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Influence of Alfalfa Plant Growth on the Multiplication Rates and Ceiling Population Density of *Meloidogyne hapla*

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Abstract: The rates of reproduction and multiplication of *Meloidogyne hapla* decreased as a result of self-regulatory, density-dependent processes with time and nematode population increase in the soil and roots of *Medicago sativa* cv. Cuf 101. Juvenile, egg, and mature female population densities increased at a maximum rate until damage to the host resulted in alfalfa yield reductions. Temporal differences in multiplication and reproduction rates of *M. hapla* were observed to be a function of initial population density (Pi), host damage, and root biomass, indicating increased levels of competition for a constant but limited number of feeding sites. Over time, a log linear relationship emerged between multiplication rate of *M. hapla* and Pi. Slopes of −0.90953 for combined eggs and juveniles and −0.71349 for mature females indicated a gradual approach to ceiling densities. Reproductive rates decreased exponentially from an initial maximal value of 200 to a relatively constant rate of 53 eggs per female.

Key words: density dependence, intraspecific competition, reproduction, population dynamics, root-knot nematode, *Medicago sativa*.

Seasonal variations in populations of plant and soil nematodes have been correlated with many density-independent climatic, edaphic, and agronomic factors (1,13,14,17,18,24,26,29,32,36). Other studies of nematode population ecology show that initial population density (Pi) influences both the growth and development of the plant, and, reciprocally through the damage inflicted to the plant, the size and nutritive quality of the root system available as food to the nematode (2,14, 19,24,25,27,31,32). Changes induced in plant roots by nematodes can influence the rate of multiplication or reproduction and constrain further population growth through intraspecific competition for limited food resources (1,20,33). Many studies involving both monospecific and multispecific nematode communities suggest the existence of self-regulatory, density-dependent, negative feedback processes. Competitive ability is explained as a function of Pi and virulence of a given nematode species in relation to a host plant (11). The suitability of a host is defined by two parameters: a, the maximum rate of population increase, and E, the equilibrium density, an asymptote value where final population density (Pf) = Pi (19). Equilibrium density when equated with time and food supply is closely analogous to the concept of carrying capacity or ceiling density. This similarity among terms occurs when total root space available for colonization and capable of providing sustenance to the nematode population is occupied and remains unchanged over time (15,19,32).

If density-dependent factors limit population growth, there should be a relationship between the rate of change of the population and population size. In annual crops, with relatively short season growth cycles, logistic type depressions in nematode multiplication rates are observed (15,19,31–34). The objective of multiplication rate analysis is to explain and reliably predict final population size even though the dynamic interactions between nematode and host plant have been essentially ignored. In contrast to annual crops where nematodes build to high levels and populations decline because of plant senescence and a collapse of food supplies, a continuous, ongoing host–parasite interaction can be expected to evolve through continuous or periodic cycles of renewed root and vegetative growth in perennial crops. The objective of this study was to examine reproduction and population development of *Meloidogyne hapla* through time in relation to growth and yield response of a perennial alfalfa crop.

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Materials and Methods

The location, time, and method of planting, harvesting, data collection, *M. hapla* inoculation techniques, and degree day (DD) calculations employed in this study were described previously (22). Alfalfa (*Medicago sativa* cv. Cuf 101) was planted in 106 microplots (0.89 m^2), and a series of initial *M. hapla* juvenile population densities (0, 4, 43, 217, 434, 1,085, 2,170/1,000 cm^3 soil) was introduced into the appropriate microplots on 6 January 1983. The microplots were harvested on 11 different occasions between 19 May 1983 and 7 July 1984. Following each alfalfa harvest, density determinations for the various *M. hapla* life stages were obtained by removing a single random soil and root sample from each microplot. Each soil sample consisted of a composite of five random soil cores (2.54 cm d) removed to 45 cm deep. Each sample was placed in a plastic bag and transported to the laboratory in an insulated chest for processing. Holes in plots were filled with steam pasteurized loamy sand (87.8% sand, 1.5% silt, 10.7% clay). Each sample was weighed (mean = 972 g) and the entire sample processed for nematode recovery.

