Influence of *Meloidogyne hapla* on Alfalfa Yield and Host Population Dynamics

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Abstract: Self-thinning in alfalfa, a dynamic process involving the progressive elimination of the weakest plants, was enhanced by *Meloidogyne hapla*. Alfalfa stand densities decreased exponentially with time and were reduced 62% ($P = 0.05$) in the presence of *M. hapla*. As stand densities decreased over time, mean plant weights increased at a rate 2.52 times faster in the absence of *M. hapla*. In a stepwise multiple regression analysis, 65% of the total variation in yield could be explained by changes in stand density and 85% by average weight of individual stems. Alfalfa yields were suppressed ($P = 0.05$) by *M. hapla*, with suppression generally increasing with time and as the nematode population density increased. Yield suppression was attributable primarily to the decline in plant numbers and to suppression in individual plant weights.

Key words: self-thinning, plant competition, plant survival, yield determinant, root-knot nematode, *Medicago sativa*, growth suppression.

The environment is important in determining the abundance, size, and distribution of plants in a population (6). Phytoparasitic nematodes in the soil alter the environment in which plants grow and interact with other plants. Stresses induced by nematode parasitism may directly and indirectly influence plant yield and survival by damaging the roots and reducing plant size and vigor, thus placing parasitized plants at a competitive disadvantage. Self-thinning of plant populations (6,20), a dynamic process involving the progressive elimination of the weakest individuals, may be enhanced by nematode stress. The sequence of competitive events ·over time that determines if a seedling will develop to a mature plant is often overlooked when plant productivity is assessed.

Many studies (1,8,11,12) have examined the relationship between final yield and preplant population (Pi) of phytoparasitic nematodes. These studies, however, have not addressed the influence of nematodes on host population dynamics and yield phenology of perennial crops. In alfalfa, yield is determined by both plant population density and by the average plant size. The total growth an individual plant ultimately achieves is determined by the time and level of initial nematode infection and by the cumulative effect of stresses imposed on the plant. Crop loss is defined, using a reference nematode-free control, as the difference in yield of a crop grown in the presence and absence of phytoparasitic nematodes.

This study examines i) the long-term effects of three moisture regimes and seven initial population densities of *Meloidogyne hapla* (Chitwood) on alfalfa yield and stand densities and 2) the influence of *M. hapla* on alfalfa yield components. Yield is expressed as the product of average stem number per plant, average stem weight per plant, and stand density.

METHODS AND MATERIALS

A long-term experiment was conducted in 106 microplots (0.89 m²) at the University of California Citrus Experiment Station, Riverside. The microplots were concrete drainage tiles (1.2 m long × 1.1 m i.d.) buried on end to 1.0 m deep and filled with 7.2 m³ loamy sand (87% sand, 2% clay, 11% silt). Each microplot was fumigated with 454 g methyl bromide on 12 December 1982. Before the introduction of *M. hapla*, an inoculation template containing 60 equally spaced 20-cm nails was forced into the soil, carefully withdrawn, rotated 5 degrees and inserted again to create 120 inoculation holes.

M. hapla populations, originally obtained from a San Bernardino County alfalfa field, were a single egg mass cultured on tomato (*Lycopersicon esculentum* Mill. cv. Tropic). Nematode eggs and juveniles for inoculum were extracted from roots (9). A series of initial population densities (0, 4, 43, 217, 434, 1,085, 2,170/1,000 cm³ soil) were established by introducing *M. hapla* inoculum into the microplots on 6 January 1983. The appropriate number of eggs and

juveniles suspended in 3.8 liters of water was uniformly sprinkled into each microplot. Immediately following inoculation, certified alfalfa seed (*Medicago sativa* L. cv. Cuf 101), 2.11 g/microplot, and its symbiont *Rhizobium meliloti* (Dangeard), 0.1 g/microplot, were thoroughly mixed and uniformly seeded into each microplot with a salt shaker. After planting, each microplot was covered with an additional 0.6 cm of steam-sterilized loamy sand. Microplots were individually sprinkler irrigated by 360° spot-spitter emitters.

