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PATHOGENICITY AND ECOLOGY OF MELOIDOGYNE HAPLA ASSOCIATED WITH ALLIUM CEPA

Michigan State University PH.D. 1983

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PATHOGENICITY AND ECOLOGY OF *Meloidogyne hapla* ASSOCIATED WITH *Allium cepa*

by

Ann Elizabeth MacGuidwin

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ABSTRACT

PATHOGENICITY AND ECOLOGY OF *Meloidogyne hapla* ASSOCIATED WITH *Allium cepa*

by

Ann Elizabeth MacGuidwin

Meloidogyne hapla Chitwood was the third most prevalent phytoparasitic nematode observed in Michigan onion acreage surveyed in 1980. A positive linear relationship between *M. hapla* density and the final yield of *Allium cepa* L. cv Krummery Special was observed in field trials. This nematode also inhibited the growth of *A. cepa* under greenhouse conditions when plants were exposed to 15,000 or more *M. hapla* eggs at seeding. Another cultivar, Downing Yellow Globe, was more tolerant of *M. hapla* parasitism.

M. hapla reproduced on *A. cepa* in greenhouse and field environments. One complete generation of *M. hapla* per year was observed on *A. cepa* and nematode populations increased from the beginning to end of a growing season. The increase in *M. hapla* population levels was less for nematodes associated with *A. cepa* than for *Daucus carota* L., due primarily to the low number of nematodes invading *A. cepa* root tissue rather than to decreased survival or reproduction.

Spores of vesicular-arbuscular mycorrhizal fungi were present in all sites surveyed in 1980. Several experiments were conducted to determine the life stage of M. hapla most affected by the presence of the mycorrhizal fungus, Glomus fasciculatus (Thaxter) Gerd. & Trappe. The penetration of second-stage M. hapla into roots was decreased in A. cepa colonized by G. fasciculatus. Mycorrhizal colonization slightly impeded the development of M. hapla juveniles within roots, but did not influence the number of eggs produced per adult female.

In the greenhouse tests, the growth of A. cepa colonized by mycorrhizae and nematodes was not different from that of plants inhabited by nematodes alone. Conversely, mycorrhizal A. cepa transplanted to microplots in the field were more tolerant and supported higher nematode densities than non-mycorrhizal plants. The discrepant results between similar experiments conducted in the greenhouse and field were probably due to the methodology employed, but also reflect the complexity of the relationship between nematodes and plant growth, and the need for continued research on the interaction between nematodes and other components of agroecosystems.

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INTRODUCTION

Nematodes in the genus Meloidogyne are among the most economically important pathogens of food crops (Taylor and Sasser, 1978). Collectively known as root-knot nematodes, 37 species of this genus were recognized as of 1978 (Taylor and Sasser, 1978). All Meloidogyne spp. are phytoparasites and alter the physiology and ontogeny of host plants. Reports of reduced plant growth, yield, and marketability due to parasitism by Meloidogyne spp. are abundant in nematological literature. In addition to a high level of pathogenicity, the global distribution and wide host range of Meloidogyne spp. contribute to the pest status of this nematode.

Plant stress and resulting crop loss caused by nematodes may be excessive in some years and minimal in others due, in part, to the dynamics of nematode populations. Plant growth in the presence of Meloidogyne hapla and other phytoparasitic nematodes is related to nematode density (Barker and Olthof, 1976). The abundance of M. hapla, in turn, influences the popultion dynamics and pathogenicity of many nematode species and other organisms contributing to plant damage. For example, parasitism of plants by M. hapla may increase the incidence of fungal and bacterial diseases (Griffin et al., 1968; Jenkins and Coursen, 1957; Meagher and Jenkins, 1970).

The population dynamics of M. hapla is influenced by a number of environmental factors and complex biological associations. The abundance and distribution of this nematode are related to soil type, inter-specific competition, soil biota, climatic conditions, and management practices (Norton, 1978; Wallace, 1973). To adequately assess the population dynamics of M. hapla, it is necessary to understand the nature of the association between these factors and the life history of this nematode.

Study of the ecology of nematodes was placed in highest priority by the ISCPP-ESCOP IPM Research Priority Report (1979) and will contribute to the development of economically and biologically sound nematode management programs. An understanding of events contributing to fluctuations in nematode population levels will enhance models predicting crop loss due to nematode parasitism. Knowledge concerning the association between nematode density and other biotic and abiotic components of an agroecosystem can form the basis for management paradigms; that is, the deletion, addition, or alteration of components within the agroecosystem.

The purpose of this research was to examine the growth and pathogencity of M. hapla populations associated with Allium cepa L., with particular emphasis on population phenomena occurring within a single nematode generation. The influence of one biotic component of the onion agroecosystem, vesicular-arbuscular mycorrhizae (VAM), on the ontogeny of M. hapla infecting a single A. cepa plant was assessed. Studies on the nematode-VAM association were designed to provide the basis for further investigations on the dynamics of M. hapla populations in Michigan. This research was conducted in cooperation with entomologists and plant pathologists who examined other biological components of the onion agroecosystem.

OBJECTIVES

The major goals of this research were (1) to elucidate and quantify the within-generation dynamics of M. hapla and (2) to assess the influence M. hapla in an onion agroecosystem both alone and in the presence of the VAM, Glomus fasciculatus. Specifically, the objectives of this research were to:

1. examine the occurrence and pathogenicity of M. hapla associated with A. cepa in Michigan and the suitability of this host for the increase of M. hapla populations
2. describe the within-generation dynamics of M. hapla infecting A. cepa
3. assess the influence of vesicular-arbuscular mycorrhizal fungi on the ontogeny and pathogenicity of M. hapla
4. relate the findings of this research to data from previous studies in a computer simulation model

RATIONALE AND RESEARCH APPROACH

Numerous studies have measured changes in nematode population density, but few have elucidated the forces responsible for fluctuating population levels. The density of nematode populations has most often been measured to evaluate the efficacy of various management tactics such as pesticide application, crop rotation, and tillage regimes. The inadequacy of many of the experiments purporting to address the population dynamics of nematodes is due to the exclusion of factors other than nematodes in the experimental design, and the use of sampling schemes inadequate for characterizing the spatial and temporal distribution of nematodes within a growing season. Consequently, it is not often demonstrated if the management tactic kills nematodes directly, or indirectly by altering some other component of the environment, or if the tactic influences the entire nematode population, or if only certain age classes are affected.

Information on the biology and ontogeny of nematodes is a necessary supplement to the study of nematode population dynamics. Although computer models simulating the life history of Meloidogyne arenaria (Ferris, 1976),

Pratylenchus hexincisus (McSorley,1977), and Heterodera schachtii (Caswell et al., 1981) have been developed, there is still an inadequate understanding among nematologists for population phenomena occurring within a single nematode generation; developmental rates, mortality rates, reproductive rates, over-winter survival, etc.

The within-generation population dynamics of nematodes is of interest for several reasons. Data on the stage-specific survival of nematodes can be used to estimate the optimum dates of pesticide application, harvest, etc. Information on the age structure of a nematode population throughout the growing season can guide sampling activities for more accurate population measurement. Prediction of end-of-season nematode densities can influence management decisions for the subsequent growing season, such as crop selection or fumigation.

The M. hapla-A. cepa system was used to elucidate the dynamics of phytoparasitic nematode populations. M. hapla is an appropriate species for quantitative population studies due to its residency within root tissue, discernible life stages, and habits of oviposition (e.g., eggs are contained in a single unit, or egg mass). The shallow-rooted and nonfibrous root system of A. cepa contributes to this plant's usefulness as a "laboratory rat" for nematological research.

Root-knot nematodes are only one component among many biological and environmental factors influencing the growth and yield of plants in agroecosystems. The dynamics of Meloidogyne populations are influenced by climatic conditions and soil biota surrounding their plant hosts. Previous studies have examined the impact of abiotic factors such as temperature (Wong and Mai, 1973a), moisture (Wong and Mai, 1973b), and edaphic parameters (Wallace, 1969) on the ontogeny of Meloidogyne spp. The influence of heritable attributes such

as nematode reproductive capacity (Ferris and Stuth, 1982; Tyler, 1938) and host quality (Balasubramanian and Rangaswami, 1963) on the increase of Meloidogyne populations has also been addressed, and the role of concomitant soil organisms in determining nematode population change has received some attention (Sayre, 1980).

The relationship between M. hapla and other members of the soil community is an appropriate and timely research topic. Estimates of plant damage attributable to root-knot nematodes in the presence of organisms beneficial or pathogenic to the host crop are essential for the accurate prediction of crop losses. More importantly, the identification and nurture of natural biological controls for nematode pests will become a crucial agricultural management activity as the cost of pesticide application increases.

There is a distinct need for alternatives to the many pesticide-dependent programs for nematode control. Prices of existing nematicides are rising yearly, and the cost to develop new pesticides increased three-fold from the 1960s to the 1970s (Edens, 1978). The efficacy of pesticides is more carefully scrutinized now than in the past. For example, according to Rohde et al. (1980), the use of fumigant biocides can lead to the eventual increase of Meloidogyne populations to levels equal to that of unfumigated areas.

Although fungi, bacteria, soil invertebrates, and other nematodes have been reported to influence the increase of Meloidogyne populations, attempts to utilize knowledge of nematode interactions with soil biota for nematode management are few. The scarcity of such programs is due, in part, to an insufficient understanding of the role of these soil organisms in agroecosystems. Of all possible candidates for the biocontrol of nematodes, VAM are unique in that not

only have they been implicated as a factor impacting nematode population growth, but are themselves the subject of extensive study.

The potential benefits of research investigating the interaction between the increase of M. hapla, VAM, and plant growth are impressive. VAM colonization increases the uptake of phosphorus and other soil nutrients, thus stimulating plant growth. Drought tolerance is also increased in mycorrhizal A. cepa (Nelsen and Safir, 1982a; 1982b). Added to these benefits, evidence of the suppression of nematode populations by VAM would support the implementation of management schemes to promote mycorrhizal development in agricultural production systems.

The effect of VAM on the population dynamics of root-knot nematodes has been addressed in several studies, although many aspects of the nematode-fungus relationship remain unanswered. Sikora (1979) proposed four ways that mycorrhizal fungi influence plant-parasitic nematodes: (1) altering root attractiveness, (2) reducing larval penetration, (3) impeding larval development, and, (4) retarding giant cell formation. Three additional means of decreasing nematode numbers can be added to this list: (1) reducing nematode survival, (2) altering sex ratios, and (3) reducing fecundity. No study to date has thoroughly examined these alternatives. Sikora and Schonbeck (1975) are credited with demonstrating the influence of VAM on the penetration and development of Meloidogyne juveniles. Two experiments are reported in their paper, one lasting 30 days (one harvest) and one lasting 125 days (harvests at 42, 84, and 126 days). Neither experiment successfully differentiated the contribution of decreased penetration, mortality within roots, and rates of development and reproduction to nematode loss in mycorrhizal roots.

The effect of VAM on nematode survival within roots or soil has not been examined. No study has attempted to enumerate different juvenile stages of Meloidogyne in mycorrhizal roots. Gall indices or counts of eggs, juveniles, or adult nematodes have been used in previous studies. The presence of VAM was demonstrated to retard or inhibit giant cell formation (Sikora, 1979). It is probable, therefore, that VAM contribute to nematode mortality. In fact, decreased nematode densities in several experiments could have been due to nematode mortality rather than to the delayed development of nematodes, as was interpreted from the data.

In addition to providing knowledge concerning ecological associations influencing the population dynamics of nematodes, aspects of this research have potential application for the production of muck vegetables in Michigan. M. hapla is a prevalent plant-parasitic nematode in Michigan onion acreage and has long been recognized as a serious pest of carrot and celery. The importance of M. hapla as a pathogen to onion has not been adequately assessed, nor has the relative effectiveness of onion in maintaining populations of M. hapla been characterized.

The population dynamics of nematodes is a complex phenomenon and cannot be properly assessed by the reductionist approach adopted so frequently in past studies. To truely understand the activities of nematodes or other pests, it is necessary to maintain a holistic conception of the pest, crop, and other interacting components of the agroecosystem. Constraints of time, facilities, and manpower dictate that the pest-crop agroecosystem be experimentally examined piecemeal. It is imperative, however, that the research is designed and analyzed without losing sight of the interdependence between all components

in the ecosystem. The complexity and intricacies of the association between organisms and their environment has too often led to the perception that the holistic approach is inappropriate and unrealistic for biological systems. Fortunately, the advent of high-speed computers and systems science methodology, developed by engineers to deal with complexity in the physical sciences, shows this perception to be false.

Systems science is a problem solving procedure to evaluate and make decisions concerning the structure, function, and behavior of complex systems. A system is defined as a "regularly interacting or interdependent group of items forming a unified whole"[1] and so this methodology can be applied to any system where the elements involved can be identified and their interrelationship conceptualized. The systems approach has been widely applied in studying mechanical, electrical, chemical, and other physical systems and has recently been used to examine economic, social, and biological systems.