Juveniles of *M. hapla* (J2) from soil were extracted using a modified semiautomatic elutriator and sugar flotation technique (6). Alfalfa roots recovered on a 425-μm-pore sieve from the soil during elutriation were blotted dry and weighed. The total root biomass recovered was separated into two equal weight portions. One root portion was stained (4), mounted in glycerin between line etched glass plates, and examined under a dissecting microscope. *M. hapla* developmental stages were identified (7), counted, and adjusted to reflect population densities per gram of root. *M. hapla* eggs were extracted (5) from the other root portion and egg population densities expressed in relation to root weight. *M. hapla* eggs and soil J2 were counted with the aid of a dissecting microscope in rectangular counting dishes. Resulting numbers of J2 and eggs were adjusted to reflect a 20% extraction efficiency and a 4.6% egg aliquant (20 ml) subsample, respectively.

M. hapla egg and J2 numbers were first analyzed using a two factorial, completely randomized design with unbalanced replications as a mean separation test for factor and treatment effects (35). *M. hapla* population growth was examined by plotting individual and combined densities of the various life stages against physiological time, degree days.

Microplots were sampled at seven harvests to assess soil and root nematode population densities. Pi for each *M. hapla* life stage was considered as the population density present at the beginning of the regrowth cycle (previous harvest sample), whereas Pf was that occurring at the following harvest. The Pf value from one harvest cycle was therefore the Pi value for the subsequent cycle. Observations in which Pi = 0 were eliminated from the data set in calculation of multiplication rates (Pf/Pi) (16). Average reproduction rates were calculated by dividing Pf values of the sum of eggs and soil J2 by Pf values for mature females. Population estimates of the root colonizing stages of *M. hapla* were variable because of the high numbers and differential staining of eggs, juveniles, and females. Female packing and the close proximity of smaller, less well-stained juvenile stages, made accurate assessment of the age and population density of nematodes in roots difficult. *M. hapla* reproduction rates were therefore calculated by averaging the Pf values for the sum of eggs and juveniles divided by Pf values for mature females that fell within a mature female density class (0–50, 51–100, . . . , 701–750/g root). Density-dependent effects were examined by plotting the relationship between the rates of population change (Pf/Pi) against both time and Pi (19).

Results

Root weight did not differ ($P = 0.05$) among *M. hapla* Pi over time (Fig. 1). Root biomass was initially greater in the presence than absence of *M. hapla* but decreased rapidly following an exponential decline in plant population densities during the early stand establishment phase and summer yield decline. Comparisons of *M. hapla* Pi over a year indicate that the numbers of eggs and juveniles per gram of roots were strongly related to root biomass and to the extent of host damage. Juvenile population levels (Fig. 2A) increased until damage to the host in the form of signifi-

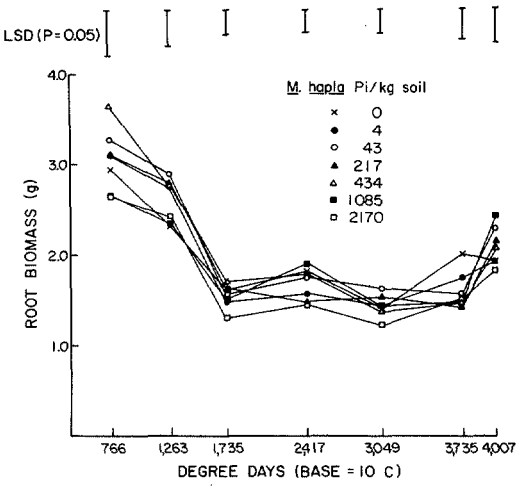

FIG. 1. Influence of *Meloidogyne hapla* on mean fresh root weight of alfalfa (cv. Cuf 101) recovered from five core soil samples (718 cm³) over time.

cant ($P = 0.05$) yield reductions was apparent on DD = 1,735. After DD 1,735, juvenile populations stabilized at a ceiling density until the final sample (DD = 4,007), when populations increased following an increase in alfalfa root and vegetative growth. Egg population densities were similarly influenced by root biomass and host damage and were related to *M. hapla* Pi (Fig. 2B). Egg populations generally increased with inoculum density at DD = 766 reaching ceiling densities, like juveniles, at DD = 1,735.

Over time, combined egg and juvenile population densities per gram of root increased rapidly and quickly converged to ceiling densities at DD = 1,735 (Fig. 2C). There was a direct relationship between initial rates of increase in numbers of eggs and juveniles and Pi at DD = 1,263 (Fig. 3). As *M. hapla* eggs and juvenile population densities increased, rates of population increase decreased from a maximum (a = 363.6) as they converged toward their ceiling density (Fig. 3). Temporal differences in the rates of increase were a function of Pi, host damage, and root biomass. The greatest rates of population increase initially occurred at the three lowest Pi, with the rate decreasing as Pi increased. After DD = 1,263, irrespective of Pi, rates of population increase were similar and generally fluctuated around a maintenance value of 1 (inset, Fig. 3).

