Factors Affecting the Infection of Alfalfa Seedlings by Ditylenchus dipsaci[1]

J. H. ELGIN, JR., D. W. EVANS, and L. R. FAULKNER[2]

Abstract: Experiments were conducted to determine the effects of plant confinement, soil type, watering practices, stage of seed germination, inoculum level, and method of applying inoculum on stem nematode (*Ditylenchus dipsaci*) infection of alfalfa (*Medicago sativa*) seedlings grown in soil. Results indicated that (i) confining seedlings together with the nematodes in small vials offered no advantage over growing plants in large flats, (ii) a very fine sandy-loam soil was superior to a fine sand for stem-nematode penetration, (iii) nematodes penetrated seedlings more readily if the soil was not watered immediately after planting and inoculation, (iv) germinating seeds with a radicle length of 0.6-1.3 cm had the highest nematode penetration, and (v) highest penetration occurred when the nematodes were placed directly upon germinating seeds. The optimum inoculum level was 50 nematodes per seedling. *Key words:* nematodes, inoculation.

Many scientists (1-6, 8-13) have developed methods to evaluate resistance and susceptibility of alfalfa, *Medicago sativa* L., to the stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, and they have described various factors which influenced the success of their methods. In particular, Bingefors (2) and Bingefors and Eriksson (4), working with both alfalfa and red clover, *Trifolium pratense* L., and Grundbacher (9) and Hanna and Hawn (10), working with alfalfa, have investigated factors affecting nematode infection of seedlings germinated in filter paper rolls and inoculated after emergence of the cotyledons.

In developing methods for evaluating stem-nematode resistance of alfalfa seedlings growing in a soil medium (6), we were confronted with variation among tests and were prompted to investigate several factors affecting nematode infection of the seedlings. Initially, we were interested in the effects of close physical confinement of the nematodes with the seedlings, and the need for isolating one seedling from another for screening and evaluation. Secondly, because Wallace (14, 15) had found that stem nematodes move quickly through soils of coarse particle sizes (100-1,300 μm) and less rapidly through those of smaller particle sizes (< 100 μm), and stem-nematode infested crop lands around Prosser, Washington, are typically fine or very fine sandy loams (average particle size < 100 μm), we needed to determine the influence of fine sand, which we had used in earlier studies, vs. a very fine sandy-loam field soil on the penetration of *D. dipsaci* into alfalfa seedlings. Thirdly, we needed to evaluate the effects of watering practices after inoculation and the stage of seed germination at time of inoculation on nematode infection. And, finally, because there were always escapes, we wanted to determine the level of inoculum needed for all plants to become infected, and the most effective method of applying inoculum. Results of studies to provide answers to these questions are reported herein.

MATERIALS AND METHODS

Nematodes for all studies were reared monoxenically in alfalfa tissue culture (7).

Plastic vials vs. a metal flat were used to determine effect of confining nematodes to the immediate vicinity of the plant on nematode infection. The vials (1.3 × 6.5 cm) were filled with steam-pasteurized fine sand, moistened, and arranged in eight groups of 25 each. One germinating seed of moderately susceptible alfalfa cultivar DuPuits was placed in each vial; 1 ml of water suspension containing about 25 stem nematodes was applied to each seed; and the seeds were covered with fine sand. A metal flat (38 × 54 × 7.5 cm) was also filled with steam-pasteurized sand, planted (eight rows 7 cm apart with 25 seeds each at 1-cm intervals), inoculated, and covered in a similar fashion. The vials and flat were placed in a growth chamber at 22 C with 16-hour photoperiod at 23,672 lux (2, 200 ft-

Received for publication 9 October 1974.

[1]Cooperative investigations of the Agricultural Research Service, U.S. Department of Agriculture, and the Washington State University College of Agriculture Research Center, Prosser. Scientific Paper No. 4226, and Washington State University Project No. 1917.

[2]Research Agronomist, Field Crops Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705 (formerly, Irrigated Agriculture Research and Extension Center, Prosser, Wash.); Associate Agronomist, and former Nematologist (now Head, Department of Plant Pathology, Kansas State University, Manhattan), Washington State University, IAREC, Prosser, WA 99350, respectively. The authors acknowledge the contributions of Fred A. Gray and Donald B. Bower for culturing the nematodes used in these studies.

c). Twenty-four days after inoculation, the seedlings were removed, stained with acid fuchsin in lactophenol (9), and the numbers of nematodes in the seedlings were determined with the aid of a stereomicroscope.

