were extremely susceptible to *M. hapla*, with an average loss of 92% and a range of 85–95%. Both alfalfa entries appeared tolerant with no observed stand loss. Among the sainfoin plants initially selected as resistant to *M. hapla*, additional

TABLE 2. Twelve-week reaction of eight sainfoin entries and one alfalfa entry to the northern root-knot nematode, *Meloidogyne hapla*, in the greenhouse.

| Entry | Root galling* | Root weight (g dry tissue/ plant) | Nematode reproduction | |
|---|---|---|---|---|
| | | | Eggs/plant | Eggs/g dry root tissue |
| Sainfoin | | | | |
| Bozeman | 3.60 a | 0.84 b–d | 1,325 a–d | 1,570 a–e |
| Creston | 3.47 a | 0.87 bc | 1,352 ab | 1,554 a–f |
| Eski | 3.71 a | 0.60 b–e | 1,423 a | 2,372 a |
| Melrose | 3.26 a | 0.42 e | 717 e–h | 1,707 a–d |
| Nova | 3.61 a | 0.60 b–e | 1,347 a–c | 2,245 ab |
| Remont | 3.52 a | 0.58 b–e | 1,096 a–e | 1,890 a–c |
| Renumex | 3.26 a | 0.94 b | 727 e–g | 773 c–h |
| W-40 | 3.60 a | 0.75 b–e | 1,066 a–f | 1,421 a–g |
| Alfalfa | | | | |
| Ladak | 1.63 b | 1.38 a | 104 i | 75 h |

Means over three replicates. Values in each column followed by the same letter are not different at $P \leq 0.05$ as determined by Duncan's multiple-range test.

* Roots were rated for galling on a scale of 1–4: 1 = no galls, 2 = 1–10 galls, 3 = 11–100 galls, 4 = > 100 galls.

TABLE 3. Sixteen-week root gall indices and mortality of nine sainfoin entries and one alfalfa entry inoculated in the greenhouse with northern root-knot nematode, *Meloidogyne hapla*.

| Entry | Root galling* | Percent mortality |
|---|---|---|
| Sainfoin | | |
| Bozeman | 2.90 a | 57.5 a |
| Creston | 2.95 a | 64.0 a |
| Eski | 2.98 a | 40.5 a |
| Melrose | 3.04 a | 44.5 a |
| Nova | 2.97 a | 36.0 a |
| Remont | 2.95 a | 64.0 a |
| Renumez | 2.81 a | 57.5 a |
| W-40 | 2.91 a | 63.5 a |
| WY-PX1-84 | 2.86 a | 64.5 a |
| Alfalfa | | |
| Ladak | 1.96 b | 7.0 b |

Means of four replicates. Values in each column followed by the same letter are not different at $P \leq 0.05$ as determined by Duncan's multiple-range test.

* Roots were rated for galling on a scale of 1–4: 1 = no galls, 2 = 1–10 galls, 3 = 11–100 galls, 4 = > 100 galls.

losses occurred between 15 May 1983 and 10 August 1984 within all entries except Remont. Bozeman had the greatest loss (39%) during this period. Of the 207 plants selected in May 1983, only 176 remained the following year (15% loss).

*Greenhouse experiments:* Although root gall ratings of sainfoin entries varied from 3.26 in Melrose and Renumex to 3.71 in Eski, differences were not significant in the first experiment (Table 2). However, all sainfoin entries had significantly more galls than Ladak (1.63).

Root weights were significantly different among sainfoin lines, ranging from 0.42 g per plant for Melrose to 0.94 g per plant for Renumex. Melrose root weight was statistically lower than Bozeman, Creston, and Renumex. The number of eggs per plant for the sainfoin entries ranged from 717 for Melrose to 1,423 for Eski. Melrose and Renumex had significantly fewer nematode eggs per plant than Bozeman, Creston, Eski, and Nova. The number of eggs per gram of dry root tissue ranged from 773 in Renumex to 2,372 in Eski. Eski had the highest number of eggs per plant and eggs per gram of dry root tissue. Ladak had significantly fewer eggs per plant (104) than any sainfoin entry and fewer eggs per gram of root tissue (75) than all except Renumex. Correlation coefficients for root gall rating and root weight, eggs per plant, and eggs per gram of dry root tissue were 0.79, 0.90, and 0.84, respectively.