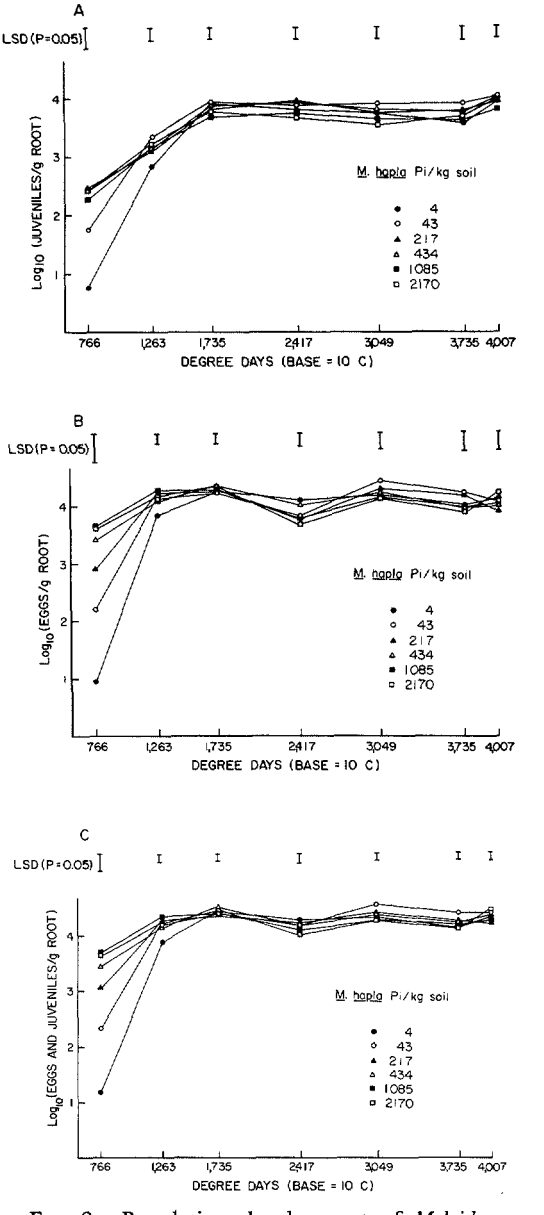

FIG. 2. Population development of *Meloidogyne hapla* juveniles (A), eggs (B), and combined eggs and juveniles (C) per gram of alfalfa roots (cv. Cuf 101) for each of six initial inoculum densities (4, 43, 217, 434, 1,085, 2,170/1,000 cm³ soil).

Mature females, like eggs and juveniles, increased with time until DD = 1,735, when ceiling population densities of 252/g root were attained (Fig. 4). An apparent temperature-dependent increase in root biomass at DD = 4,007 coincided with a decline in *M. hapla* female population densities. At DD = 766, numerous mature

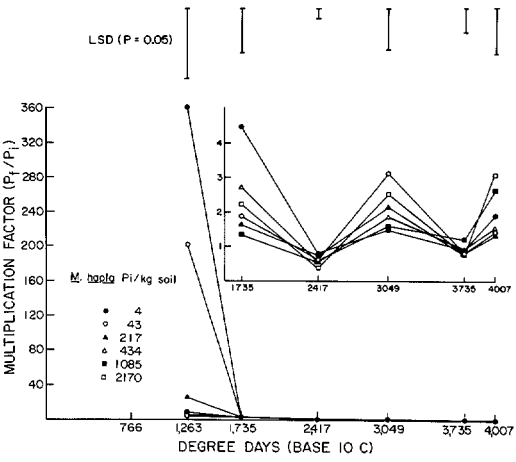

FIG. 3. Influence of initial inoculum density on the rate of population change (Pf/Pi) of *Meloidogyne hapla* eggs and juveniles over time. Inset: Exposed view DD = 1,735 to 4,007.