To elucidate the effect of soil types on infection, three metal flats (replications) were filled with steam-pasteurized fine sand (particle size, 100-250 μm) and three with a very fine sandy loam (average particle size $<$ 100 μm). Twenty-five germinating DuPuits seeds were planted at 1-cm intervals in a single row in each flat, inoculated with 25 nematodes, covered with soil, and placed in the growth chamber as described above. After 3 days, seedlings were removed from the soil, the seedlings were stained, and nematode counts were made.

To determine the effects of watering practices on nematode penetration, 24 10-cm diam pots filled with steam-pasteurized very fine sandy-loam soil were watered uniformly to moisten the soil and placed in a growth chamber as described above. Additional water was added to 12 of the pots until the soil was thoroughly saturated. Ten germinating seeds of DuPuits were planted in each of the 24 pots, inoculated with 25 nematodes, and covered with soil. Following seeding and inoculation, four pots (replicates) containing moistened soil and four containing saturated soil were watered with 0.6 cm of water over the soil surface; four pots each were watered lightly (sprinkled); and four each received no water. No pots were watered further. After 5 days, the numbers of nematodes penetrating the seedlings were determined.

To determine the effects of stage of seed germination on nematode penetration, susceptible DuPuits and resistant Lahontan were tested at five stages of seed germination: (i) dry seeds (no germination), (ii) water-imbibed seeds (before radicle protrusion), (iii) seeds with slight protrusion of the radicle, (iv) seeds with radicle length 0.6 cm, and (v) seeds with radicle length 1.3 cm. Two metal flats (replicates) were filled with steam-pasteurized very fine sandy-loam soil. Ten seeds of each cultivar and germination stage were planted at 2.5-cm intervals in rows spaced 5 cm apart, inoculated, and covered with soil. The flats were placed in the growth chamber and were watered lightly 3 days later. Seven days after planting, the numbers of nematodes in the seedlings were determined.

Twenty-eight treatments were used to determine the level of inoculum needed for all plants to become infected, and the most effective method of applying inoculum, a single or split application. Treatments consisted of combinations of four inoculum levels (25, 50, 100, or 200 stem nematodes per seedling) and seven methods of application: (i) all nematodes were applied directly to the seeds; (ii) all nematodes were applied to the soil surface after seeds were covered with 1.0 cm of soil; (iii) all nematodes were applied to the soil surface as the seedlings were emerging; (iv) half of the nematodes were applied to the seeds, and half were applied after the seeds were covered with soil; (v) half of the nematodes were applied to the seeds, and half were applied as the seedlings emerged; (vi) half of the nematodes were applied after the seeds were covered with soil, and half were applied as the seedlings emerged; and (vii) one-third of the nematodes were applied to the seeds, one-third after the seeds were

TABLE 1. Influence of inoculation method and inoculum levels on numbers of *Ditylenchus dipsaci* in alfalfa seedlings 7 days after inoculation.

Method of application	Numbers of nematodes penetrating[a] with inoculum levels (no. nematodes per seedling) of:				Mean
	25	50	100	200	
All on seeds	10.4 a	23.0 a	35.5 a	64.7 a	33.4 a
All after planting	6.0 a	10.6 b	25.8 ab	41.5 cd	21.0 c
All as emerging	2.2 a	8.5 b	18.9 b	34.8 de	16.1 d
1/2 on seeds, 1/2 after planting	9.4 a	15.7 ab	28.7 ab	58.5 ab	28.1 b
1/2 on seeds, 1/2 as emerging	7.8 a	14.5 ab	27.9 ab	46.7 c	24.2 bc
1/2 after planting, 1/2 as emerging	2.9 a	10.4 b	19.2 b	27.0 e	14.9 d
1/3 on seeds, 1/3 after planting, 1/3 as emerging	5.3 a	17.8 ab	34.4 a	51.1 bc	27.2 b
Mean	6.3 d	14.4 c	27.2 b	46.3 a	

[a]Means within a column (except the general means for inoculum levels below each column) having letters in common do not differ, $P = 0.05$. The general means are compared within their row.