Implicit to the systems approach is the consideration of all factors that contribute to the solution of problems presented by the behavior of the system--e.g., holism. Inclusion of all factors in the problem-solving process leads to the realization that "trade-offs" in benefits to individual components of the system are inevitable. In the onion agroecosystem, for example, fumigant nematicides applied to reduce nematode populations may also be detrimental to beneficial organisms such as nematophagous insects or VAM.

There are many advantages to using systems methodology when dealing with nematodes or other pests. No one person can deal with the diversity inherent to biological systems, so the efforts of a transdisciplinary team must be

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[1]Webster's New Collegiate Dictionary, 1976.

coordinated to solve the problems of increased crop production. The activities of all involved researchers are organized by the logic and methodology of systems science. A holistic approach is conducive to long-term planning and solutions to pest problems that are ecologically sound. Most importantly, the use of systems methodology has led to the development of flexible, economic pest management strategies (on-line pest management) and the realization that solutions to pest problems may lie in the redesign of the structure of agroecosystems (Edens and Haynes, 1982; Tummala and Haynes, 1977).

An established hierarchy of activities is inherent to the systems approach. The initial phase in the process is a feasibility analysis which includes a definition of needs, the formulation of the problem one wishes to solve, and the generation and examination of alternative ways to redesign the structure of the system to meet the desired needs. Next, an abstraction of the system in the form of analytical or computer simulation models is developed to analyze the behavior of the system. For complex systems, models representing only a portion of the system can be developed and later interfaced together so that the entire system is represented. This technique, referred to as the discrete component approach (Tummala et al., 1975), is particularly useful for biological systems. Finally, the details of the system structure and management strategies identified earlier are operationalized and evaluated in the real world (Allen and Bath, 1980).

The identification of the system is an insightful and valuable activity for the biologist. The "object of control", identified in the formulation of needs, determines those components or "system design parameters" to be included in the system and those considered to be exogenous to the system (hereafter

referred to as the environment). Environmental variables influencing the system can be described as controllable (e.g., plant cultivar) or uncontrollable (e.g., weather). The behavior of the system can be assessed by measuring desired (e.g., decreased nematode density) and undesired (e.g., overcrowding of plants) system output variables. To illustrate these concepts, the M. hapla-onion system is identified in Figure 1. Components other than M. hapla are characterized according to their level of involvement in the population dynamics of this nematode.

BACKGROUND

Life History

Meloidogyne hapla progresses through six developmental stages in the course of its lifetime: egg, four juvenile stages, and adult. Eggs are deposited into a gelatinous matrix extruded from specialized glands of the female. Individual eggs are retained in this egg mass, which adheres to the root surface or lays amid soil particles. Within the egg, the embryo develops into a first- and then a second-stage juvenile. Specific environmental stimuli elicit mechanical and chemical responses from the second-stage juvenile that initiate egg hatch (Bird, 1971).

The second-stage juvenile moves through the soil in search of a host plant. Movement of the juvenile is not random, but rather is directed by stimuli associated with a developing plant (Green, 1971). Piercing the root with its stylet, the nematode migrates intercellularly in the root cortex in search of an appropriate feeding site. Hyperplastic responses of the infected plant, hypertrophy and hyperplasia, can be detected soon after the juvenile begins feeding.

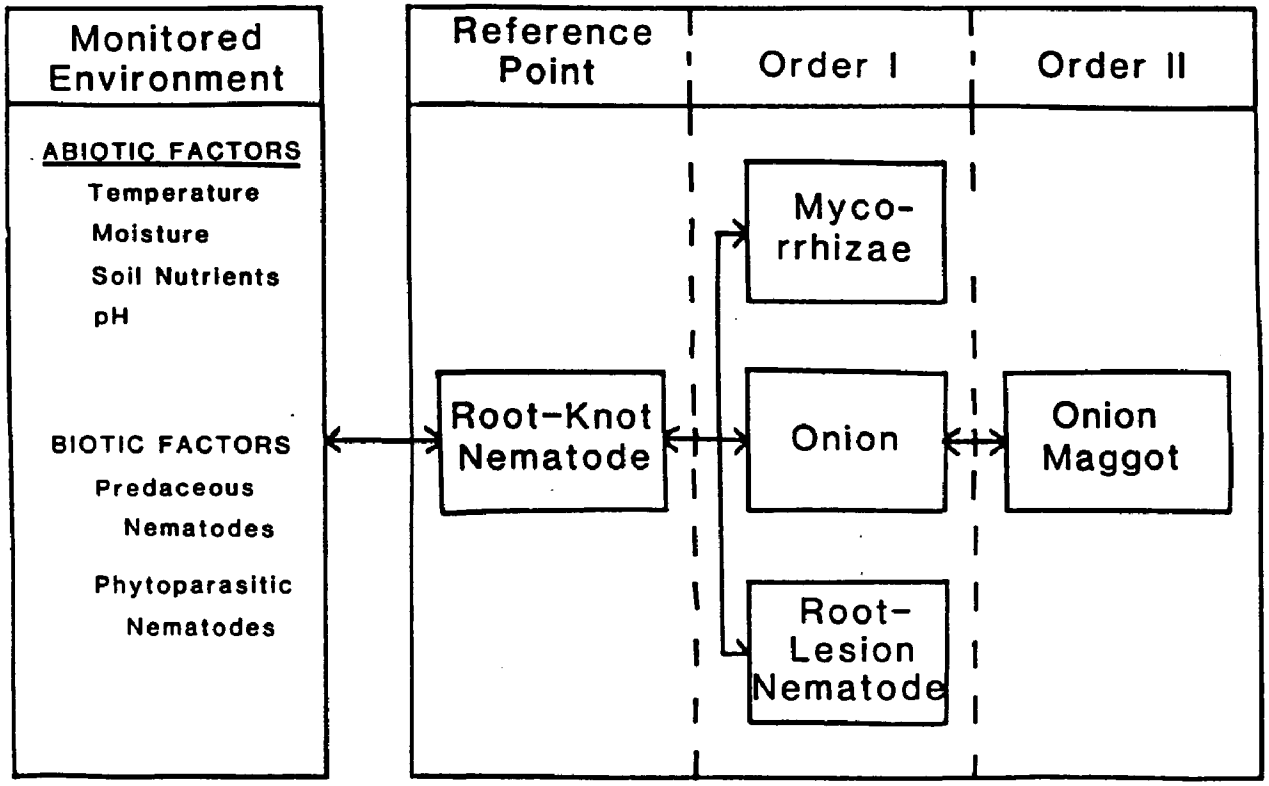


Figure 1. Conceptualization of the M. hapla - A. cepa ecosystem with M. hapla as the reference point.

Disagreement arises in the literature as to the sequence of developmental changes occurring for root-inhabiting juveniles. According to Nagakura (1930) and Ritter and Ritter (1958), nematodes molt from the second stage to the third stage soon after feeding commences. Other authors (Triantaphyllou and Hirschmann, 1960; Bird, 1959) report that considerable growth of the second-stage juvenile and sexual differentiation occur before the molt to the third stage. The latter view is generally accepted. An extimated 50 - 70% of the time spent in the development of M. hapla from root penetration to oviposition is passed within the second juvenile stage (Ferris and Hunt, 1979; Triantaphyllou and Hirschmann, 1960; Tyler, 1933; Vrain et al., 1978; Wong and Mai, 1973a).

The third and fourth juvenile stages occur in relatively rapid succession. The cuticle of the second stage is retained as the juvenile molts to the third stage. Similarly, both the second- and third-stage cuticles are retained in the fourth-stage juvenile. Third- and fourth-stage juveniles do not feed as a result of this cuticular encasement and a degeneration of the stylet, although maturation continues. Females retain a saccate shape throughout the developmental period, but males undergo a metamorphosis in the fourth juvenile stage to the eelworm shape of soil-inhabiting nematodes.

Following a fourth molt, the adult female resumes feeding and increases substantially in size. The adult male does not feed and after mating migrates away from the root system. There is a considerable delay between the molt to adulthood and commencement of oviposition of the female. Approximately 30% of the time necessary for development of the female, from root penetration until the appearance of eggs, is passed as a preovipositing adult (Bird and Wallace, 1965; Wong and Mai, 1973a).

M. incognita females produce ca 150 - 500 eggs per egg mass (Carlson and Rothfus, 1978). Tyler (1933), working most probably with M. hapla (Thomason and Lear, 1961), estimated that one egg was produced per 12 degree hours (base 10 C). The duration of the oviposition period of M. hapla has not been reported, but according to Carlson and Rothfus (1978) M. incognita females remain alive and lay eggs through at least one additional generation. The age of the female does not seem to influence egg production; de Guiran and Ritter (1979) observed no differences in the weekly rate or total egg production of M. incognita over a four-week period.

Factors determining the ratio of females to males is not well understood. No sex chromosomes have been observed in plant-parasitic nematodes and sexual differentiation in Meloidogyne spp. seems dependent on environmental factors. Under conditions favorable to nematode development (suitable temperature, moisture, host, etc.) all or most juveniles develop into females. Under unsuitable conditions, males as well as females occur. The sex of M. hapla and other Meloidogyne spp. is determined in the second juvenile stage. Second-stage juveniles developing as females may revert to males if unfavorable conditions arise before the second-stage is completed. Undifferentiated juveniles which become males, and females which undergo sex reversal are referred to as "true males". In M. javanica male intersexes, i.e., males with female sex characteristics, also occur.

The role of Meloidogyne males in reproduction is variable among the species. No obligatory amphimictic species have been described for this genus. M. hapla populations show variation in chromosome number and mode of reproduction. Populations described as Race A reproduce by amphimixis, or

meiotic parthenogenesis, or both. Populations described as Race B reproduce by mitotic parthenogensis (Triantaphyllou, 1966).

The number of generations of M. hapla occurring per year depends on the host crop and climatic conditions. In Michigan muck soils two generations are observed in an average year, with peak nematode densities evident in early August. M. hapla overwinters in the egg stage.

Several adaptations enable M. hapla to survive conditions unsuitable to nematode development. Eggs retained within the protective covering of the gelatinous matrix successfully withstand freezing temperatures. Many nematodes, probably including M. hapla juveniles, exhibit decreased metabolic activity when exposed to climatic extremes (Cooper and Van Gundy, 1971). Quiescent states for many nematode genera are evoked by low soil moisture, oxygen, and temperature. In addition, cryptobiosis, the almost complete shutdown of metabolism, allows nematodes to survive long periods of low soil moisture or temperature.

Mortality rates of M. hapla due to biotic, abiotic, and human factors have not been clearly defined. Examination of data from several studies (Dropkin, 1963; Griffin and Elgin, 1977; Wong and Mai, 1973b) indicate that ca 38% of M. hapla eggs eventually become root-inhabiting nematodes. Estimates of the mortality of juveniles within roots are not available.

Pathogenicity of Meloidogyne hapla

M. hapla has a wide host range, including crop plants and weeds. General symptoms of all plants to infection by Meloidogyne spp. include stunting, yellowing, and the proliferation of lateral roots. The most obvious symptom of

root-knot infection is root galling, although galls formed by M. hapla are usually smaller than those produced by the other most common Meloidogyne spp. Pathogenicity of M. hapla varies with the host crop, but this nematode is generally considered to be a detriment to plant growth and yield (Franklin,1979).

Meloidogyne infection induces mechanical and physiological changes that can alter the relationship between some plants and other pathogens. Parasitism of plants by M. hapla may increase the incidence and severity of both fungal (Jenkins and Coursen, 1957; Meagher and Jenkins, 1970) and bacterial (Griffin et al., 1968; Hunt et al., 1971) diseases. Moreover, the resistance of plants to fungal disease can be broken by root-knot infection (Sidhu and Webster, 1977). Little is known concerning the relationship between nematodes, insects, and plant growth, although it has been hypothesized (Wallace, 1973) that nematode infection might influence the susceptibility of a plant to insect attack.

Onion, Allium cepa L., is listed as a host for M. hapla, M. incognita, and M. javanica (Chitwood, 1951; Martin, 1961; Tyler, 1933). Some reports indicate that economic losses of onion may result from M. hapla infection (Kotcon, 1979; Olthof and Potter, 1972; Sherf and Stone, 1956; Smith, 1964). According to other sources (Chitwood, 1951; Tyler, 1933) onion is relatively resistant to M. hapla and only limited development of nematodes and resulting yield losses occur. Discrepencies in the literature may be due to variability in the damage and reproduction of M. hapla associated with different onion cultivars (Franklin and Hooper, 1959; Kotcon, 1979; Sasser, 1954).

The relative effectiveness of onion in maintaining populations of M. hapla has not been definitively characterized. Chitwood (1951) found that M. hapla was unable to reproduce on Downing Yellow Globe onions. Observations of M.

hapla reproduction on Yellow Globe (Kotcon, 1979; Lewis et al., 1958; Olthof and Potter, 1972) and Aristocrat (Smith, 1964) varieties were published subsequent to Chitwood's report. These studies, conducted under laboratory conditions, did not determine if M. hapla levels can increase on onion to the point where succeeding onion crops are damaged under field conditions.