Three water regimes, high (15 cb), moderate (35 cb), and low (65 cb) were superimposed on the seven Pi levels of *M. hapla*. The experimental site consisted of eight rows each containing 18 tiles. The 106 microplots available for use in this study were divided 35, 36, and 35 among the high, moderate, and low moisture regimes, respectively. Two full rows of 18 tiles each were randomly assigned the moderate moisture regime and the remaining six rows randomly assigned either high or low. Each tile within a moisture regime was then randomly assigned a *M. hapla* Pi level. Tensiometers were installed 45 cm deep in the soil in each of three microplots within each row of tiles and monitored at 2–3-day intervals. Irrigations were initiated following the first harvest when average tensiometer readings reached -0.15 for the high, -0.35 for the moderate, and -0.65 b for the low moisture regime. Water was individually metered to each row of microplots through individual water lines. The total water applied to each row during an irrigation was recorded.

Plants in the microplots were first harvested on 19 May 1983, 134 days after planting, and subsequently harvested on days 170, 196, 229, 258, 308, 381, 422, 466, 477, and 496 after planting. The population dynamics of *M. hapla* and the growth of the alfalfa plant were expressed on a physiological time scale, degree days (DD), by calculating the number of degrees above the developmental threshold temperature of 10 C (16). The number of degree hours contributed above the threshold temperature for each day were added, divided by 24, and then summed across time to obtain a degree-day estimate for each harvest following planting. Soil temperatures always exceeded 10 C, so degree-day conversions using other basal thresh-

old temperatures (14,19) can be obtained easily. Plants were cut 5 cm above the soil level at harvest. Two-centimeter bud regrowth and 10% plant flowering criteria were used to dictate harvest cycles (18).

Before each harvest, five random stand height measurements, taken as the distance from soil surface to longest stem with extended leaflet, were obtained from the plants in each microplot. At alternate harvests, the number of stems more than 5 cm long per plant, measured from the soil surface, were counted for five randomly selected plants in each microplot. At each harvest, in addition to total plant weight per microplot, five randomly chosen stems were collected, their lengths measured, and both leaf and stem fresh and dry weights determined. The number of plants was monitored nondestructively by tossing a 23-cm-d ring randomly into each microplot and counting plants within the ring. Average plant densities were based on the mean number of plants in four tosses of the rings, representing an assessment of 25% of the surface area of each microplot.

The experiment was terminated between 7 and 25 May 1984 in a time series fashion, allowing determination of alfalfa regrowth within a cutting cycle. Each microplot within the moderate and high moisture regime was flooded and all plants carefully removed. Tap root diameters were measured with a micrometer 1 cm below the last regrowth bud for each plant. Plants were separated into five tap root diameter categories—0.0–0.25, 0.26–0.50, 0.51–0.75, 0.76–1.0, > 1.0 cm. Plants in each category were counted, and foliage, crown, and tap root fresh weights were recorded. From each category, a single randomly selected representative plant was chosen for further analysis. From the randomly selected plants, stem numbers and tap root lengths were also recorded. The last harvest of plants in microplots within the moderate moisture regime (35 cb) was 7 May 1984, 12 days after the previous harvest. In microplots in the high moisture regime (15 cb), the last harvest was 29 days after the previous harvest on 25 May 1984.

An analysis of variance using a two factorial, completely randomized experimental design with unbalanced replications was initially conducted as a means separation test to determine differences among factor and treatment level means (13). Simple lin-
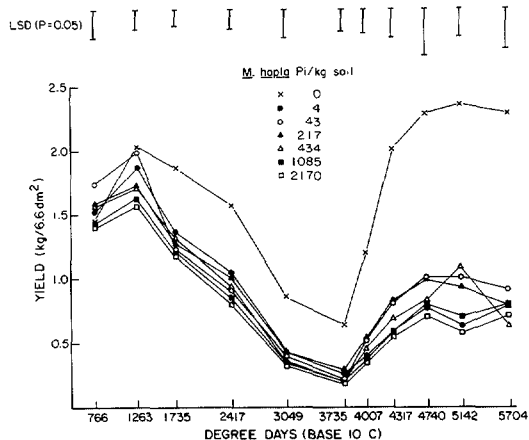
FIG. 1. Influence of initial inoculum densities of *Meloidogyne hapla* on consecutive harvest yields of alfalfa (cv. Cuf 101).
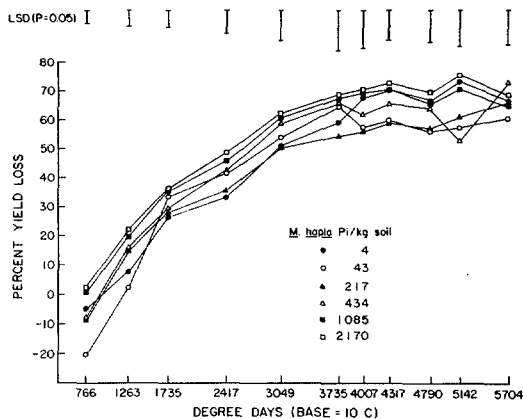