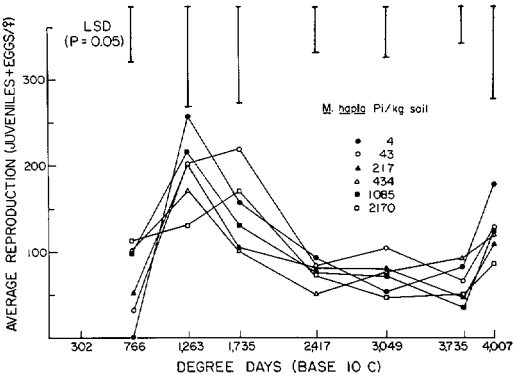

FIG. 5. Average number of eggs and juveniles per mature female of *Meloidogyne hapla* over time in roots of alfalfa (cv. Cuf 101) for each of six initial inoculum densities (4, 43, 217, 434, 1,085, 2,170/1,000 cm³ soil).

females were present, with many having only recently initiated egg production, as indicated by submaximal average reproduction rates (Fig. 5). Average reproduction rates decreased with time as female population densities increased and yield and root biomass decreased. As root biomass increased and population densities of mature females per gram of root decreased at DD = 4,007, average reproduction rates increased (Fig. 5). As mature female population densities increased, combined densities of eggs and juveniles increased rapidly (Fig. 6). Further increases in the number of females produced no additional changes in egg or juvenile population densities, indicating a density-dependent reduction in reproduction rate per female (inset, Fig. 6). Reproduction rates per female decreased exponentially from an initial maximal value of 200 to a relatively constant rate of 53 eggs per female.

The rates of change in *M. hapla* populations as they converged on their ceiling density were investigated by logarithmically plotting both Pf and Pf/Pi against Pi (20) (Fig. 7). As food supply (root biomass) was expanding, nematode multiplication rates were related to Pi and were limited only by apparent food constraints at the highest Pi. Over time, a log linear relationship formed between the rates of population increase of eggs and juveniles and

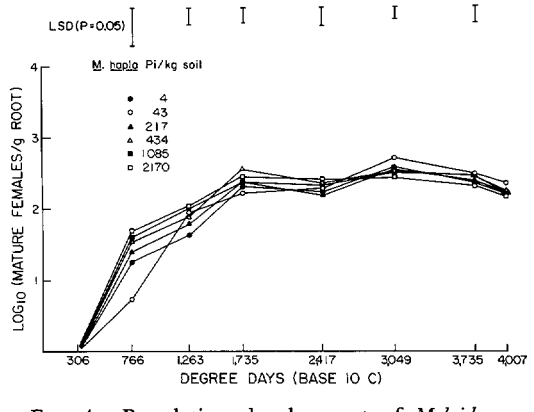

FIG. 4. Population development of *Meloidogyne hapla* mature females per gram of alfalfa root (cv. Cuf 101) for each of six initial inoculum densities (4, 43, 217, 434, 1,085, 2,170/1,000 cm³ soil).

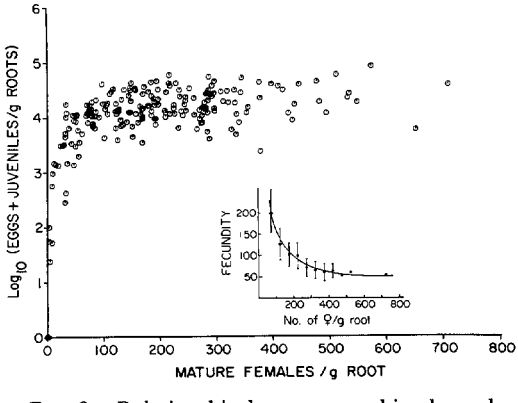

FIG. 6. Relationship between combined number of eggs and juveniles and the numbers of *Meloidogyne hapla* females per gram of alfalfa roots (cv. Cuf 101). Inset: Influence of population density of *M. hapla* mature females on average fecundity (eggs + juveniles/♀) in alfalfa roots (cv. Cuf 101).