Interaction Between Meloidogyne hapla and Abiotic Factors

Temperature

The effect of temperature on Meloidogyne spp. has received much attention. The range of optimal temperature differs between species (Bird and Wallace, 1965; Dropkin, 1963; Vrain and Barker, 1978). M. hapla has lower thermal optima than do most other species. Differential growth responses to temperature have also been reported between populations of M. javanica (Daulton and Nusbaum, 1961) and between races of M. naasi (Michell et al., 1973). Although variability in thermal tolerance has not been tested for M. hapla, a comparison of the results reported by Wong and Mai (1973a) in New York and Vrain et al. (1978) in North Carolina suggest that such differences do exist. Among populations of Meloidogyne spp. differential effects of temperature on each life stage have been noted (Bird and Wallace, 1965; Wallace, 1971). In general, soil-inhabiting stages have lower temperature ranges than do the root-inhabiting stages. A similar phenomenon has been reported for Heterodera schachtii (Wallace, 1973).

Temperature affects all life stages of Meloidogyne spp. Eggs of M. hapla hatch optimally at ca 20 C (Vrain and Barker, 1978). Wuest and Bloom (1965), however, found that the thermal optima for M. hapla eggs decreased with time.

The temperature for the maximum survival of eggs incubated three and 30 days was 27 C and 12 C, respectively. Temperature influences the movement of M. hapla juveniles in soil and the ability of juveniles to invade plant roots. Bird and Wallace (1965) found that the movement of juveniles through a sand column was fastest at 20 C and mobility was severly limited by temperatures of 30 and 35 C. Consequently, these authors observed that significantly greater numbers of juveniles penetrated roots under a 15 - 20 C temperature regime than did juveniles reared under 20 - 25 C and 25 - 30 C regimes. Vrain and Barker (1978) demonstrated that the minimum temperature for root penetration by M. hapla is 8.8 C. Development of M. hapla juveniles inside roots proceeds at temperatures as low as 12 C (Vrain et al., 1978) and as high as 32.3 C (Wong and Mai, 1973a). The optimal temperature for juvenile development is ca 25 C (Wong and Mai, 1973a).

Heat units are generally used to relate temperature and developmental time. One heat unit (= one degree hour) equals one degree above the developmental threshold acting for one hour. Based on a developmental threshold of 10 C (data were adjusted where necessary) estimates of ca 9500, 6500, 7500, and 8000 heat units were reported by Wong and Mai (1973a), Starr and Mai (1976), Vrain et al. (1978), and Tyler (1933), respectively, as the developmental time necessary for second-stage juveniles of M. hapla within roots to become egg-producing females. An additional period of 4000 - 5000 heat units is required for egg development and hatch (Tyler, 1933). The above estimates are similar to those reported for other Meloidogyne spp. (Bird and Wallace, 1965; Ferris and Hunt, 1979; Milne and Duplessis, 1964). It should be noted that these estimates of M. hapla development are based on the first appearance of eggs and

do not represent the average developmental time of all observed nematodes. Considerable variation between the developmental rates of the fastest and the average nematode occur (Ferris and Hunt, 1979).

Temperature is one of the factors influencing sex determination of Meloidogyne spp. Davide and Triantaphyllou (1967) found that at temperatures of 20 - 35 C only one percent of M. incognita reared on tomato developed into males while at 15 C, 6.7% developed as males. The hypothesis that differential mortality between the sexes rather than sex expression was responsible for the results was rejected, because no dead juveniles were observed. Laughlin et al. (1969) reported even higher male/female ratios for M. graminis reared under high temperatures; at 16 and 21 C, no males were observed; at 27 C, 4% of the nematodes were males; at 32 C, 80% of all nematodes developed as males. No information is available regarding the effect of temperature on the sex determination of M. hapla.

The optimal temperature for reproduction by M. hapla is from 25 - 30 C (Griffin and Elgin, 1977; Thomason and Lear, 1961). The maximum and minimum temperature for reproduction by this species on tomato are 32.6 and 15.0 C (constant temperature), respectively. Wong and Mai (1973a) reported that under a temperature regime of 21.1 - 26.7 C, 1662 eggs and juveniles per gram of root were produced by M. hapla infecting lettuce. During the same time span, under a higher temperature regime of 26.7 -32.2 C, 3019 eggs and juveniles per gram of root were produced from the initial inoculum of 400 juveniles/plant.

Meloidogyne spp. differ in their susceptibility to high and low temperature extremes. M. hapla can survive lower temperatures than M. javanica or M. incognita (Bergeson, 1959; Daulton and Nusbaum, 1961; Vrain et al., 1978).

Conversely, M. hapla are less able to survive high soil temperatures (Daulton and Nusbaum, 1961).

Several factors influence the ability of M. hapla to withstand freezing temperatures. Longer exposure times to low temperatures are necessary to kill eggs in dry soil than in wet soil (Daulton and Nusbaum, 1961). The stage of the nematode affects survival ability. Unhatched juveniles survive freezing temperatures better than the embryonic stages within eggs of M. hapla (Vrain et al., 1978). The habitat of the nematode is also important. All stages of M. hapla survive freezing temperatures when protected by root tissue (Vrain et al., 1978). The tolerance of M. hapla to freezing indicates the presence of a mechanism for withstanding low temperatures (Sayre, 1964). There is evidence that an acclimation period to low temperature enhances survival. Nusbaum (1962) found that egg masses buried outdoors in August show greater survival the next spring than do egg masses buried in November.

Moisture

Soil-inhabiting nematodes are essentially aquatic organisms. Soil moisture directly influences the developmental rates of Meloidogyne eggs and infectivity of second-stage juveniles (Ogunfowora, 1978; Wallace, 1968b; Wong and Mai, 1973b). Water provides a medium for movement of nematodes within the soil. Differences in tolerance of soil moisture stress by geographically isolated populations of Meloidogyne spp. occur (Daulton and Nusbaum, 1961). As with temperature, soil moisture content and resulting O_2 concentrations have differential effects on each stage of nematode development (Godfrey and Morita, 1929; Wallace, 1966; 1968a).

Soil moisture influences the rate of development and hatch of Meloidogyne eggs. Decreased O_2 levels associated with high soil moisture conditions affect developing embryos more severely than juveniles within eggs of M. javanica (Wallace, 1968a). Egg mortality results if embryonated eggs are subjected to anaerobic conditions for one week (Ferris and Van Gundy, 1979). The effect of O_2 on egg hatch can be mediated by corresponding CO_2 levels and it may be the ratio of O_2/CO_2 rather than O_2 alone that is responsible for the inhibition of egg hatch (Wong and Mai, 1973b). Egg development and hatch are also decreased under low soil moisture conditions. Dehydration of eggs seems to affect second-stage juveniles more severly than developing embryos and first-stage juveniles, due to the increased permeability of the egg shell prior to hatch (Wallace, 1966). Optimal egg hatch and development occurs when the soil moisture level is at field capacity.

Meloidogyne eggs exhibit mechanisms for surviving low soil moisture extremes. High osmotic concentrations, simulating moisture stress, inhibit the hatch of M. arenaria eggs, although exposure to even high concentrations (1M solutions of NaCl, KCl, $CaCl_2$ or dextrose) for 33 days does not prevent hatch when eggs are transferred to water (Dropkin et al., 1958). Soil moisture levels have a greater effect on the activity of M. hapla juveniles in the soil. No penetration of lettuce roots occurs in water-saturated soil (Wong and Mai, 1973b) and flooding has long been used to reduce nematode populations (Dropkin, 1980). Low soil moisture impedes nematode movement due to the redistribution of the water surrounding soil particles. Soil moisture levels unfavorable to nematode activity most often delay rather than prevent infection.

The effect of soil moisture on the root-inhabiting stages of Meloidogyne and other endoparasitic nematodes is not known. The influence of soil moisture

on nematodes reproducing within plant roots is certainly mediated through the host plant. It has been observed that decreased plant vigor limits reproduction by Meloidogyne spp., although the specific effects of water stress on this phenomenon are not known.

Soil texture

Soil texture and composition affect the temperature conductivity and moisture relations of the soil environment. The size of soil particles determines the number and size of pore spaces and consequently, the ability of nematode juveniles and water to move through the soil. Both very small and large pore spaces tend to inhibit Meloidogyne movement. Invasion and reproduction of M. javanica infecting tomato is greater in fine sand (150 - 250 u) than in coarse (500 - 600 u) or medium (250 - 500 u) sand (Wallace, 1969). Soils with many pore spaces, however, limit the reproduction (as measured by the number of second-stage juveniles recovered from soil) of M. hapla infecting sugarbeet (Santo and Bolander, 1979).

Soil pH

The effect of pH on the hatch of Meloidogyne eggs and invasion of roots by second-stage juveniles appears to be minimal (Watson and Lownsbery, 1970). Lowenberg et al. (1960) found that the optimal pH for egg hatch and survival of M. incognita was 6.4 in Heller's medium. It seems likely that the range of pH found in most agricultural soils is favorable for the development of the soil-inhabiting stages of Meloigodyne spp. Once infection has occurred, the influence of pH on nematode development is probably mediated through the host plant.

Interaction Between *Meloidogyne hapla* and Biotic Factors

Predators and parasites

The association between plant-parasitic nematodes and other soil organisms has often received low priority in nematology research programs. Consequently, primarily descriptive accounts of interactions between nematodes and their predators and parasites have been published.

The egg and second juvenile stages of *M. hapla* are susceptible to predation by a variety of organisms (Esser and Sobers, 1964; Sayre, 1971; 1980). Predaceous fungi, ubiquitous in most soils, trap *Meloidogyne* and other nematodes by specialized mechanisms such as adhesive networks, sticky knobs, or constricting hyphal rings (Barron, 1977). Some predaceous nematode genera including *Mononchus*, *Mononchoides*, *Buterius*, *Anatonchus*, *Diplogaster*, *Tripyla*, *Seinura*, and some species of *Dorylaimus*, *Discolaimus*, and *Actinolaimus* also prey upon *Meloidogyne* (Esser and Sobers, 1964). Other invertebrate predators of nematodes include tardigrades (Doncaster and Hooper, 1961), tubellarians, mites, protozoans, oligochaetes, and Collembola (Gilmore, 1970).

The soil-inhabiting stages of *M. hapla* are parasitized by several organisms (Sayre, 1971). Certain endozoic fungi parasitize *Meloidogyne* spp., including *Dactylella* (Stirling and Mankau, 1978), *Haptoglossa* (Esser, 1976), and *Catenaria* (Birchfield, 1960). Only one report of a virus disease in nematodes (*M. incognita*) exists in the literature (Sayre, 1971). The nematode parasite receiving the most attention has been the bacteria *Bacillus penetrans* (earlier classified as a protozoan). *Bacillus penetrans* has been identified infecting *Meloidogyne* spp. (Sayre and Wergin, 1979) and work investigating the viability of this pathogen as a biological control agent is now in progress.

Other phytoparasitic nematodes

The increase of M. hapla is related to nematode density. Low density levels are inhibitory to populations in which reproduction depends on the location of the opposite sex (Seinhorst, 1968). High nematode density inhibits root colonization and leads to competition for food reserves or feeding sites. Damage by nematodes reduce plant growth, thereby limiting the number of nematodes the plant can support (Seinhorst, 1967).

The population dynamics of M. hapla is also influenced by other nematode genera. As discussed previously, predatory nematodes may reduce levels of the soil-inhabiting stages. Competition with other root-inhabiting genera of Meloidogyne spp. affects levels of M. hapla within roots (Chapman, 1966; Estores and Chen, 1972; Gay and Bird, 1973).

Inter- and intra-specific competition regulate the penetration of roots by M. hapla juveniles. Decreased infectivity of M. hapla was observed in roots simultaneously inoculated with M. javanica and the penetration of tomato roots by M. hapla is negatively correlated with inoculum level (Kinloch and Allen, 1972). Conversely, the penetation of tomato roots by M. javanica is not density dependent. Kinloch and Allen (1972) hypothesized that in contrast to M. javanica, M. hapla is not attracted to, or cannot penetrate galled tissue. This hypothesis is supported by the higher incidence of terminal root galls and the lower mean number of cohabiting nematodes per gall for this species.

Pratylenchus spp. (migratory endoparasitic nematodes) can also inhibit root penetration by root-knot nematodes. Simultaneous inoculation or previous inoculation of P. penetrans (Turner and Chapman, 1972) or P. brachyurus (Gay and Bird, 1973) decreased root penetration by M. incognita. Penetration by

Pratylenchus was unaffected in both studies. In the presence of M. incognita, numbers of P. brachyurus were increased on cotton, decreased on tomato, and unaffected on alfalfa or tobacco. The Gay and Bird study illustrates the complexity of interactions between nematode species.

The effect of nematode density and mixed populations on the development of M. hapla within roots has received little attention. Studies using several Meloidogyne spp. are impeded by the difficulty of identifying pre-adult stages to the species level. The effects of intra-specific competition on nematode development are more easily discernible. Davide and Triantaphyllou (1967) found that high infection density reduced the rate of development of M. incognita and M. javanica. Wallace (1969) attributed the decrease of M. javanica adult females after 20 days with increasing inoculum levels, to delayed development resulting from competition for feeding sites. His conclusions were based on findings that the rate of root invasion was unaffected by population level; root growth was unaffected by population density; there was no indication of differential mortality; and the effect disappeared in plants reared for 42 days.