FIG. 2. Influence of initial inoculum densities of *Meloidogyne hapla* on yield reduction compared to nematode-free controls over consecutive harvest of alfalfa (cv. Cuf 101).

ear regression analysis was used for continuous variables to quantify the relationship between various plant parameters and the influence of *M. hapla* Pi. Multiple regression analysis was used to study the contribution of various yield-determining components to individual harvest yields. A linear regression analysis of self-thinning over time was performed to examine the relationship between stand density and mean plant weight, both on logarithmic scales (20). As crown diameters and subterranean crown branching increased, the ability to differentiate individual plants in a clump using a nondestructive sample decreased. Alfalfa stand density estimates derived from average ring tosses were significantly biased in the nematode-free microplots. Plant numbers were underestimated by 44%, compared with complete enumeration at the end of the experiment. The inability to assess plant densities accurately was assumed to increase log-linearly as plant size increased over time. Adjusted stand density (ASD) was derived from the correction equation: $ASD = 1.0 + (-0.1602 + 0.000128 * DD) * \text{stand density}$.

RESULTS

There were no significant ($P = 0.05$) differences among moisture regimes for any plant population or growth parameter; therefore the influence of soil moisture was disregarded in all further analysis. Following an initial stimulation (DD = 766), yields were reduced ($P = 0.05$) at the two highest

Pi levels and at each subsequent harvest by *M. hapla* (Fig. 1). Although no significant ($P = 0.05$) differences in plant yields related to Pi were observed, alfalfa yield reductions at each harvest generally increased to maximum levels of 65% with time (Fig. 2).

Alfalfa stand densities decreased exponentially with time, and were reduced by 62% ($P = 0.05$) in the presence of *M. hapla* (Fig. 3). Irrespective of Pi, stand decline rates did not differ, averaging 2.21 plants/DD, during the first 170 days after planting. After 1,263 DD, stand decline rates, like yield losses, were greatest with *M. hapla* occurring progressively at or near a constant rate of 0.1 plant/DD.

As stand density decreased over time, mean plant weights increased (Fig. 4). Inverse changes in stand density and mean plant weight formed a strong negative log-linear relationship over time, with significantly differing slopes of -1.25 for plants in control and -0.49 for plants in *M. hapla*-infested microplots. The difference in slopes of the two regression lines indicates that in the presence of *M. hapla* the rate of growth increase of individual plants is significantly reduced compared to nematode-free plants.

The final plant-size frequency distribution (DD = 4,790) indicated a reduction ($P = 0.05$) in numbers of plants with tap root diameters less than 1.0 cm in *M. hapla*-infested microplots (Fig. 5). When plant frequencies were expressed as a proportion of the total plant population, the dis-
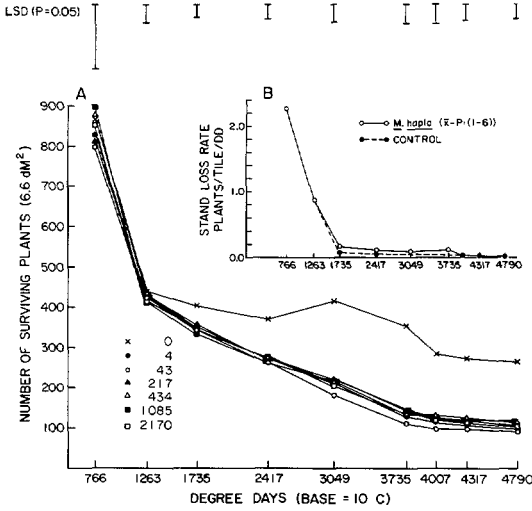
FIG. 3. *Meloidogyne hapla* inoculum density and plant survival. A) Influence of seven initial inoculum densities of *M. hapla* on alfalfa plant survival over time. B) Average rate of plant population decline per degree day for nematode-free and *Meloidogyne hapla*-infested microplots.