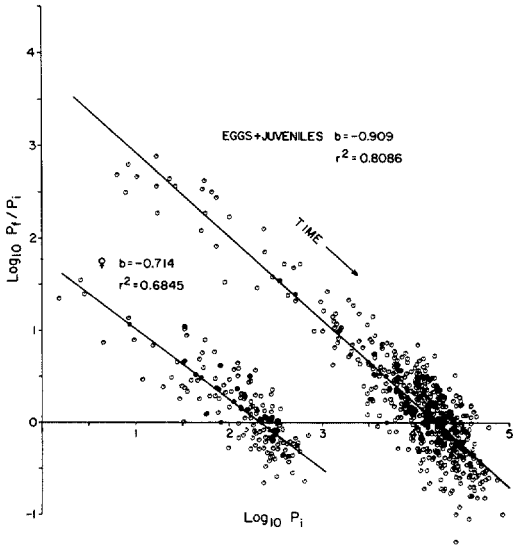

FIG. 7. Relationship between population log_{10}multiplication factor (Pf/Pi) and log_{10}initial population density (Pi) for combined eggs and juveniles and for mature females of *Meloidogyne hapla*. The general influence of time is indicated; b = slope of regression line.

mature females against their respective initial preharvest population densities. Slopes of −0.90953 for combined eggs and juveniles and −0.71349 for mature females indicated a gradual approach to their respective ceiling densities of 20,069 eggs and juveniles and 252 mature females per gram of root.

DISCUSSION

Food supply for nematodes in the form of root biomass expands as individual plants establish deep taproots and an extensive fibrous root system develops during initial seedling establishment of an alfalfa stand. A self-thinning process ensues (22) whereby plant population density and concomitantly root biomass decline to relatively stable levels. As plant population densities decline, individual plant size increases, compensating for the effect of elimination of alfalfa plants on reducing total root biomass. Following the same time course, *M. hapla* population densities responded initially to an apparent unlimited food source by increasing exponentially at rates decreasing with increasing Pi. As plant growth, stand density, and root biomass decreased and population increased with time, there was a corresponding reduction in both multiplication and reproduction rates from their maximum values. Multiplication rates, constrained by a stable but limited food supply, were maintained at or near a maintenance value of 1.0. Maintenance of root biomass, through an apparently continuous root growth process, allowed populations of *M. hapla* to gradually attain ceiling density. At this density, Pf equaled Pi and sufficient food was available to support 252 mature females per gram of root.

Over time, average reproduction rates of *M. hapla* decreased and then stabilized as damage to the host increased to an asymptote level. As the number of mature females increased in the root, the number of available feeding sites decreased and began to overlap resulting in an observed reduction in female size as competition intensified and a logistic type reduction in the fecundity consistent with other studies (3,8,9,11,12,20–22,30–32,34,36). In the present study, the quantity and (or) quality of the available food supply (root biomass) acted as a density-dependent regulatory factor, governing unlimited population growth of *M. hapla*.

The logic of negative feedback systems implies that the population density of the present generation negatively influences growth of the root, and consequently food supply for the following nematode generation; the effect is delayed by the average generation time of *M. hapla* (ca. 500 DD). Interpretation may be confounded by the plotting process in which Pf/Pi values (multiplication rates) are equated with final populations. Periodic cycles of increased root growth would, however, lead to new feeding sites. Considering *Meloidogyne* developmental rates, cyclic root growth would allow renewed periods of nematode multiplication and an increase in population growth rates in the following generation. Population increase is thus dependent upon population levels of females in the root and the amount of new unattacked root biomass. Severe damage to the crop thus reduces the rate of nematode reproduction which in turn sets a limit on the numbers of eggs and juveniles produced.

Observed maximum *M. hapla* reproduction rates were below values reported in other crops (38). Nematode reproduction is generally studied in controlled green-

house or laboratory experiments. In these experiments eggs are separated from the gelatinous matrix and the number of eggs per female assessed. In long-term field studies with overlapping nematode generations and continuous egg production, female fecundity values are obtained by averaging across females of different ages and reproductive capacities (37). Averaging would lower reproductive rates from reported maximum values. The life span, female size, and fecundity values may also be reduced from one generation to the next because of a deterioration of food quality or insufficient food supply (3,10,19,30).

Our study contributes additional information to an increasing body of theoretical and experimental evidence on self-regulatory, density-dependent mechanisms governing nematode population growth. We illustrate how reproductive rates vary with nematode population density and food supply and how it can be subsequently modified by other biotic or abiotic factors. We also show that ceiling population densities of nematodes are not necessarily constant values, but are regulated through time within finite limits by the relative growth rates of the host (10,31), crop management practices (17,19,20,24,28,32,34), and edaphic conditions (23,27,32,36). For perennial crops at least, yield loss models linking the population dynamics of phytoparasitic nematodes with the long-term yield performance of the crop are needed before nematode population growth parameters can be employed in crop management predictive systems.

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