In the second experiment, no statistical differences were detected among sainfoin entries for either percentage of mortality or root galling (Table 3). Ladak alfalfa had significantly lower root galling (1.96) and less mortality (7%) than any of the sainfoin entries.

Shoot weights varied from 45.8 mg per plant for Creston to 97.7 mg per plant for WY-PX1-84 (Table 4). Remont and WY-PX1-84 had significantly higher shoot weights than Creston in the inoculated group. In the noninoculated control, Nova had significantly higher shoot weight (187.3 mg) than all other sainfoin entries except Renumex. Six of the nine entries had significant decreases in shoot weight caused by *M. hapla*, when compared with their respective control, but Bozeman, Remont, and WY-PX1-84 did not.

Root weights among sainfoin entries in the noninoculated control were not significantly different, but there were differences in the inoculated group (Table 4).

TABLE 4. Sixteen-week means for dry shoot weight, dry root weight, and shoot : root ratio for nine sainfoin entries in the greenhouse.

| Entry | Shoot weight (mg/plant) | | Root weight (mg/plant) | | Shoot : root ratio | |
|---|---|---|---|---|---|---|
| | Inoculated | Noninoculated | Inoculated | Noninoculated | Inoculated | Noninoculated |
| Bozeman | 77.8 a–c A | 113.8 b A | 61.2 a–c A | 55.0 a A | 1.3 ab A | 2.1 ab A |
| Creston | 45.8 c A | 101.1 b B | 52.5 a–c A | 58.7 a A | 1.0 b A | 1.7 b A |
| Eski | 66.6 a–c A | 113.7 b B | 59.9 a–c A | 60.3 a A | 1.2 b A | 2.3 ab B |
| Melrose | 78.0 a–c A | 126.2 b B | 69.5 a–c A | 60.0 a A | 1.3 ab A | 2.2 ab A |
| Nova | 72.9 a–c A | 187.3 a B | 87.0 a A | 73.8 a A | 0.9 b A | 2.8 a B |
| Remont | 85.6 ab A | 114.4 b A | 79.7 ab A | 67.4 a A | 1.1 b A | 1.8 b A |
| Renumex | 71.0 a–c A | 143.3 ab B | 42.1 c A | 67.4 a A | 2.1 a A | 2.3 ab A |
| W-40 | 56.1 bc A | 106.5 b B | 67.2 a–c A | 55.7 a A | 0.9 b A | 1.9 ab B |
| WY-PX1-84 | 97.7 a A | 119.8 b A | 61.0 a–c A | 49.3 a A | 1.7 ab A | 2.6 ab A |

Means of four replications. Values in each column followed by the same lower-case letter are not different at $P \leq 0.05$ as determined by Duncan's multiple-range test. Values in each row within headings (shoot weight, root weight, and shoot : root ratio) followed by the same capital letter are not different at $P \leq 0.05$ as determined by Duncan's multiple-range test.

Nova had significantly higher root weight (87.0) than did Renumex (42.1). There were no statistical differences for root weight between the inoculated and noninoculated treatments within an entry.

Significant differences were detected for shoot : root ratios among entries within both the inoculated and noninoculated treatments (Table 4). Shoot : root ratio was decreased in all entries due to the presence of *M. hapla.* Eski, Nova, and W-40 had significant decreases in shoot : root ratios of inoculated plants compared with their respective noninoculated control.

Root gall ratings for the plant introduction accessions ranged from 2.75 to 3.72 (Table 5). Six entries had one or no plants and are not included in the results. Seventeen entries had root gall means that were numerically less than 3.0; however, the means for six of these entries were based on 10 or fewer plants. The cultivar Eski had a 3.09 gall rating based on 39 plants. None of the plant introductions were significantly better ($P \leq 0.05 = 0.47$) than Eski for root gall rating.

## DISCUSSION

The field study demonstrated the extreme susceptibility of sainfoin to *M. hapla.* Although isolations were not made from plants randomly removed from plots, symptoms of other diseases were not present, suggesting *M. hapla* was the primary pathogen associated with the stand failure. Although Ladak is reported to be susceptible to *M. hapla* (5), no visual damage to plants, such as stunting, or stands was observed, indicating a greater tolerance level than sainfoin. Perry appeared equally tolerant. The possibility of an interaction between *M. hapla* and low temperature stress in sainfoin is suggested by losses to stands only after plots had gone through the winter. Both the lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven (18) and the stem nematode, *D. dipsaci* (1,13) have been reported to predispose alfalfa to winterkill. Such an interaction may exist between *M. hapla* and sainfoin and could be an important factor in stand depletion in areas of sainfoin production where *M. hapla* occurs.