FIG. 5. Frequency distribution of average alfalfa plant sizes in five root diameter categories for nematode-free and *Meloidogyne hapla*-infested microplots.

tribution of plants was skewed toward plants with crowns exceeding 0.50 cm as stands are thinned by *M. hapla* (Fig. 6). Total foliage weights of plants within each crown size category increased with time (Fig. 7). The contribution that plants in each crown size category made to total yield increased with crown size in plants in the *M. hapla*-infested microplots (Fig. 8), in contrast to the more normal distribution of plant sizes in the control microplots. In the controls, average plant weights and plant densities were greater in the intermediate size categories (0.26–1.0 cm) and contributed over 78% of the total plant.

As crown size increased, the average number of harvestable stems (> 5 cm long) per plant increased (Fig. 9). In relation to *M. hapla* Pi, there were no differences ($P = 0.05$) in stem numbers of plants in any crown size category when compared to the nematode-free plants. Average weight of individual harvestable stems was reduced ($P = 0.05$) an average 38% in the presence of *M. hapla* for plants with crowns > 0.25 cm (Fig. 10). The influence of *M. hapla* is
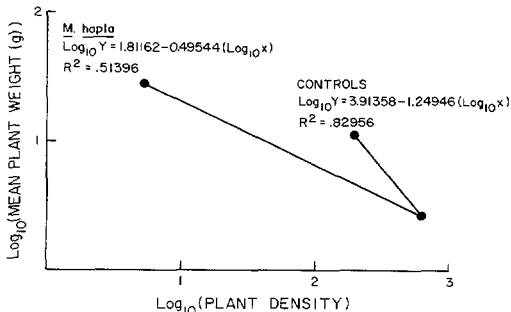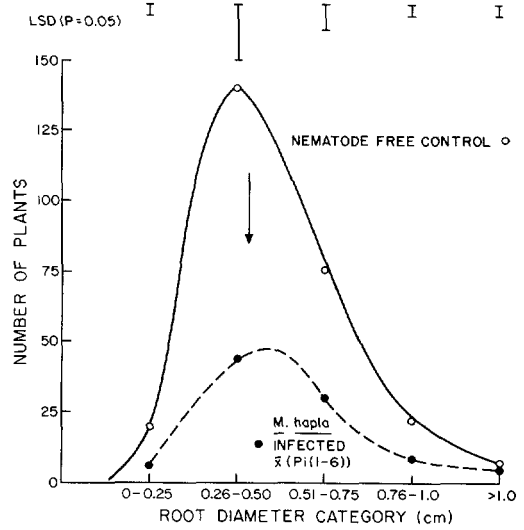

FIG. 4. Relationship between the average number of surviving alfalfa plants and mean plant weight over time for nematode-free and *Meloidogyne hapla*-infested microplots.
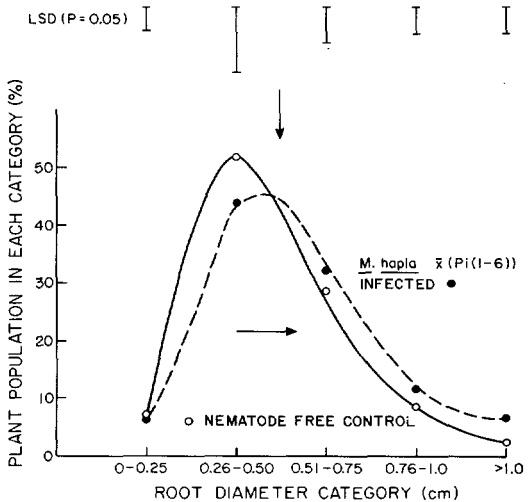

FIG. 6. Proportion of total plant population density at the final harvest (DD = 5,704) in five root diameter categories for nematode-free and *Meloidogyne hapla*-infested microplots.
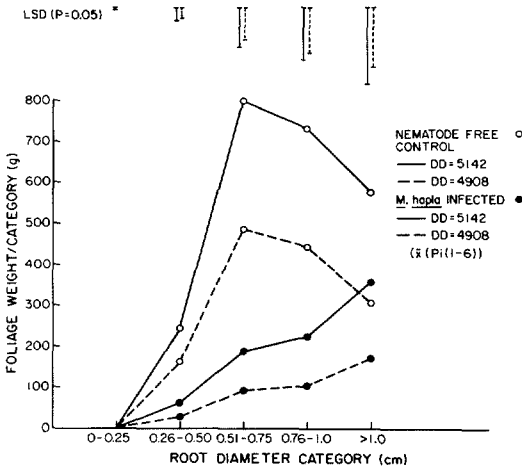
FIG. 7. Relationship between average foliage weight distribution for five root diameter categories during a single alfalfa regrowth cycle for nematode-free and *Meloidogyne hapla*-infested microplots.

also reflected in a reduction in mean weights of plants with crowns > 0.50 cm (Fig. 11). In a multiple regression analysis, 65% of the total variation in yield could be explained by changes in stand density and 85% by the average weight of individual stems. Differences in the number of stems per plant contributed less than 3% to variations in yield.