Decreased reproduction by root-knot nematodes at high densities or with concomitant infection of other Meloidogyne or Pratylenchus spp. has been noted. A correlation between decreased egg production by M. javanica infecting tomato and inoculum level was observed by Wallace (1969). The reproduction of M. hapla on tobacco was reduced by the presence of M. incognita or P. brachyurus (Johnson and Nusbaum 1970). Greenhouse temperatures in this study may have been less favorable for M. hapla and thus partially responsible for the reported effect. Again, the complexity and difficulty of assessing nematode-nematode interactions is illustrated.

High nematode density can also alter the sex ratios of some Meloidogyne spp. Davide and Triantaphyllou (1967) found that inoculum levels of 350 and 50,000 M. incognita juveniles per tomato plant resulted in populations with 0.5% and 53.5% males, respectively, although high nematode density had no effect on the proportion of males in populations of M. javanica.

Vesicular-arbuscular mycorrhizae

The beneficial effects of vesicular-arbuscular mycorrhizae (VAM) on plant growth are well documented (Atilano et al., 1981; Bird et al., 1974; Hussey and Roncadori, 1978; Rich and Bird, 1974). Stimulation of plant growth due to a VAM association has been attributed to increased water and nutrient uptake and a reduction of pathogenic organisms. Several reports indicate that mycorrhizal colonization negates the deleterious effects of nematodes on plant growth (Kellam and Schenck, 1980; Hussey and Roncadori, 1978, 1982; Roncadori and Hussey, 1977). The mechanisms by which VAM limit nematode damage to plants has not been determined. The hypothesis tested most often in previous studies is that nematode density, and hence damage, is reduced as a consequence of VAM colonization.

Kellam and Schenck (1980) found that M. incognita produced fewer galls on mycorrhizal soybeans, both on a per plant and per gram of root basis. Bagyaraj et al. (1979) also observed fewer galls on tomato plants jointly inoculated with M. incognita or M. javanica and Glomus fasciculatus (VAM) than on plants inoculated with the nematode alone. A decrease in the number of root-knot nematodes infecting mycorrhizal tomato, tobacco, oat, and carrot was reported by Sikora and Schonbeck (1975). It should be noted, however, that differences in

nematode levels between mycorrhizal and non-mycorrhizal plants decreased towards the end of the Bagyaraj and Sikora studies.

Conversely, several authors concluded that VAM colonization enhances the growth of nematode populations (Atilano et al., 1981; Kotcon, 1979; Roncadori and Hussey, 1977; Schenck et al., 1975). Roncadori and Hussey (1977) extracted more M. incognita eggs from mycorrhizal cotton than from non-mycorrhizal plants. The same authors (Hussey and Roncadori, 1978) observed that mycorrhizal cotton supported greater numbers of Pratylenchus brachyurus than did non-mycorrhizal plants. In both studies, however, mycorrhizal roots supported fewer nematodes on a per gram unit basis. A positive correlation between the density of Meloidogyne spp. and VAM colonization has also been observed on onion (Kotcon, 1979), soybean (Schenck and Kellam, 1978), and grape (Atilano et al., 1981).

Differences in the methodology of previous experiments may account for inconsistant results regarding the nematode-VAM interaction. The association between root-knot nematodes and VAM is dependent on the host plant, levels of nematode and fungal inoculum, and the timing of inoculation (Schenck, et al., 1975). More importantly, examination of data from previous studies shows that the interaction between plant, nematode, and VAM is dynamic. Time has been considered as a variable in only four studies (Bagyaraj et al., 1979; Kellam and Schenck, 1980; Kotcon, 1979; Sikora and Schonbeck, 1975). In two of these studies (Bagyaraj et al., 1979; Kellam and Schecnk, 1980) gall indices rather than nematode numbers were recorded. Galling does not necessarily reflect population levels of M. hapla, since the number of nematodes inhabiting each gall is variable (Kinloch and Allen, 1972). Of the remaining studies, only adults were

measured in one experiment (Sikora and Schonbeck, 1975) and the other experiment was biased by very poor plant growth (Kotcon, 1979).

Little is known concerning the influence of VAM on the ontogeny of M. hapla and other plant-parasitic nematodes. It has been hypothesized that fewer M. hapla juveniles penetrate mycorrhizal roots (Sitaramaiah and Sikora, 1980). Root extracts from mycorrhizal roots, however, do not repel nematodes (Sikora and Schonbeck, 1975). It has been speculated that VAM alter the physiology of the plant making it less susceptible to nematode attack (Bagyaraj et al., 1979; Sikora, 1979) or compete with nematodes for colonization sites within the root (Fox and Spasoff, 1972; Kellam and Schenck, 1980; Hussey and Roncadori, 1978). The development of root-knot nematodes seems to be impeded in mycorrhizal tissue. Baltruschat et al. (1973) reported a 75% reduction in the number of M. incognita juveniles that developed into adults on mycorrhizal tomato. Similarly, Sikora and Schonbeck (1975) observed fewer adult M. incognita on mycorrhizal tobacco, oat, and tomato than on nonmycorrhizal plants after 30 days. These authors report similar findings for M. hapla infecting carrot.

Only two studies have examined the influence of VAM on nematode reproduction. Roncadori and Hussey (1977) found greater numbers of M. incognita eggs on mycorrhizal cotton plants maintained under low or high fertility conditions. Calculations of eggs per g of root tissue, however, showed that egg numbers were lower on mycorrhizal plants grown under low fertility conditions, compared to non-mycorrhizal controls, due to differences in the size of the root systems. These authors did not count the number of adult females responsible for egg production. The influence of Glomus mosseae on the fecundity of Rotylenchulus reniformis was examined by Sitaramaiah and Sikora

(1980). Adult females produced fewer egg masses and laid fewer eggs per egg mass on mycorrhizal tomato.

Conceptual Model of Meloidogyne hapla Associated with Allium cepa

Discrete component models of Meloidogyne hapla and other components of the onion agroecosystem were constructed by myself and other researchers at Michigan State University. My objectives in developing the M. hapla model were to: (1) assimilate information previously published on the biology of M. hapla, (2) identify information gaps concerning the interaction between M. hapla and A. cepa, (3) develop a mathematical description of the population dynamics of M. hapla that could incorporate data from my research projects to an existing data base, and (4) provide a means for comparing laboratory and field data.

The model simulates the population dynamics of M. hapla associated with a single onion plant. The daily development of seven nematode life stages is predicted by the model according to soil temperature, using information from studies cited previously. Other abiotic factors such as soil moisture, texture, nutrient levels, and pH are not currently included in the model, due to a lack of quantitative data, but can be added when an appropriate data base is developed. Similarly, little information on the relationship between M. hapla and the growth of A. cepa was available when the model was first developed. Data from my experiments were used to describe the influence of M. hapla on the growth of A. cepa and to estimate the amount of root substrate available for nematode ingress and nourishment.

Overview of the Model

Figure 2 shows a simplified diagram of the M. hapla model. The nematode population is divided into egg, juvenile, and adult stages. Juveniles are further characterized according to a soil or root habitat. Flows from one stage to another are dictated by temperature-dependent developmental rates and by constraints imposed by the onion plant. Onion root growth determines the substrate available for nematode penetration. Nematode feeding inhibits root growth, thereby limiting the number of nematodes eligible for root penetration.

A flow chart of the model is presented in Figure 3. The user is prompted for initial egg density, planting date, length of the growing season, the time increment for the simulation, and whether or not onion plants have a VAM association. Field soil temperature is read from a tape at each daily iteration. Degree hour (base 9 C) accumulation is computed and the various subroutines are called. Model output is stored at the end of each daily iteration.

Description of Subroutines

Subroutine Onion (Figure 4)

A simple regression model of onion root growth was substituted for a more complex onion plant model. Presently, root growth from planting until 54 days after planting is described by the equation:

$$0.0095537 * e^{0.0924842 * \text{numday}} * DT \quad (\text{numday} = \text{day since planting})$$

and from 54 days after planting until harvest by the equation:

$$0.000002694 * \text{numday}^{3.340064} * DT$$

This value for root growth under optimal conditons is then modified by temperature according to the function:

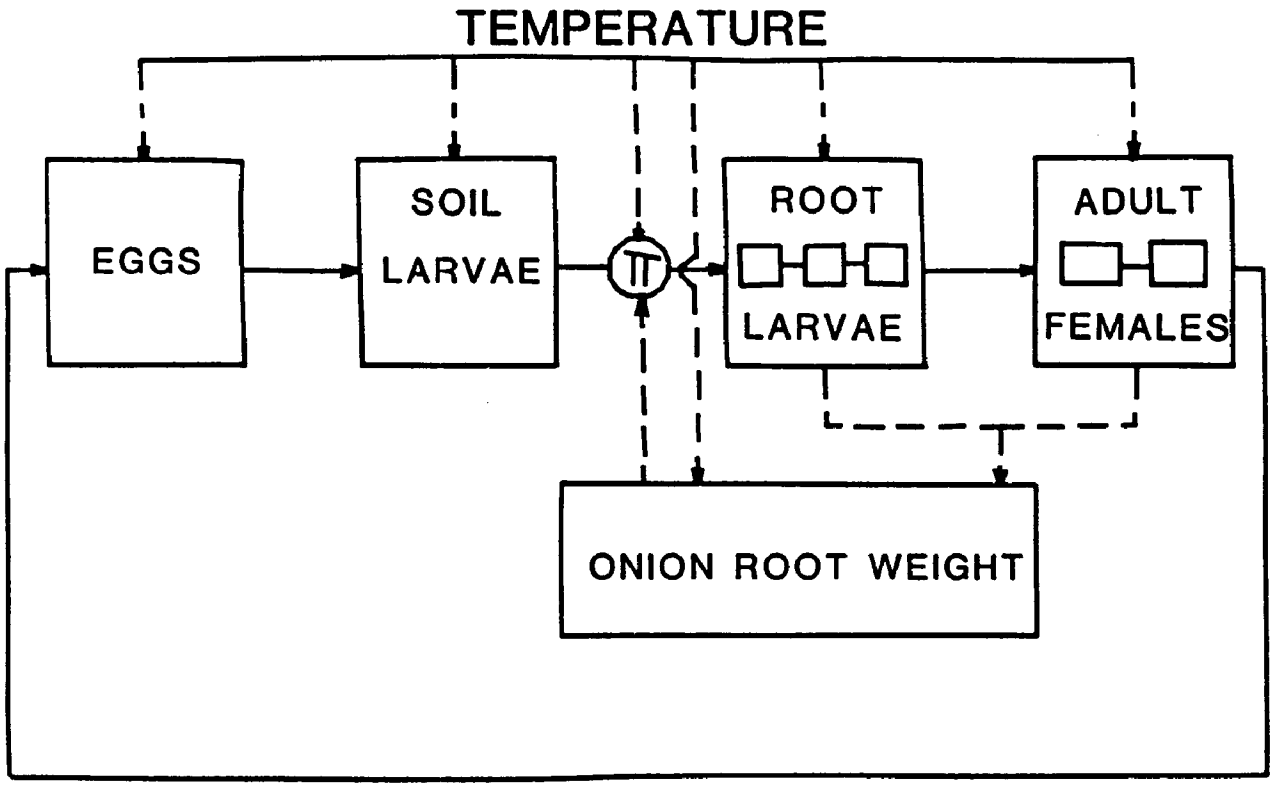


Figure 2. Overview of a computer model simulating the population dynamics of Meloidogyne hapla associated with Allium cepa.

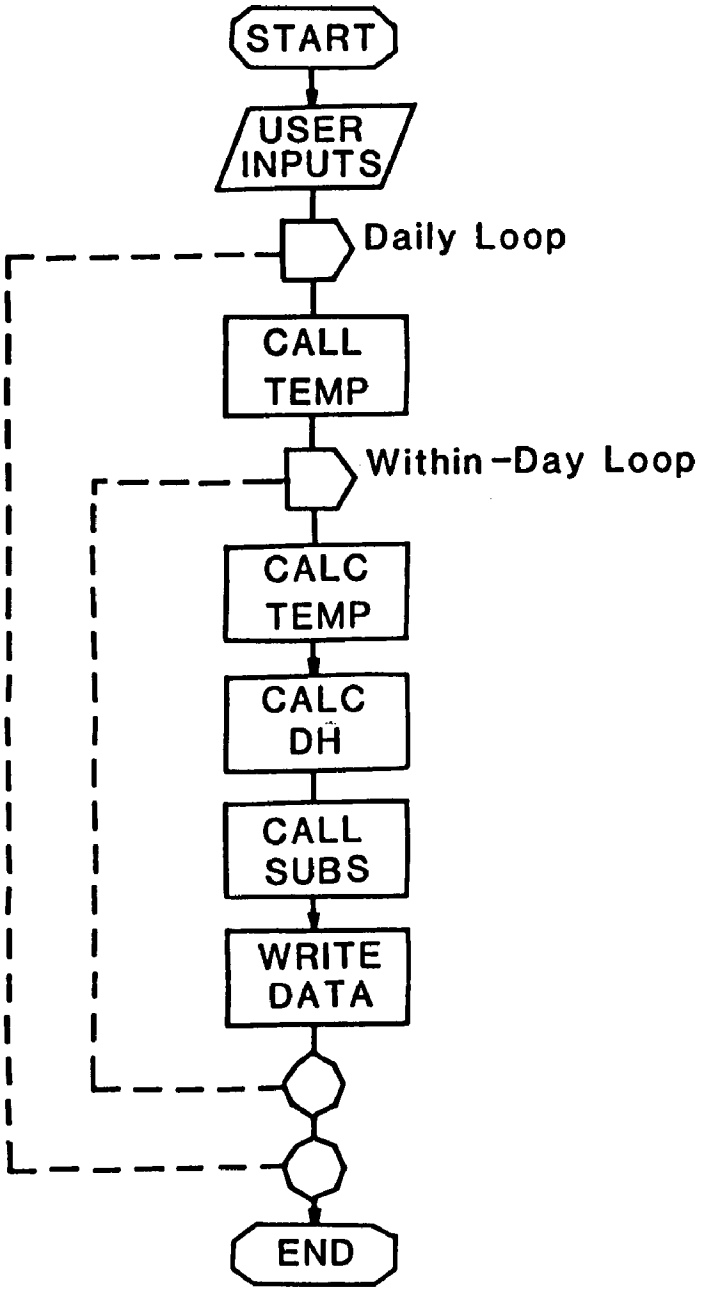


Figure 3. Flow chart of a computer model simulating the population dynamics of Meloidogyne hapla associated with Allium cepa.