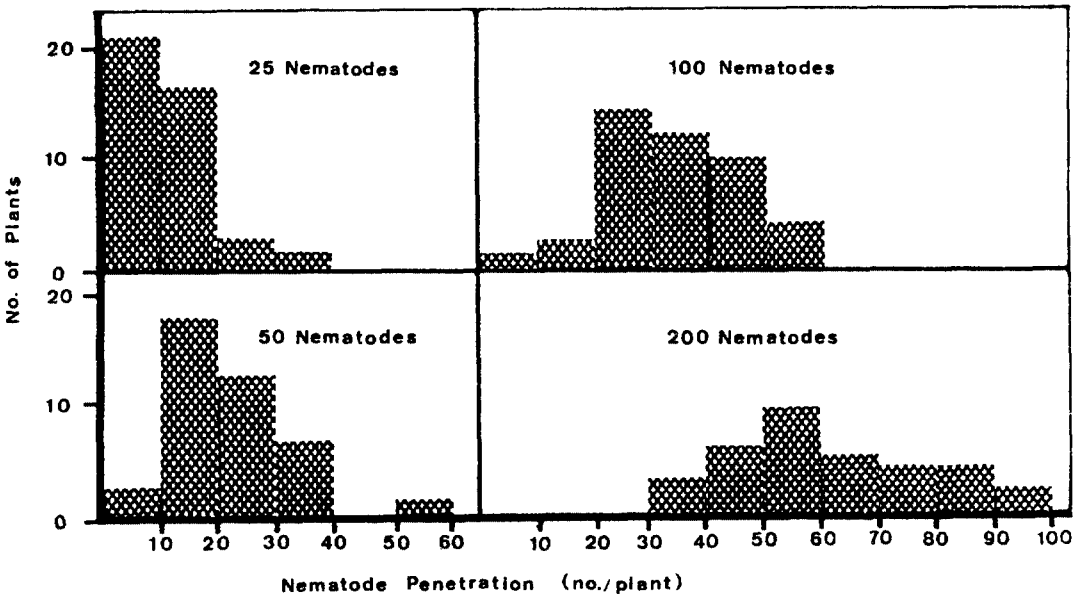

FIG. 1. Frequency of nematode penetration in soil-grown alfalfa seedlings 7 days after inoculation of germinating seeds (radicle length, 0.6 cm) with 25, 50, 100, and 200 *Ditylenchus dipsaci*. Inoculum was placed directly on the seeds at planting just before they were covered with soil.

covered with soil, and one-third as the seedlings emerged. Each of 112 10-cm diam pots filled with steam-pasteurized very fine sandy-loam soil was seeded with 10 germinating S_1 seeds (radicle length 0.6 cm) of a highly susceptible clone selected from DuPuits alfalfa. Pots were distributed into four equal groups (replicates), and a single treatment was applied to each pot. The experiment was conducted in a greenhouse at about 23 C with a 14-hour photoperiod. No water was applied until 2 days after planting, and then only as needed. Most seedlings had emerged after 3 days. Seven days after planting, all seedlings were removed and the numbers of nematodes within the tissues determined.

RESULTS AND DISCUSSION

Greater numbers of nematodes were found in seedlings grown in flats (42.4 nematodes/seedling) than in vials (21.6 nematodes/seedling). Evaluating alfalfa seedlings for stem-nematode infection was easier and faster when they were grown in flats than in vials. Confining the nematodes to the plants in small vials offered no apparent advantage over the grouping of plants grown in flats.

More stem nematodes penetrated seedlings grown in very fine sandy-loam soil (5.6 nematodes/seedling) than those grown in fine sand (0.5 nematode/seedling). Based on Wallace's (14, 15) observations, these nematodes are less mobile in media of small than of coarse particle size. With less mobility in the very fine sandy-loam soil than in the fine sand, more nematodes would remain near and penetrate developing seedlings. Nematodes may have been moved from the area of the seedlings in the fine sand by water percolation.

No significant difference in numbers of nematodes which penetrated seedlings was found between the soils moistened and saturated before seeding and inoculation (6.2 vs. 6.4 nematodes/plant). However, significant effects were observed for watering practices applied after seeding and inoculation (3.1, 6.2, and 9.6 nematodes/plant for 0.6 cm of water, sprinkled, and no additional water, respectively). Apparently, watering after seeding should be avoided because it moves the nematodes away from the plants before they penetrate.