DISCUSSION

M. hapla is generally considered a minor pest of alfalfa in the cool temperate regions of its primary distribution (3,5,8). The results of numerous studies evaluating the damage relationship have been variable, suggesting that diverse geographical, edaphic, and physiological differences in plant and nematode populations are im-
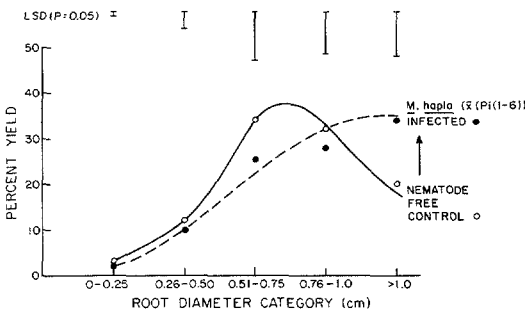

FIG. 8. Percent of total yield at the final harvest (DD = 5,704) in five root diameter categories for nematode-free and *Meloidogyne hapla*-infested microplots.
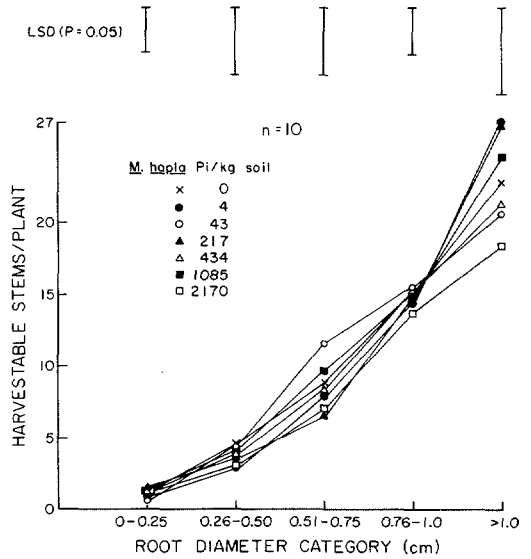

FIG. 9. Influence of initial inoculum densities of *Meloidogyne hapla* on the average number of harvestable stems (> 2.0 cm) per plant at final harvest (DD = 5,704) in five root diameter categories.

portant in determining pathogenicity (5,8). When yield reductions occurred, they were generally associated with reductions in plant numbers during early stand establishment (8,10,15), increased plant over-
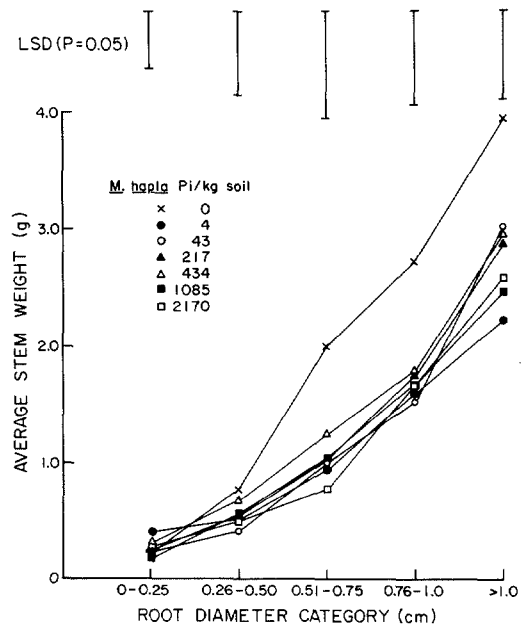