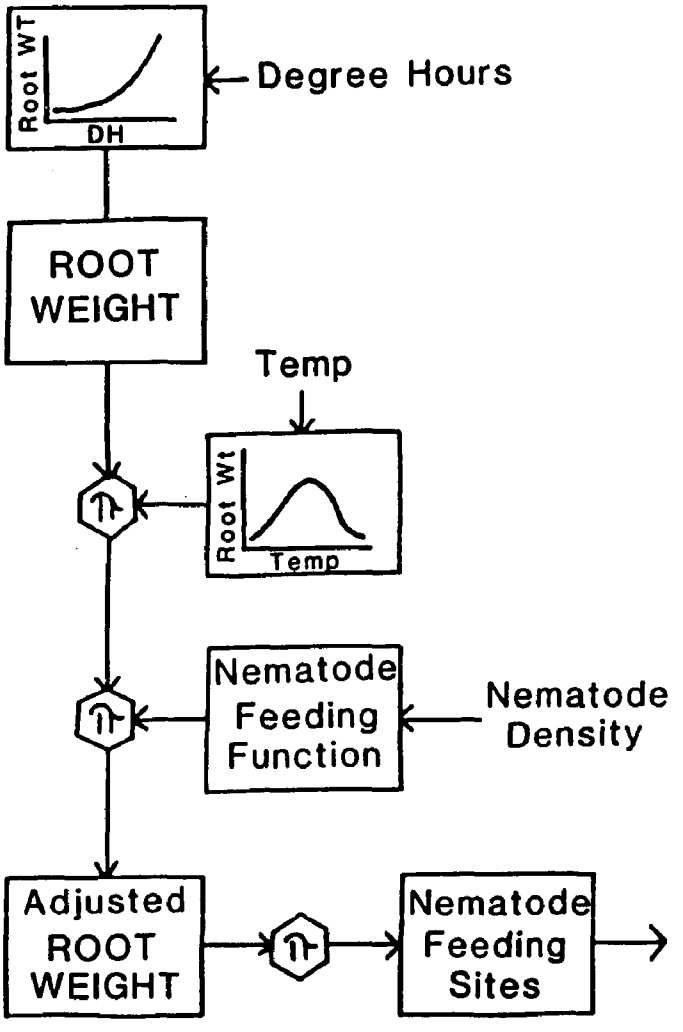


Figure 4. Flow chart of Subroutine Onion.

$$tempfac = -9.4925 + (1.8461*temp) - (0.1115*temp^2 + (0.00265*temp/D/D3/U/U) - (0.00002092*temp^4)$$

No root growth is considered to occur below 10 C or above 30 C. Nematode density is also used to modify the estimate of root growth from what would be expected under optimal edaphic conditions. The adjusted root weight is then passed as an input back to Subroutine Nema.

Subroutine Nemfeed

The feeding function used in the model was determined from the experiment described on pages 69-101 . A decreased influence of each individual nematode on plant root weight with increasing population levels was observed in this study. In the model, the percent root reduction attributed to each nematode is described by the following equation:

$0.0005019 + 0.65623 / feednem * DT$ (feednem = total no. nemas feeding).

The impact of nematode feeding on plant root weight is decreased after the accumulation of 8000 degree hours, to reflect the differential tolerance of new seedlings and established plants to nematode parasitism.

Subroutine Nema (Figures 5, 6, 7)

Nematodes in the model are divided into seven life stages; eggs, soil juveniles, early second-stage juveniles, late second-stage juveniles, third/fourth-stage juveniles, pre-ovipositing adults, and ovipositing adults. The true life stages of M. hapla were subdivided or combined to more accurately estimate nematode development and survival. Early second-stage juveniles, for example, are expected to have the greatest mortality level, since it is this stage that must

select and establish a feeding site within host root tissue. In contrast, third- and fourth-stage juveniles do not feed and develop rapidly. Currently, rates of nematode development are computed solely from soil temperature data. The survival of early second-stage juveniles is considered to be 80%, and 100% for the other root-inhabiting stages. These values were obtained from the experiment described on pages 124-133.

In the model, eggs develop into soil juveniles (Figure 5). A developmental subroutine is used to progress soil juveniles to death rather than to an advanced developmental stage. Soil juveniles die in 14 days when soil temperature is less than 20 C, in 7 days when soil temperature is above 20 C, and in 1000 days when soil temperature is less than 9 C. The latter condition was imposed to approximate the delayed development of nematodes in a state of cryptobiosis. All juveniles in the soil are available for root ingress. The number of juveniles sucessfully penetrating roots, however, is a function of several factors. The mobility of juveniles in the soil is greatest at 20 C and is reduced to negligible distances when temperatures are less than 10 C or greater than 30 C (Wallace, 1965). The carrying capacity of onion root systems is estimated to be 5000 when plants are less than 54 days old and 1000 when plants are greater than 54 days old. These esitmates are based on observations and were not confirmed experimentally. The number of juveniles penetrating roots is reduced by 25% in plants with a mycorrhizal association, according to an experiment described on pages 161-173.

Within the root, juveniles progress through three developmental stages (Figure 6). Second-stage juveniles are subdivided into early and late second stages. Early second-stage juveniles are nematodes that have recently pene-

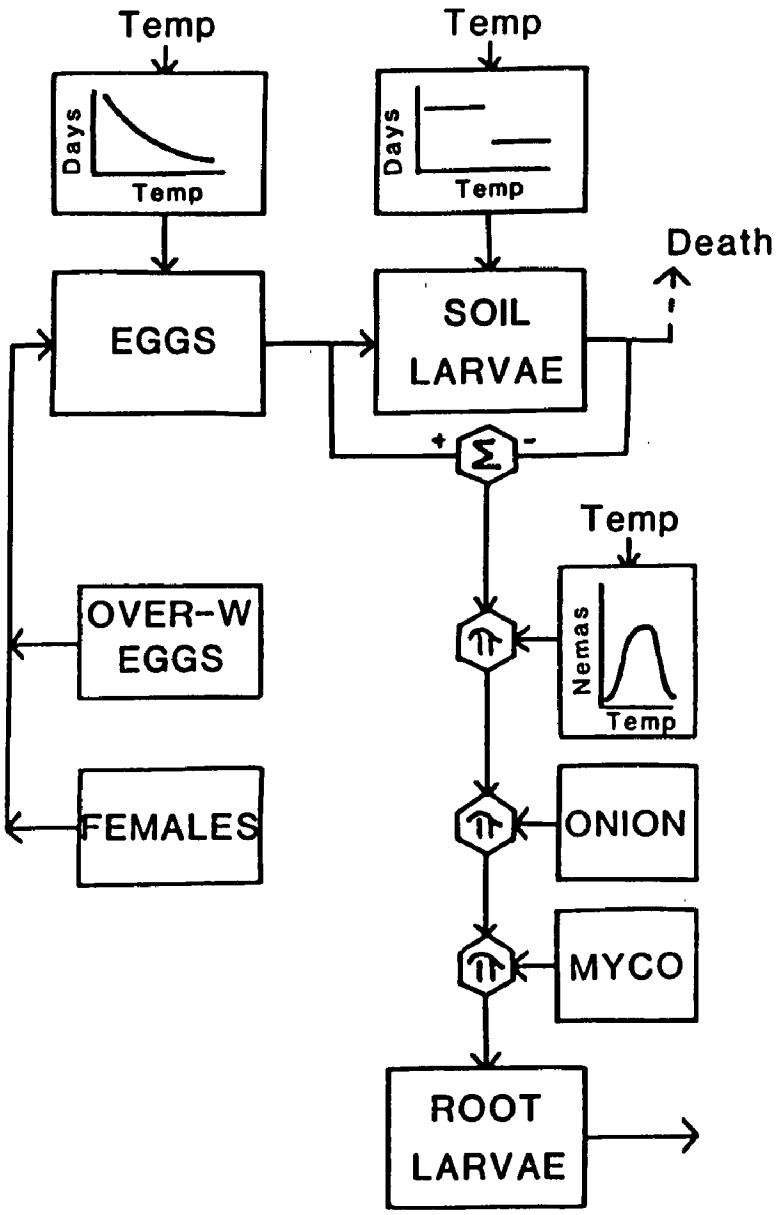


Figure 5. Flow chart of Subroutine Nema from the egg stage until the penetration of Allium cepa roots by Meloidogyne hapla juveniles.

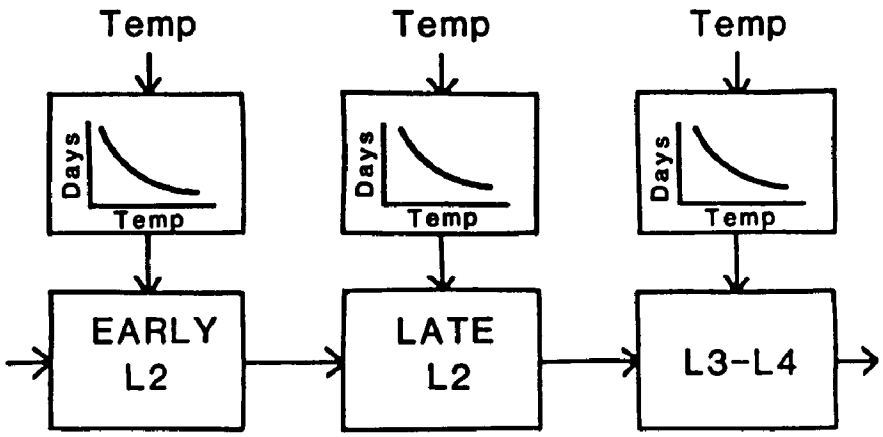


Figure 6. Flow chart of Subroutine Nema: the root-inhabiting stages of Meloidogyne hapla.

trated root tissue and have not begun feeding. Late second-stage juveniles are sexually-differentiated and feeding nematodes. The third and fourth juvenile stages are combined. Developmental rates for the juvenile stages were obtained from the literature (Tyler, 1933; Vrain et al, 1978; Wong and Mai, 1973a). The minimal rate of development, considered to occur at 22 C, is 10 days for early second-stage juveniles, 6 days for late second-stage juveniles, and 2 days for the combined third- and fourth-stage juveniles.

All juveniles successfully maturing are assumed to become adult females (Figure 7). Pre-ovipositing females are distinguished from ovipositing females, due to the relatively long period of time required for reproduction to commence. The minimal rate of development for pre-ovipositing females is 8 days when soil temperature is 25 C. Egg production is determined by the number of ovipositing females (i.e., is density dependent). An optimal egg output of 14 eggs/female /day is modified according to soil temperature and nematode density.

Subroutine Soiltemp

This subroutine reads and converts bihourly soil temperature data to an average daily temperature value. If a time step less than one day is used in the simulation, the average temperature for each iteration is calculated using function FNL. The model may also be executed using a constant temperature input.