The uniform susceptibility of all sainfoin lines in the first greenhouse experiment is in agreement with earlier reports (9,21). The experimental lines Bozeman, Creston, and W-40 were all derived from Eski which had been exposed to natural selection over 8–10 years at three different locations in Montana (R. L. Ditterline, pers. comm.). Apparently there was no change in *M. hapla* susceptibility since all three lines had approximately the same root galling as Eski (Table 2). There was, however, a reduction in nematode reproduction (eggs per gram of dry root tissue) in the three lines as compared to Eski. Renumex had the best over-

TABLE 5. Root gall indices of the plant introduction lines of *Onobrychis viciifolia* inoculated in the greenhouse with northern root-knot nematode, *Meloidogyne hapla*.

| Plant introduction no. | Origin | Total number of plants rated* | Root galling† |
|---|---|---|---|
| 110397 | Soviet Union | 32 | 3.27 |
| 110400 | Turkey | 10 | 3.20 |
| 110404 | Soviety Union | 28 | 3.00 |
| 167236 | Turkey | 10 | 3.25 |
| 170582 | Turkey | 13 | 2.99 |
| 170583 | Turkey | 31 | 3.00 |
| 170585 | Turkey | 7 | 3.10 |
| 171725 | Turkey | 13 | 3.25 |
| 171726 | Turkey | 21 | 3.32 |
| 178988 | Turkey | 6 | 3.63 |
| 182247 | Turkey | 7 | 2.75 |
| 186520 | Spain | 11 | 3.38 |
| 192993 | Spain | 20 | 3.07 |
| 192994 | Spain | 17 | 3.00 |
| 192995 | Spain | 5 | 3.16 |
| 200872 | Turkey | 12 | 3.00 |
| 204595 | Turkey | 6 | 3.00 |
| 205201 | Turkey | 13 | 3.24 |
| 205202 | Turkey | 3 | 3.25 |
| 206458 | Turkey | 5 | 3.50 |
| 206459 | Turkey | 6 | 3.50 |
| 206577 | Greece | 34 | 3.15 |
| 212241 | Washington, U.S.A. | 10 | 3.10 |
| 223389 | Iran | 13 | 3.10 |
| 225728 | Turkey | 2 | 3.00 |
| 227038 | Iran | 27 | 3.33 |
| 228156 | Soviet Union | 4 | 3.00 |
| 228289 | Iran | 4 | 3.00 |
| 227373 | Iran | 38 | 3.04 |
| 228156 | Soviet Union | 3 | 3.00 |
| 228289 | Iran | 3 | 3.50 |
| 228352 | Iran | 7 | 3.50 |
| 228402 | Iran | 8 | 3.00 |
| 229612 | Iran | 8 | 2.87 |
| 229613 | Iran | 39 | 3.05 |
| 234644 | Spain | 4 | 3.25 |
| 234822 | Switzerland | 13 | 3.30 |
| 234823 | Switzerland | 10 | 3.10 |
| 236486 | Turkey | 4 | 3.50 |
| 237089 | Turkey | 8 | 3.13 |
| 239957 | Iran | 9 | 3.30 |
| 239958 | Iran | 11 | 3.72 |
| 239959 | Iran | 5 | 3.50 |
| 239960 | Iran | 9 | 3.33 |
| 243227 | Iran | 17 | 3.08 |
| 250024 | Iran | 2 | 3.50 |
| 251160 | Yugoslavia | 11 | 3.00 |
| 251669 | Yugoslavia | 3 | 3.00 |
| 251840 | Italy | 6 | 3.13 |
| 258767 | Soviet Union | 8 | 3.15 |
| 258768 | Soviet Union | 17 | 3.25 |
| 258769 | Soviet Union | 12 | 3.09 |
| 258770 | Soviet Union | 22 | 3.09 |
| 258771 | Soviet Union | 7 | 3.00 |

TABLE 5. Continued.