FIG. 10. Influence of initial inoculum densities of *Meloidogyne hapla* on average stem weight per plant at final harvest (DD = 5,704) in five root diameter categories.
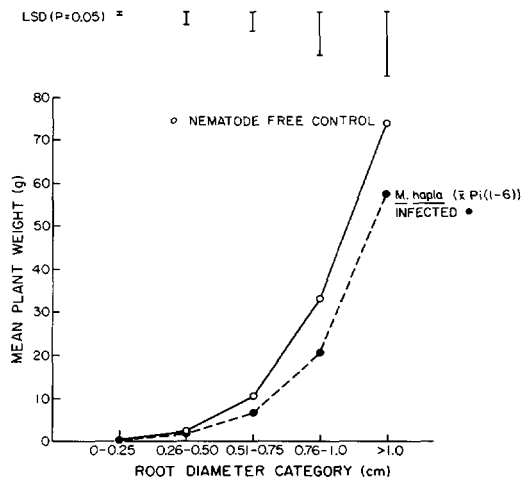
FIG. 11. Mean alfalfa plant weight at final harvest (DD = 5,704) in five root diameter categories for nematode-free and *Meloidogyne hapla*-infested microplots.

wintering mortality (10), or interaction with other pathogens (5,7,10). Griffin (4,5) and Inserra (8) have suggested that, at least in the cooler regions of the northwest United States, suppression of growth of resistant cultivars is of limited duration in the field because of the inverse relationship between increasing plant age and decreased root galling and population development of *M. hapla*.

In our study, *M. hapla* played a major role in reducing alfalfa yields and stand densities over time. The insignificant differences in alfalfa growth and yield in relation to different moisture regimes may be related to the drought tolerance and deep rooting habit (> 1.0 m) of plants within the microplots, even though moisture fluctuations were noted at 45 cm deep. Seasonal alfalfa yield reductions during the summer and fall of 1983 may be partially attributable to the rise and fall of atmospheric pollution concentrations. Data obtained from the California South Coast Air Quality Management District showed that air pollutants frequently exceeded levels injurious to alfalfa (17). Even during these periods of high pollutant concentrations, *M. hapla* had a negative impact on alfalfa yield. Alfalfa yield reductions by *M. hapla* increased in magnitude during each subsequent harvest to an asymptote level of about 65% of the yield in microplots where *M. hapla* was absent.

Stand decline rates, like yield losses, were greatest in the *M. hapla*-infested microplots, decreasing exponentially with time to similar low levels. Final stand density reductions of 62% in the presence of *M. hapla* were not affected by increasing Pi, as observed by Townshend and Potter (15). Plants died continuously over time with higher death rates in microplots with *M. hapla*. Plant survival seems to be determined primarily during early growth and stand establishment, with increased stand losses from *M. hapla* occurring progressively at or near a constant rate of 0.1 plant/DD. Spatial and temporal changes in alfalfa stand densities affect the degree of mutual interference between individual plants. At high plant densities, competition constrains individual plants from exhibiting their maximum growth potential. Plant competition effects are described in the 3/2 thinning law (20) which suggests that rates of increase in mean plant weight as plant densities decrease over time occurs at a constant rate of -1.5 when expressed on a double log scale (i.e., $W = Cp - 1.5$, where W = log mean weight, p = log plant density, C = constant).

Larger, faster growing, more vigorous plants produce a greater density stress and a greater rate of increase in mean plant weights when stands are thinned. This is in contrast to the stunted, slower growing *M. hapla*-infected plants, which had reduced rates of growth and correspondingly of competitive stresses. As stand densities decreased in nematode-free microplots, mean plant weights increased 2.52 times the rate of plants grown in the presence of *M. hapla*. The increased growth of individual plants more than compensated for the effect of decline in plant density. In contrast, plants in microplots with *M. hapla* did not exhibit compensatory increased growth rates.

Description of yield as a multiplicative product of stem number, average stem weight, and plant density was poor ($r^2 = 0.4289$) due to difficulty in assessment of these components by nondestructive sampling. When yield-determining components can be measured accurately, as in the complete enumeration study, 65% of the variation in yield can be explained by changes in the number of plants and 85% by the average weight of individual stems

per plant. Differences in the number of stems per plant contributed an average of only 3% to total variation explained in the yield model. Differences in stem weights were due primarily to a significant ($P = 0.05$) reduction in stem lengths.

As the smaller and weaker individuals were eliminated in *M. hapla* microplots, the frequency distribution of alfalfa plant sizes shifted, resulting in an increase in average plant size in relation to the controls. Even though average plant size was greater, average plant weights for each *M. hapla* root size category were lower. Vegetative regrowth following each harvest was delayed and ultimately reduced by *M. hapla*. The largest plant-size categories (> 0.75 cm) represented 61% of total yield, compared with the controls in which 79% of yield was explained by the smaller and intermediately sized categories (0.25–1.0 cm).