Subroutines Model 1, Model 2, Model 3

These subroutines were developed by Dr. R. L. Tummala of Michigan State University to model the development of Oulema melanopus. Each stage is

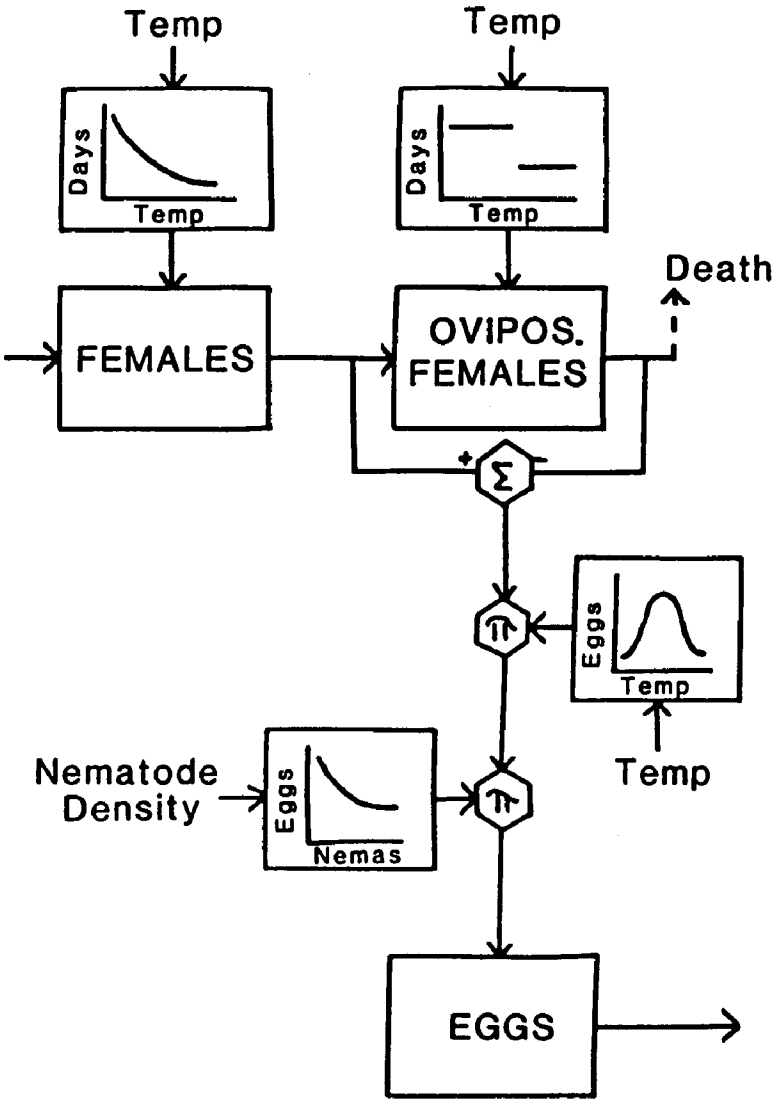


Figure 7. Flow chart of Subroutine Nema: adult stages of Meloidogyne hapla.

divided into ten age classes in the subroutines and both intra- and inter-stage development are calculated. For a further discussion see Tummala et al. (1974).

Model Output

The density of eggs, soil-inhabiting nematodes, and the five nematode stages occurring within plant roots are computed daily. Data on the total number of feeding sites within root tissue, the total number of feeding nematodes, and root weight are also available. The Julian day and accumulated degree hours (base 9 C) are printed with the above information at each daily iteration. (The computer code for the model is presented in Appendix I.)

PROBLEM FORMULATION

The study of population dynamics deals with the abundance and distribution of organisms. The abundance of M. hapla is determined by the survival of these nematodes through time, the rate at which surviving nematodes complete their life cycle, and the fecundity of female nematodes. These population attributes, in turn, are influenced by the quality and quantity of food reserves, climatic conditions, and concomitant soil biota.

It is difficult to evaluate the population dynamics of M. hapla in onion production systems without a preliminary understanding of the biology and ecology of this nematode. For this reason, only a precursory examination of the population dynamics of M. hapla under agricultural conditions was attempted. The majority of my efforts were directed toward research needs that can be addressed in controlled laboratory studies, but yet are useful for investigations on the population dynamics of M. hapla. The identified needs were as follows:

1. Study the response of the onion agroecosystem to M. hapla, determining the occurrence, pathogenicity, and persistence of M. hapla associated with Allium cepa in Michigan.
2. Describe the suitability of A. cepa for the increase of M. hapla populations.
3. Examine the within-generation dynamics of M. hapla contributing to the increase of this nematode in Michigan.
4. Determine the influence of other components of the onion agroecosystem on population attributes of M. hapla.

SYSTEM IDENTIFICATION AND MENSURATION OF SYSTEM COMPONENTS

A subset of all M. hapla in Michigan, those infecting A. cepa, was the object of concern for this research. M. hapla associated with other crops were not considered in order to minimize variability in measurements of populaion attributes. The rationale for this decision was based on observations that the host plant is the primary factor influencing the increase of Meloidogyne populations. Oviposition by M. javanica, for example, is almost twice as great on tomato as on sugarcane (Balasubramanian and Rangaswami, 1963).

For field experiments, a population of M. hapla was defined to be those nematodes infecting areas of onion plants that could be spatially demarcated from other areas containing onions. Movement between populations was assumed to be minimal, since only one or two generations of M. hapla normally occur during one growing season in Michigan. Some passive dispersal of nematodes due to wind, water, or machinery may have occurred prior to planting and harvest, since soil-inhabiting juveniles are most prevalent at these times. The passive

relocation of M. hapla, however, was assumed to be a random event and of negligible importance to the ontogeny of nematodes during the experimental period.

The onion agroecosystem consists of many components that influence the population dynamics of M. hapla (Figure 1). The research discussed here considered only two components affecting M. hapla levels; the first order interaction with A. cepa and the second order interaction with the VAM Glomus fasciculatus. Abiotic components of the environment which were monitored during experimentation were soil temperature and moisture.

The association of M. hapla, A. cepa, and G. fasciculatus was characterized in greenhouse experiments. Population attributes of M. hapla were determined using nematodes infecting individual A. cepa plants. Data from the greenhouse studies were used to supplement observations from field studies and to provide the basis for further experimentation.

One of the major goals of this research was to measure the influence or pathogenicity or M. hapla to A. cepa. The pathogenicity of any organism to a plant is assessed by measuring the ability of that organism to incite disease. Disease, in turn, can be defined as "any disturbance brought about by a pathogen or an environmental factor which interferes with manufacture, translocation, or utilization of food, mineral nutrients, and water in such a way that the affected plant changes in appearance and/or yields less than a normal, healthy plant of the same variety" (Agrios, (1978). Parameters of plant growth are generally measured to assess the extent of disease exhibited by a plant, since "growth represents the excess of constructive over destructive metabolism" (Sinnot and Wilson, 1955).

Parameters of total plant weight and weight of component plant parts were used most often in my analyses. Weight measurements were easy to obtain and standardize. In many of the experiments, particularly those conducted in the greenhouse, the weight of fresh rather than dry plant material was obtained, because the plants tended to be small and the mensuration of dry plant material would have required specialized equipment and expertise among employees.

Fresh weight is generally not as reliable an indicator of plant growth as dry weight, since fluctuations in the water content of plants can cause changes in weight that are not due to the increase of plant tissue. A significant (P=0.05) linear relationship between fresh and dry weights was obtained for my experiments, however, demonstrating that fresh weight was suitable as at least a relative indication of plant growth (Figure 8). The relationship between the fresh and dry weight of the A. cepa grown under field conditions was more variable, but yet still statistically significant (Figure 9).

I also measured the size of plant components in some experiments. Leaf area was assessed by a Li-cor leaf area meter. The same apparatus was used to measure root area for one experiment, but the practice was discontinued because of the difficulty of accurately measuring individual roots. Root length, measured by hand with a ruler, proved to be a more satisfactory indicator of the size of the root system. The number of growing tips in a root system was also recorded for some experiments. The volume of onion bulbs was assessed by measuring the volume of water displaced when the bulbs were immersed.

Procedures used for the inoculation and quantification of vesicular--arbuscular mycorrhizal (VAM) fungi were adapted from previously published techniques (Schenck, 1982). Fungal chlamydospores were used as inoculum in all

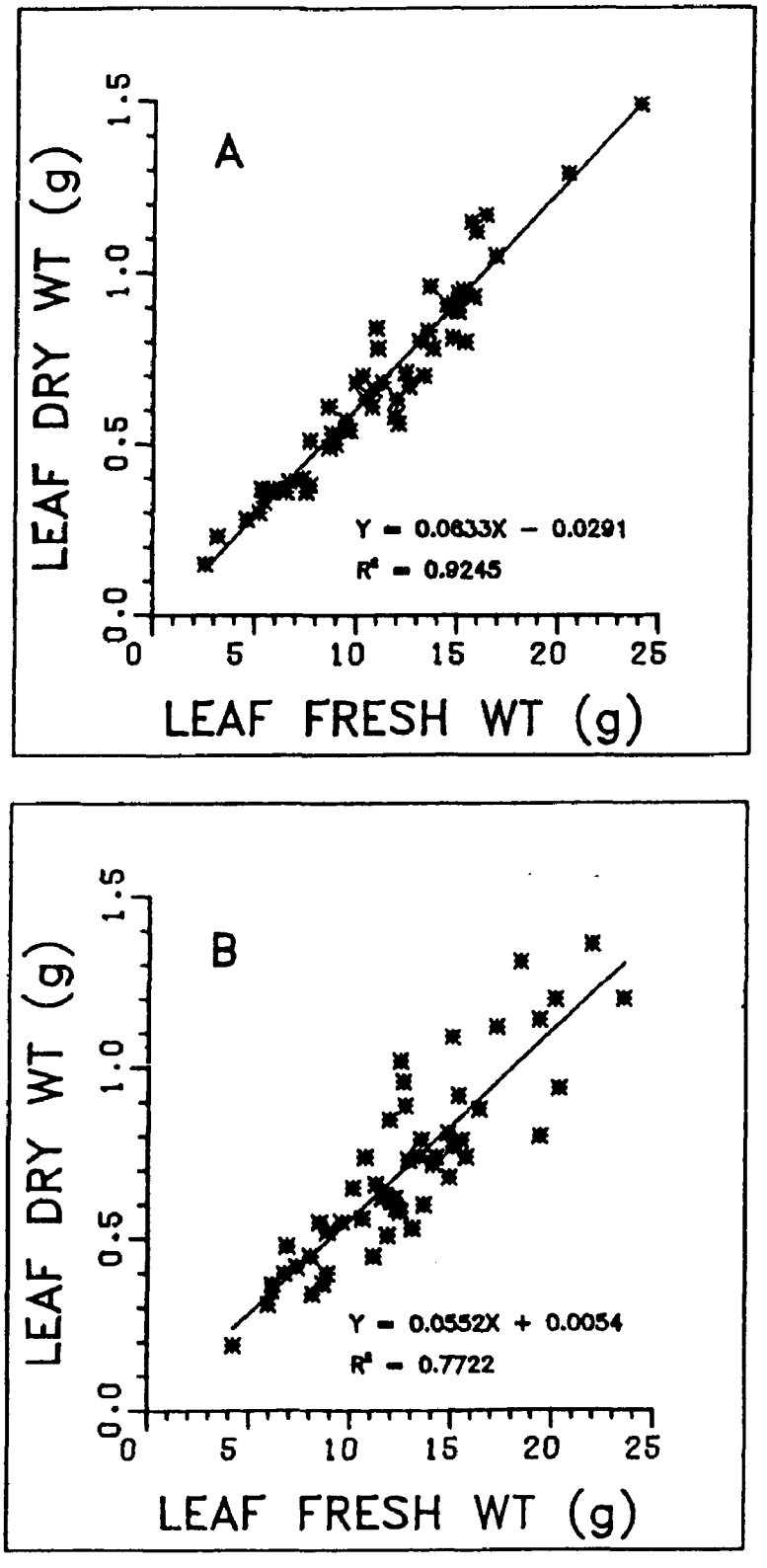


Figure 8. Relationship between fresh and dry leaf weights of Krummery Special (A) and Downing Yellow Globe (B) Allium cepa grown in the greenhouse.