| Plant introduction no. | Origin | Total number of plants rated* | Root galling† |
|---|---|---|---|
| 258772 | Soviet Union | 14 | 2.93 |
| 258773 | Soviet Union | 15 | 3.11 |
| 258774 | Soviet Union | 20 | 3.08 |
| 258775 | Soviet Union | 13 | 3.07 |
| 258776 | Soviet Union | 12 | 3.25 |
| 258777 | Soviet Union | 13 | 3.09 |
| 258778 | Soviet Union | 6 | 3.00 |
| 259491 | United Kingdom | 20 | 3.13 |
| 259492 | United Kingdom | 32 | 3.10 |
| 259493 | United Kingdom | 6 | 3.00 |
| 259494 | United Kingdom | 9 | 3.00 |
| 263158 | Soviet Union | 18 | 3.00 |
| 263159 | Soviet Union | 15 | 3.00 |
| 273784 | Soviet Union | 12 | 3.38 |
| 273785 | Soviet Union | 24 | 3.22 |
| 273786 | Soviet Union | 17 | 3.32 |
| 273787 | Soviet Union | 13 | 2.99 |
| 273788 | Soviet Union | 8 | 3.10 |
| 273789 | Soviet Union | 26 | 3.51 |
| 273790 | Soviet Union | 6 | 2.80 |
| 273791 | Soviet Union | 18 | 3.17 |
| 302936 | Spain | 6 | 3.00 |
| 302938 | Spain | 4 | 2.75 |
| 302939 | Spain | 19 | 3.25 |
| 306693 | Italy | 13 | 3.70 |
| 311467 | Spain | 17 | 3.00 |
| 311468 | Spain | 21 | 3.11 |
| 311469 | Spain | 13 | 2.75 |
| 311470 | Spain | 2 | 3.00 |
| 311471 | Spain | 17 | 3.04 |
| 313046 | Spain | 29 | 3.35 |
| 313047 | Germany | 30 | 3.59 |
| 313048 | Soviet Union | 27 | 3.30 |
| 313049 | Poland | 24 | 3.38 |
| 313050 | Soviet Union | 34 | 3.03 |
| 313051 | Switzerland | 33 | 3.25 |
| 313052 | Soviet Union | 41 | 3.00 |
| 313053 | Soviet Union | 18 | 3.11 |
| 313054 | Soviet Union | 24 | 3.26 |
| 313055 | Soviet Union | 27 | 3.35 |
| 313056 | Norway | 28 | 3.22 |
| 313057 | Soviet Union | 25 | 3.00 |
| 313058 | Soviet Union | 33 | 3.32 |
| 313059 | Germany | 27 | 3.11 |
| 313060 | Soviet Union | 30 | 3.00 |
| 313061 | Soviet Union | 22 | 3.00 |
| 313062 | Soviet Union | 34 | 3.15 |
| 313063 | Soviet Union | 29 | 3.26 |
| 313064 | Italy | 25 | 3.09 |
| 313065 | Soviet Union | 32 | 3.25 |
| 313066 | Bulgaria | 21 | 3.14 |
| 314099 | Soviet Union | 5 | 2.84 |
| 316296 | Soviet Union | 38 | 3.29 |
| 318602 | Switzerland | 7 | 2.88 |
| 318604 | Switzerland | 25 | 3.06 |
| 318605 | Switzerland | 33 | 3.19 |
| 318606 | Switzerland | 23 | 3.19 |

(Continued on next page)

TABLE 5. Continued.

| Plant introduction no. | Origin | Total number of plants rated* | Root galling† |
|---|---|---|---|
| 319059 | Spain | 4 | 3.00 |
| 319061 | Spain | 2 | 3.00 |
| 319062 | Spain | 9 | 3.20 |
| 319713 | Romania | 7 | 3.45 |
| 338651 | Morocco | 34 | 3.05 |
| 368034 | Turkey | 18 | 3.10 |
| 368035 | Turkey | 34 | 2.96 |
| 368036 | Turkey | 25 | 3.08 |
| 372828 | Czechoslovakia | 26 | 3.04 |
| 372829 | Czechoslovakia | 25 | 3.25 |
| 372830 | Czechoslovakia | 35 | 3.23 |
| 372831 | Czechoslovakia | 22 | 3.19 |
| 372832 | Czechoslovakia | 34 | 3.15 |
| 372833 | Czechoslovakia | 34 | 3.27 |
| 372835 | Czechoslovakia | 22 | 3.09 |
| 380948 | Iran | 43 | 2.95 |
| 380949 | Iran | 46 | 3.05 |
| 383713 | Turkey | 44 | 3.00 |
| 383714 | Turkey | 14 | 3.17 |
| 383715 | Turkey | 24 | 3.29 |
| 383716 | Turkey | 31 | 3.06 |
| 383717 | Turkey | 46 | 2.98 |
| 400305 | Rhodesia | 33 | 2.92 |
| 400306 | Rhodesia | 32 | 3.00 |
| 401419 | Unknown | 21 | 3.03 |
| 401467 | Romania | 26 | 2.92 |
| 401468 | Romania | 25 | 2.97 |
| 401715 | Soviet Union | 39 | 3.17 |
| 440575 | Soviet Union | 42 | 2.97 |
| 440576 | Soviet Union | 24 | 3.00 |