Plant stunting also has an indirect effect on thinning of stands. When stunted plants are harvested over numerous cutting cycles, root reserves are depleted, stands decline, yields decrease, and productive longevity of the crop is shortened. In addition, the ability of the plant to compete for resources is reduced, accelerating stand decline rates in the presence of *M. hapla*.

This study showed that alfalfa yield losses from *M. hapla* are primarily attributable to reductions in plant densities and individual plant growth rates. With apparently wide thermal tolerances, soil populations of *M. hapla* at or near nondetectable Pi levels ($4/1,000$ cm^3) are capable of inflicting serious losses to alfalfa, at least in southern California.

Population densities of *M. hapla* eggs, juveniles, and root parasitic stages, which were assessed at each harvest, increased over time to equilibrium levels of 20,000 eggs and juveniles and 250 adult females per gram of root. The influence of plant damage and a reduction in plant densities on *M. hapla* population development is addressed in a subsequent paper.

LITERATURE CITED

1. Ferris, H., W. D. Turner, and L. W. Duncan. 1981. An algorithm for fitting Seinhorst curves to the relationship between plant growth and preplant nematode densities. Journal of Nematology 13:300–304.

2. Frakes, R. V., R. L. Davis, and F. L. Patterson. 1961. The breeding behavior of yield and related variables in alfalfa. II. Associations between characters. Crop Science 1:207–209.

3. Griffin, G. D. 1969. Effects of temperature on *Meloidogyne hapla* in alfalfa. Phytopathology 59:599–602.

4. Griffin, G. D., and O. J. Hunt. 1972. Effect of plant age on resistance of alfalfa to *Meloidogyne hapla*. Journal of Nematology 4:87–90.

5. Griffin, G. D. 1980. Interrelationship of *Meloidogyne hapla* and *Ditylenchus dipsaci* on resistant and susceptible alfalfa. Journal of Nematology 12:287–293.

6. Harper, J. L. 1977. Population biology of plants. New York: Academic Press.

7. Hunt, P. J., G. D. Griffin, J. J. Murray, M. W. Pedersen, and R. N. Peaden. 1971. The effects of root knot nematodes on bacterial wilt in alfalfa. Phytopathology 61:256–259.

8. Inserra, R. N., J. H. O'Bannon, M. DiVito, and H. Ferris. 1983. Response of two alfalfa cultivars to *Meloidogyne hapla*. Journal of Nematology 15:644–646.

9. McClure, M. A., T. H. Kruk, and I. Misaghi. 1973. A method for obtaining quantities of clean *Meloidogyne* eggs. Journal of Nematology 5:230.

10. Norton, D. C. 1969. *Meloidogyne hapla* as a factor in alfalfa decline in Iowa. Phytopathology 59:1824–1828.

11. Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Mededelingen Lanbouwhogeschool, Wageningen 66:1–46.

12. Seinhorst, J. W. 1965. The relation between nematode density and damage to plants. Nematologica 11:137–154.

13. Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. Ames: Iowa State University Press.

14. Starr, J. L., and W. F. Mai. 1976. Predicting onset of egg production by *Meloidogyne hapla* on lettuce from field soil temperatures. Journal of Nematology 8:87–88.

15. Townshend, J. L., and J. W. Potter. 1978. Yield losses among forage legumes infected with *Meloidogyne hapla*. Canadian Journal of Plant Disease 58:939–943.

16. Tyler, J. 1933. Development of the root-knot nematode as affected by temperature. Hilgardia 7:391–415.

17. University of California Statewide IPM Project. Integrated Pest Management for Alfalfa Hay. 1981. Publication 4104. UC Agricultural Sciences Publications, Richmond, California.

18. United States Environmental Protection Agency. 1976. Diagnosing vegetation injury caused by air pollution. Contract 68-02-1344. Applied Science Associates, Inc. Air Pollution Institute, Research Triangle Park, North Carolina.

19. Vrain, T. C., and K. R. Barker. 1978. Influence of low temperature on development of *Meloidogyne incognita* and *M. hapla* eggs in egg masses. Journal of Nematology 10:311–313.

20. Yoda, K., T. Kira, H. Ogawa, and K. Hozumi. 1963. Self-thinning in overcrowded pure stands under uncultivated and natural conditions. Journal of Biology 14:107–129.

21. Zadoks, J. C., and R. D. Schein. 1979. Epidemiology and plant disease management. New York: Oxford University Press.