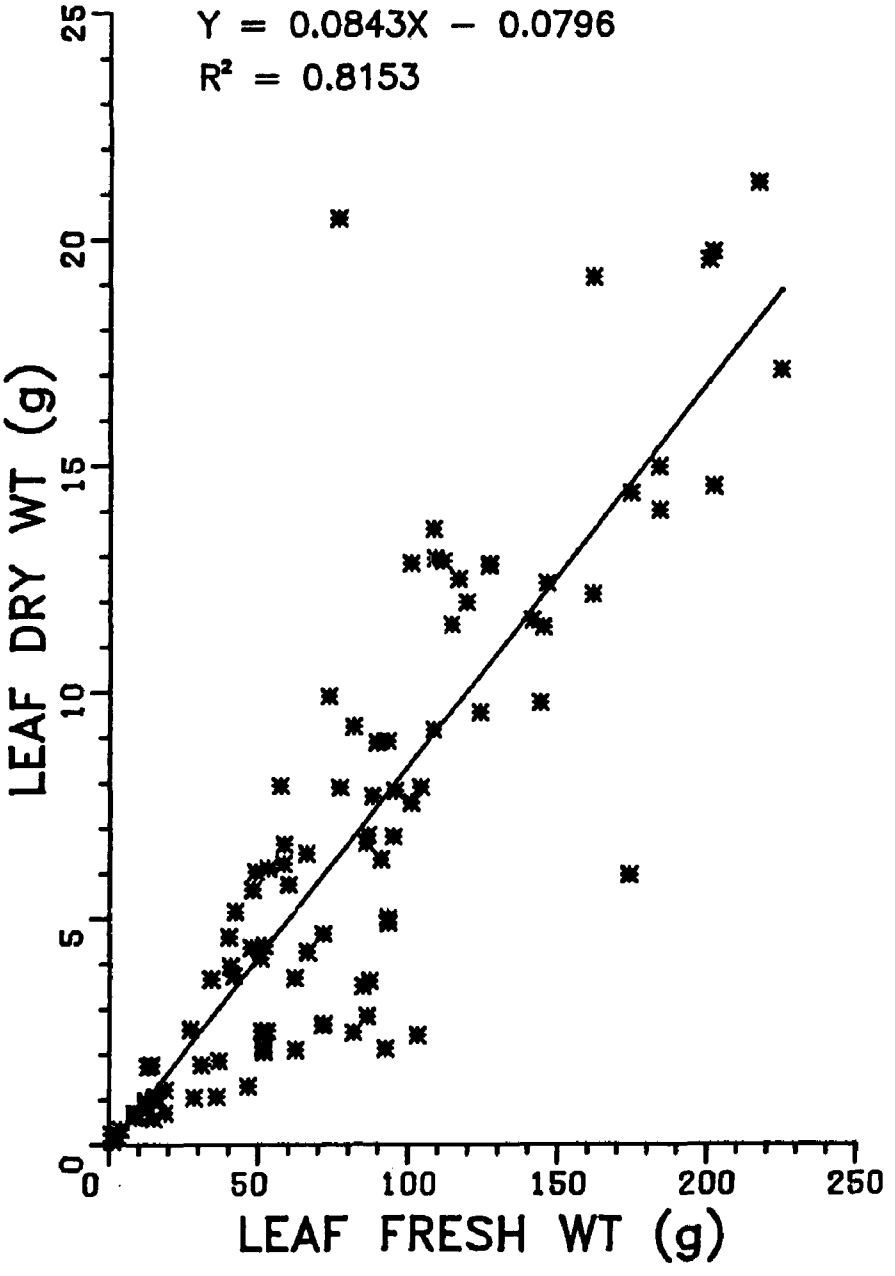


Figure 9. Relationship between fresh and dry leaf weights of Krummery Special Allium cepa grown under field conditions.

experiments for several reasons: (1) it was easy to quantify and standardize the dose received by each plant, (2) spores could by extracted from soil samples simultaneously with nematodes, and (3) the possibility of contamination of inoculum by other organisms was minimal. Spore density in the soil was used to confirm the colonization of roots by VAM and to characterize the development of the fungal association. The presence of spores is not as reliable an indicator of VAM colonization as is the presence of fungal hyphae, vesicles, and arbuscules in root tissue, but does demonstrate whether or not colonization has occurred. Enumeration of spores provides a gross measure of the extent of colonization.

I also attempted, unsuccessfully, to quantify VAM colonization in root tissue. To properly visualize VAM it is necessary to clear roots of all internal structures. Unfortunately, all clearing procedures destroyed the nematodes present in root tissue. To minimize the loss of M. hapla within roots, ten 2 cm sections from each root system were stained for VAM, and the remainder of the root system was stained for nematodes. This amount of root tissue proved to be too little for assessing the extent of fungal colonization in large root systems, and too large in proportion to the size of root systems produced in most of my greenhouse experiments. In fact, the small A. cepa root systems examined in the greenhouse experiments, particularly in the first six weeks after seeding, were not amenable to mycorrhizal analyses, since both the fungus and nematode entered roots near the growing root tips. Removing any tissue at all destroyed a significant proportion of the nematodes inhabiting that root system. Nor was it possible to increase the size of greenhouse experiments to include plants for mycorrhizal analysis alone, due to the size of the controlled-temperature facilities available. The limitations imposed on experimental design by assessing

VAM only through the quantification of soil spore levels were not serious for the experiments described here, since the goal of these experiments was to ascertain what, if any, life-stage or process of M. hapla is influenced by the presence of VAM. Simultaneous quantification of mycorrhizae and nematode colonization will be essential to the further elucidation of the relationship between these two organisms and plant growth, and should be a high research priority.

The accurate assessment of M. hapla population levels was important to my research. The aggregate distribution of nematodes in the field and the difficulty of extracting nematodes from the soil medium are constraints common to all nematological research. To minimize the variability associated with the pattern of nematode distribution, experiments in the field were conducted in an area not larger, and generally smaller, than 15.4 x 30.8 m. Microplots were also used, providing the benefits of a small confined area and a variable climatic environment approaching actual field conditions. In all field experiments a large number of soil cores relative to the sample area were obtained for each soil sample. The samples were thoroughly mixed before a subsample of 100 cm^3 was removed for the assay of nematodes and mycorrhizal spores. Roots were carefully removed from each sample and stained to reveal the nematodes within.

Entire root systems were stained for the observation of nematodes in the greenhouse experiments, in order to reduce the sampling error associated with patterns of nematode distribution within root tissue. Soil from each pot was thoroughly mixed before a subsample was removed for nematode analysis. The size of the pots was varied in each experiment, depending on the duration and expected level of nematodes within the soil. For example, small pots were used when the presence of many second-stage juvenile M. hapla was anticipated. The

duration of most experiments, however, was not sufficient for the production of a second nematode generation and levels of M. hapla in the soil remained low.

Sampling error associated with those M. hapla life stages inhabiting root tissue was small, since entire root systems and all root fragments in the soil were examined for nematodes. In contrast, there was sampling error in enumerating second-stage M. hapla in the soil. Error in estimating soil levels of nematodes is introduced in the (1) collection of samples, (2) storage of samples, and (3) extraction of nematodes from the soil. The process of extracting nematodes from soil is variable between nematology facilities and is subject to many sources of error (Kotcon, 1979; Moriarty, 1960; Skellam, 1962).

I conducted an experiment to assess the error and variability associated with extracting M. hapla juveniles from a muck soil (Appendix II). The efficiency of my extraction procedure, a modified centrifugation-flotation technique (Jenkins, 1964), was 35% and independent of nematode density. The data presented in this document has not been adjusted by this extraction efficiency factor and so reflect the actual number of nematodes obtained from the soil extraction procedure.

CHAPTER I.

Studies on the Occurrence, Pathogenicity, and Increase of *Meloidogyne hapla* Associated With *Allium cepa*

Meloidogyne hapla is a parasite of many plant species and is an economically damaging pathogen of a wide range of agricultural crops. It is one of the most important pests in Michigan and has been the subject of much research. I conducted several studies to clarify the interaction between *M. hapla* and *Allium cepa*. The goals of this research were twofold: (1) to provide information useful to Michigan growers specializing in muck vegetable production and (2) to explore the ecological relationship between a nematode pathogen and its plant host. The specific objectives of the experiments described here were: (1) to determine the incidence of *M. hapla* associated with *A. cepa* in Michigan, (2) to assess the pathogenicity of *M. hapla* to *A. cepa*, and (3) to assess the suitability of *A. cepa* for the increase of *M. hapla* populations.

Survey of Michigan Onion Acreage for *Meloidogyne hapla* and Other Phytoparasitic Nematodes

INTRODUCTION

Seventy-eight genera of plant-parasitic nematodes have been observed from agricultural sites in Michigan (Knobloch and Bird, 1981). Many of these genera are associated with *Allium cepa* and other vegetables grown in muck soils. B. G. Chitwood, in a 1953 report, listed *Ditylenchus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Helicotylenchus* spp., and *Trichodorus* spp., as the most important pests of *A. cepa* in Michigan. Since little is known on the suitability of *A. cepa* as a host to phytoparasitic nematodes, and in particular to *Meloidogyne hapla*, I conducted a survey of *ca* 5% of the onion acreage in Michigan. Each site in the survey was sampled three times to detect changes in the population levels of phytoparasitic nematodes throughout the growing season.

MATERIALS AND METHODS

Farms from the following counties were selected for the survey: Ingham, Calhoun, Lapeer, Ottawa, and Newaygo (Figure 10). Survey participants were selected from lists of growers provided by the Michigan Cooperative Extension Service District Horticultural Agents serving these areas. Onion acreage was not a criterion for selection. Consequently, farms of all sizes with onion acreage ranging from 10 to 150 acres were included in the survey. Each grower provided information on 1) onion cultivar, 2) preceding crop, and 3) pesticides used. Observations on soil type, use of windbreaks, etc. were recorded when the samples were collected.

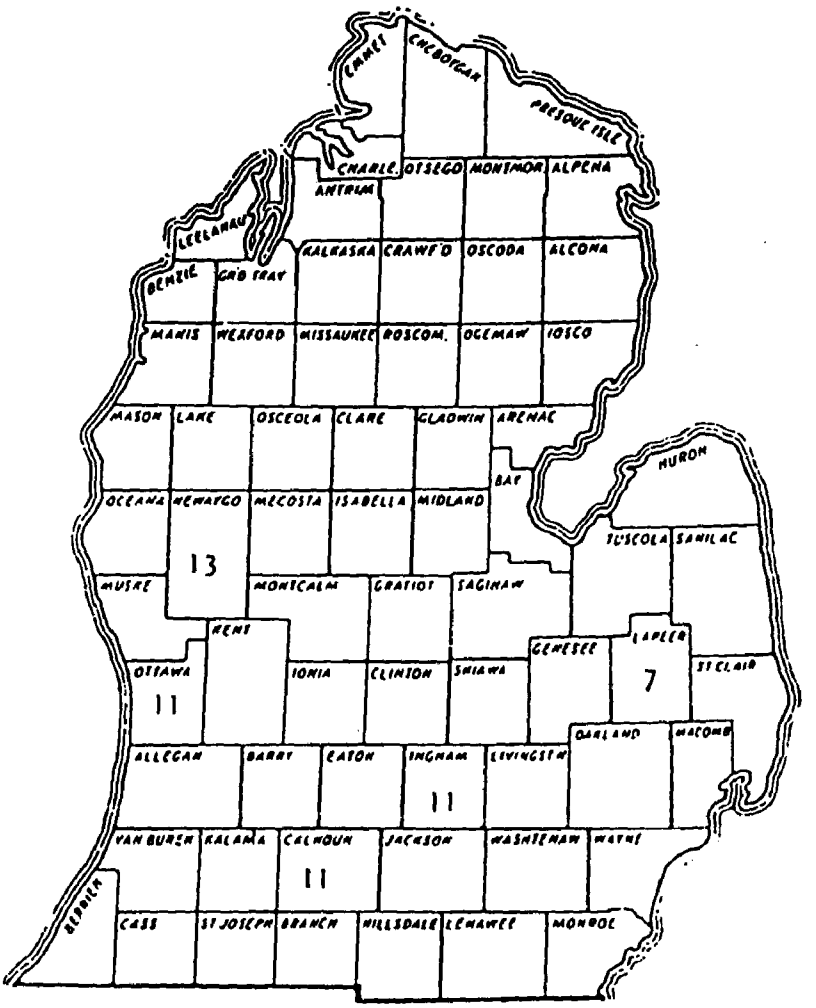


Figure 10. Location and number of sites in five counties in Michigan surveyed for phytoparasitic nematodes in 1980.

One to five sites, each ca five acres, were sampled on each farm. A minimum of 20 soil cores and five plants comprised each sample. The cores were collected in a random pattern at a depth of 15 cm. The location of sites contained within larger fields was mapped for future visits. Each site was sampled once in May, July, and September.

Both soil and roots were assayed for nematodes. A 100 cm^3 subsample was removed from each soil sample and processed by a sugar flotation-centrifugation technique (Jenkins, 1964), using a sugar solution with 1.37 specific gravity. The final product, a suspension of nematodes and debris in water, was poured onto a glass slide and observed through a dissecting microscope at 150X. All plant-parasitic nematodes and spores of vesicular- arbuscular mycorrhizal (VAM) fungi on one half of the slide were enumerated, and the remaining portion of the slide scanned. Two subsamples of 0.25 g each were removed from each root sample, which consisted of the roots from collected plants and root fragments found in the soil sample. Each root subsample was processed and stained in a solution of lactophenol with 0.01% acid fuchsin for observation of either nematodes or VAM colonization.

Nematode counts from the soil samples collected in the survey were used to determine the nematode genera present, the proportion of sites infested with a particular nematode genus, and the density of each genus per sample. The similarity of sites within counties in regard to the nematode genera represented was determined using two methods:

1. Sorensen's (1948) Method:

   $IS_s = 2c/a + b$

where:

c = no. of genera two counties have in common

a = no. of genera in county a

b = no. of genera in county b

2. Bray and Curtis' (1957) Method

$$C_n = 2jN/aN + bN$$

where:

jN = the sum of the lesser values for the genera common to both counties

aN = total no. of nematodes in county a

bN = total no. of nematodes in county b

A tomato bioassay was also used to detect the soil-inhabiting stages of Meloidogyne hapla. Approximately 1500 cm^3 of soil from each sample was planted with a one-month-old tomato seedling (cv Rutgers). The tomato plants were harvested after ca 8000 degree hours (base = 9 C). After the roots were weighed, cut, and mixed, a 0.25 g subsample was stained for the observation of nematodes. In addition, a 100 cm^3 soil sample was processed as described previously for nematode detection.

Data from samples containing M. hapla were classified according to the crop planted in the sample site the preceding year and the A. cepa cultivar planted. The soil samples were submitted to the Michigan State University Soils Diagnostic Laboratory for nutrient analysis.