Significant differences were found for nematode penetration among stages of seed germination. Seeds with radicle length of 0.6

(3.4 nematodes/plant) and 1.3 cm (3.1 nematodes/plant) had more nematode penetration than dry (0.5 nematode/plant) or imbibed (1.5 nematodes/plant) seeds. Those with slight radicle protrusion were intermediate (2.4 nematodes/plant). No difference in penetration was found between DuPuits and Lahontan, and no cultivar × stage of germination interaction was indicated. Under conditions of our test, germinating seeds with radicle lengths of approximately 0.6-1.3 cm were most suitable for nematode penetration.

Inoculum levels and methods of application also influenced penetration by *D. dipsaci* (Table 1). Generally, placing all of the inoculum directly on the germinating seeds was the most efficient method of application. Placing one-half or one-third of the inoculum directly on the germinating seeds was second, applying the nematodes after covering with soil was third, and inoculating as the seedlings were emerging was fourth in efficiency. Significant inoculum levels × methods of application interactions were obtained.

Ideally, the optimum inoculum level should result in penetration of at least 10 nematodes into most plants, which would provide for fairly rapid population increase in the plants through reproduction, while avoiding severe initial damage from penetration by large numbers of nematodes. Figure 1 reveals the effects of increasing inoculum levels on the frequencies of plants with various levels of infection when all nematodes were placed on the germinating seeds. At the 25-nematode inoculum level, 21 of 40 plants contained fewer than 10 nematodes. When the 50- or 100-nematode inoculum levels were used, only 2 and 1 plants, respectively, had fewer than 10 nematodes. There were no plants with fewer than 10 nematodes at the 200-nematode level, but seedlings were damaged severely. Based on the number of plants with 10-20 nematodes, applying 50 nematodes directly to the germinating seed is most efficient.

The data show that even when nematodes were applied directly to germinating seeds, only about 40% of the nematodes were recovered in young seedlings. Similar rates of recovery have been obtained in our other studies. Remaining nematodes apparently were lost into surrounding soil.

LITERATURE CITED

1. BARKER, K. R., and J. N. SASSER. 1959. Biology and control of the stem nematode, Ditylenchus dipsaci. Phytopathology 49:664-670.
2. BINGEFORS, S. 1957. Studies on breeding red clover for resistance to stem nematodes. Växtodling 8:1-123.
3. BINGEFORS, S. 1961. Stem nematode in lucerne in Sweden. II. Resistance in lucerne against stem nematode. K. Lantbrukshögsk. Ann. 27:385-398.
4. BINGEFORS, S., and K. B. ERIKSSON. 1968. Some problems connected with resistance breeding against stem nematodes in Sweden. Z. Pflanzenzücht. 59:359-375.
5. DIJKSTRA, J. 1956. Experiences with the breeding of red clover resistant to the stem eelworm. Euphytica 5:298-307.
6. ELGIN, J. H., JR., D. W. EVANS, and L. R. FAULKNER. 1975. Evaluation of alfalfa for stem nematode resistance. Crop Sci. 15:275-276.
7. FAULKNER, L. R., D. B. BOWER, D. W. EVANS, and J. H. ELGIN, JR. 1974. Mass culturing of Ditylenchus dipsaci to yield large quantities of inoculum. J. Nematol. 6:126-129.
8. GRIFFIN, G. D. 1967. Evaluation of several techniques for screening alfalfa for resistance to Ditylenchus dipsaci. Plant Dis. Rep. 51:651-654.
9. GRUNDBACHER, F. J. 1962. Testing alfalfa seedlings for resistance to the stem nematode Ditylenchus dipsaci (Kühn) Filipjev. Proc. Helminthol. Soc. Wash. 29:152-158.
10. HANNA, M. R., and E. J. HAWN. 1965. Seedling inoculation studies with the alfalfa stem nematode. Can. J. Plant Sci. 45:357-363.
11. SHERWOOD, R. T., J. W. DUDLEY, T. H. BUSBICE, and C. H. HANSON. 1967. Breeding alfalfa for resistance to the stem nematode Ditylenchus dipsaci. Crop Sci. 7:382-384.
12. SMITH, O. F. 1958. Reactions of some alfalfa varieties to the stem nematode. Phytopathology 48:107.
13. WYNNE, J. C., and T. H. BUSBICE. 1968. Effects of temperature and incubation period on the expression of resistance to stem nematode in alfalfa. Crop Sci. 8:179-183.
14. WALLACE, H. R. 1961. The orientation of Ditylenchus dipsaci to physical stimuli. Nematologica 6:222-236.
15. WALLACE, H. R. 1962. Observations on the behavior of Ditylenchus dipsaci in soil. Nematologica 7:91-101.