* Total number of plants in two replications.

† Average root gall rating for two replications on a scale of 1–4: 1 = 0, 2 = 1–10, 3 = 11–100, 4 = > 100.

all resistance based on nematode reproduction (eggs per gram of dry root tissue). Renumex was derived from intermating individuals selected from Eski, Remont, and a composite germplasm of the world collection of sainfoin (16). The slightly enhanced resistance of Renumex may trace to the world collection, since both Eski and Remont displayed lower levels of resistance.

The second greenhouse experiment differed from the first greenhouse experiment in the following two ways: plants were grown in flats rather than pots, and plants were inoculated with nematode eggs at the time of planting rather than 3 and 6 weeks after planting. Previous findings (10) have shown root galling is normally more severe when plants are inoculated at the time of planting than when plants are inoculated after planting. This occurred with Ladak alfalfa where root gall ratings were 1.96 and 1.63 when inoculated at planting and after planting, respectively. However, sainfoin root gall ratings were higher when plants were inoculated after planting and grown in a less confined system. The difference is probably due to the high mortality of sainfoin when inoculated at planting. The high level of mortality in all sainfoin lines resulted in the loss of plants that would have increased the root gall rating averages had they remained alive.

The mortality percentage of sainfoin was lower in the greenhouse than in the field. This possibly can be explained by two facts: yield trials were longer in duration, and greenhouse tests were not exposed to the extremely low winter temperatures that occurred with the field trial. Nonetheless both studies demonstrated the highly susceptible nature of sainfoin to *M. hapla*. Alfalfa suffered very little mortality in the field or greenhouse experiments compared with sainfoin, which supports previous work (22). Griffin (9), however, reported that sainfoin and alfalfa show a similar response to *M. hapla*. Differences in the environmental conditions, inoculum density, and (or) genetic background of the alfalfa and sainfoin entries used may explain the different results.

Shoot weights of inoculated plants were significantly less than the noninoculated controls for six of the nine sainfoin entries. The largest decline was in Nova which is reported to have greater first growth yield than Melrose, Eski, and Remont (12). In the control group, Nova yielded significantly more than all entries except Renumex.

The experimental line (WY-PX1-84), derived from the intermating of selected survivors of the field trial, had the highest shoot weight of all sainfoin entries in the presence of *M. hapla* (Table 4). This, in addition to a lack of significant difference in shoot weight between inoculated and noninoculated control, may indicate a slight

increase in resistance to *M. hapla* was obtained in the field selection.

The plant introduction accessions were not significantly more resistant than the cultivar Eski. Eski's root gall rating mean of 3.09 was considerably below that obtained in the first greenhouse experiment (3.71), probably because of differences in inoculum between the two studies. The 11 plant introduction lines with root gall averages of less than 3.0, based on more than 10 plants, originated from Iran 1, Soviet Union 3, Spain 1, Turkey 3, Rhodesia 1, and Romania 2. None of these appear to be better sources of resistance to *M. hapla* than currently available cultivars.

In conclusion, *M. hapla* was shown to be a devastating pathogen of sainfoin in the field which may help explain the severe stand decline of sainfoin in the United States. All commercially available cultivars and experimental lines were susceptible except Renumex, which showed a low level of resistance. No source of resistance was identified in the plant introduction collection of *O. viciifolia.* Undoubtedly, considerable effort will be required to develop cultivars with resistance to *M. hapla.* If sainfoin is to reach its potential as a forage crop in the United States and Canada, continued effort must be made to identify damaging pathogens and to develop cultivars with multiple pest resistance, as has been done for alfalfa.

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