RESULTS

Fifty-three sites, representing ca 105 hectares, were monitored for nematodes. Forty-eight of the 53 sites contained at least one genus of plant-parasitic

nematodes. The genera found most often were Pratylenchus, Tylenchorhynchus, and Meloidogyne, with absolute frequencies of 73.58, 50.94, and 28.30%, respectively (Table 1). Nematode genera found less frequently included Trichodorus, Paratylenchus, and Heterodera. Predatory nematodes (family Mononchidae) were observed in 20.75% of the samples. Spores of vesicular-arbuscular mycorrhizal (VAM) fungi (Glomus and Gigaspora spp.) were observed in 100% of the samples. Not all genera observed within a site on one sampling date were observed on other sampling dates (Table 2). Densities of each nematode genera fluctuated throughout the growing season.

Pratylenchus and Tylenchorhynchus spp. were observed in September in all counties sampled (Table 3). The highest nematode densities were generally observed in samples from Newaygo and Ottawa counties, although even in those sites there tended to be less than 10 nematodes per sample. According to Sorensen's Index of Similarity (1948), Calhoun and Lapeer counties were most similar in terms of the nematode genera present (Table 4). Other counties which shared a high proportion of nematode genera were Ingham and Calhoun, Ingham and Lapeer, Ingham and Ottawa, and Ingham and Newaygo. When Bray and Curtis' (1957) Index was used to compare the number and levels of nematode genera between counties, however, Ingham and Lapeer counties were more similar than any other combination of counties. Ingham and Ottawa counties, according to this index, did not contain a comparable variety or density of nematode genera.

Fifteen sites contained Meloidogyne hapla. This nematode was detected in soil and root samples from 12 of these sites and in the tomato bioassay only, from three sites. Both the relative density of M. hapla in soil samples and the mean number of M. hapla in root samples increased during the 1980 growing

Table 1. Incidence of phytoparasitic nematodes in Michigan onion acreage surveyed in 1980.

Nematode genus	No. of sites containing ea. genus (total = 53)	Absolute frequency
Meloidogyne spp.	15	28.30
Pratylenchus spp.	39	73.58
Tylenchorhynchus spp.	27	50.94
Trichodorus spp.	12	22.64
Paratylenchus spp.	10	18.87
Heterodera spp.	3	5.67
Predaceous genera	11	20.75

Table 2. Incidence of six nematode genera in Michigan onion acreage in May, July, and September of 1980.

Sample period and genus	Mean no. nematodes per 100 cm^3 soil and 0.25 g roots + (S.E.) n = 53		Percent sites infested
May			
Tylenchorhynchus spp.	0.53	(0.1695)	30.19
Pratylenchus spp.	1.62	(0.4775)	24.53
Meloidogyne spp.	0.55	(0.2633)	15.09
Trichodorus spp.	0.		0.
Heterodera spp.	0.		0.
Paratylenchus spp.	0.17	(0.1166)	7.55
July			
Tylenchorhynchus spp.	2.52	(0.6332)	33.96
Pratylenchus spp.	14.70	(5.1914)	56.60
Meloidogyne spp.	0.77	(0.2742)	16.98
Trichodorus spp.	0.34	(0.1765)	7.55
Heterodera spp.	2.68	(1.8222)	7.55
Paratylenchus spp.	0.38	(0.1708)	11.32
September			
Tylenchorhynchus spp.	6.38	(1.8957)	43.40
Pratylenchus spp.	7.81	(2.4177)	52.83
Meloidogyne spp.	1.87	(0.8373)	16.98
Trichodorus spp.	1.04	(0.3418)	22.64
Heterodera spp.	0.80	(0.6450)	5.67
Paratylenchus spp.	0.22	(0.1282)	7.55

Table 3. Incidence of six nematode genera in five Michigan counties in September, 1980.

Nematode genera and county	No. sites sampled (n)	Percent sites infested	Mean density per 100 cm^3 soil and 0.25 g roots (S.E.)	Percent sites with given density				
				0	1-10	11-25	26-50	51-100
Pratylenchus								
Ingham	11	55	6.36 (2.3482) ab	45	55	0	0	0
Calhoun	11	36	3.09 (1.3912) b	64	36	0	0	0
Lapeer	7	43	4.86 (3.3766) ab	57	29	14	0	0
Ottawa	11	64	2.82 (0.9127) b	36	64	0	0	0
Newaygo	13	54	18.85 (8.9635) a	46	23	0	23	8
Meloidogyne								
Ingham	11	0	0. a	100	0	0	0	0
Calhoun	11	0	0. a	100	0	0	0	0
Lapeer	7	14	0.57 (0.3780) a	86	14	0	0	0
Ottawa	11	0	0. a	100	0	0	0	0
Newaygo	13	62	7.31 (3.003) b	38	46	8	8	0
Tylenchorhynchus								
Ingham	11	36	1.45 (0.5455) a	64	36	0	0	0
Calhoun	11	45	4.55 (1.7496) a	55	45	0	0	0
Lapeer	7	43	2.57 (1.4286) a	57	43	0	0	0
Ottawa	11	82	21.45 (7.3365) b	18	46	18	18	0
Newaygo	13	8	1.38 (1.3846) a	92	8	0	0	0

Table 3. continued

Nematode genera and county	No. sites sampled (n)	Percent sites infested	Mean density per 100 cm^3 soil and 0.25 g roots (S.E.)		Percent sites with given density				
					0	1-10	11-25	26-50	51-100
Trichodorus									
Ingham	11	9	0.18 (0.1818)	a	91	9	0	0	0
Calhoun	11	55	1.64 (0.7042)	ab	45	55	0	0	0
Lapeer	7	0	0.	a	100	0	0	0	0
Ottawa	11	45	3.09 (1.2753)	b	55	45	0	0	0
Newaygo	13	0	0.	a	100	0	0	0	0
Paratylenchus									
Ingham	11	9	0.18 (0.1818)	a	91	9	0	0	0
Calhoun	11	9	0.18 (0.1818)	a	91	9	0	0	0
Lapeer	7	14	0.29 (0.2857)	a	86	14	0	0	0
Ottawa	11	0	0.	a	100	0	0	0	0
Newaygo	13	8	0.46 (0.4615)	a	92	8	0	0	0
Heterodera									
Ingham	11	0	0.	a	100	0	0	0	0
Calhoun	11	0	0.	a	100	0	0	0	0
Lapeer	7	0	0.	a	100	0	0	0	0
Ottawa	11	0	0.	a	100	0	0	0	0
Newaygo	13	23	0.31 (0.1748)	a	92	8	0	0	0

[a] Values within a column followed by the same letter are not significantly (P = 0.05) different according to Duncan's New Multiple Range Test.

Table 4. Similarity in the incidence of phytoparasitic nematode genera present in five Michigan counties in September, 1980 according to Sorensen's (A) and Bray and Curtis' (B) Index of Similarity.

A. Sorensen's Index of Similarity

	County				
County	Ingham	Calhoun	Lapeer	Ottawa	Newaygo
Ingham	1.0000	0.9231	0.9231	0.8333	0.8571
Calhoun		1.0000	1.0000	0.7273	0.7692
Lapeer			1.0000	0.7273	0.7692
Ottawa				1.0000	0.6667
Newaygo					1.0000

B. Bray and Curtis' Index of Similarity

	County				
County	Ingham	Calhoun	Lapeer	Ottawa	Newaygo
Ingham	1.0000	0.4783	0.6667	0.1412	0.5775
Calhoun		1.0000	0.5897	0.4127	0.3243
Lapeer			1.0000	0.1657	0.3125
Ottawa				1.0000	0.1172
Newaygo					1.0000

season (Table 5). Onion cultivars grown during the survey in the M. hapla-infested sites were Spartan Banner, Trapp, Krummery Special, and Harris. More than one cultivar or unknown cultivars were present in three sites (Table 6). The crop grown in the year preceding the survey was carrot in seven sites, onion in six sites, and mint or potato in one site each (Table 7).

Sites not containing M. hapla were planted with Spartan Banner (6 sites), Downing Yellow Globe (6 sites), Krummery Special (3 sites), Trapp (3 sites), Sentinel (2 sites), Fiesta (2 sites), Cima (1 site), Abbot (1 site), or unknown (14 sites) cultivars. Of the sites with no M. hapla present, five had been planted with carrot the preceding year, three with onion or an unknown crop, two each with potato or radish, and one site each with sod, soybean or lettuce.

Phosphorus, potassium, calcium, and magnesium averaged 224.26, 488.53, 10283.73 and 732.13 ppm in those sites containing M. hapla (Table 8). M. hapla density in September was most highly correlated with soil pH and, to a lesser degree, with phosphorus, potassium, and calcium levels (Table 9). M. hapla density was not related to soil levels of magnesium.

Seventy-three percent of the sites containing M. hapla also contained Pratylenchus spp. Levels of Pratylenchus in soil and root samples averaged 1.62 nematodes in May, 14.70 nematodes in July, and 7.81 nematodes in September (Table 2). There was no correlation between population densities of Pratylenchus spp. and M. hapla (Table 9).

Tylenchorhynchus spp. occurred concomitantly with M. hapla in 47% of the sites surveyed. Mean soil levels of Tylenchorhynchus were 0.53, 2.52, and 6.38 nematodes per 100 cm^3 soil in May, July, and September, respectively (Table 2). There was a weak negative correlation between population densities of Tylenchorhynchus and M. hapla in the September sample (Table 9).

Table 5. Incidence of *Meloidogyne hapla* in Michigan onion acreage surveyed in 1980.

Sample [a]	May		July		September	
	No.[b]	R.D.[c]	No.	R.D.	No.	R.D.
1	1	6.25	0	0.	0	0.
2	6	3.33	0	0.	4	18.18
3	0	0.	2	2.50	0	0.
4	0	0.	0	0.	12	85.71
5	0	0.	2	1.11	8	36.36
6	12	50.00	0	0.	8	28.57
7	2	50.00	2	2.50	22	100.00
8	0	0.	0	0.	6	42.86
9	0	0.	6	50.00	2	20.00
10	2	50.00	0	0.	8	66.67
11	0	0.	2	1.11	2	10.00
Mean	2.09	14.51	1.27	5.20	6.55	37.12
S.E.	0.1318	6.8987	0.5574	4.4897	1.9276	10.1971
Mean no. *M. hapla* per 0.25 g root	0.09		2.18		2.45	
S.E.	0.0909		0.9612		1.5097	

[a] Four sites where the presence of *M. hapla* was detected only in roots or by a tomato bioassay are not included in this table.

[b] Number of *M. hapla* per 100 cm^3 soil

[c] Relative density of *M. hapla* per sample R.D. = $\frac{\text{No. } M. \text{ hapla}}{\text{Total no. of all nematode genera}}$

Table 6. *Allium cepa* cultivar planted in sites infested with *Meloidogyne hapla*.

Cultivar	*M. hapla* / 100 cm^3 soil + 0.25 g roots		
	May	July	September
Spartan Banner	0.	0.	0.
Spartan Banner	0.	10.	17.
Spartan Banner	0.	4.	14.
Spartan Banner	12.	3.	8.
Spartan Banner	2.	6.	38.
Spartan Banner	0.	0.	6.
Spartan Banner	0.	6.	2.
Spartan Banner	2.	0.	8.
Krummery Special	1.	0.	0.
Harris	0.	0.	0.
Trapp	0.	2.	0.
Trapp	1.	0.	0.
Unknown	0.	0.	0.
Unknown	6.	0.	4.
Unknown	0.	2.	2.

Table 7. Crop planted in 1979 in sites infested with *Meloidogyne hapla* in 1980.

	M. hapla / 100 cm^3 soil + 0.25 g root		
Previous crop	May	July	September
Onion	0.	0.	0.
Onion	0.	2.	0.
Onion	0.	0.	6.
Onion	0.	6.	2.
Onion	1.	0.	0.
Onion	2.	0.	8.
Carrot	0.	0.	0.
Carrot	0.	0.	0.
Carrot	0.	10.	17.
Carrot	0.	4.	14.
Carrot	12.	3.	8.
Carrot	2.	6.	38.
Carrot	0.	2.	2.
Potato	6.	0.	4.
Mint	1.	0.	0.

Table 8. Nutrient analysis for soil samples containing Meloidogyne hapla.

Sample	pH	Nutrient			
		P	K	Ca	Mg
1	5.7	227	552	11093	800
2	5.5	445	616	8533	785
3	5.1	288	456	7467	457
4	6.1	280	368	10240	698
5	6.7	338	792	10027	1600
6	6.3	82	432	12587	1034
7	6.0	445	624	11947	914
8	7.6	27	584	13029	684
9	7.6	8	424	12373	505
10	7.0	259	448	9173	684
11	7.1	179	648	12587	800
12	6.1	78	384	6827	423
13	5.9	349	424	7680	480
14	7.3	42	232	10240	434
15	5.6	317	344	10453	684
Mean	6.37	224.3	488.5	10283	732.1
Standard Error	0.20	38.14	37.26	520.95	77.85
Range	5.1	8.0	232.0	6827.0	423.0
	7.6	445.0	792.0	13029.0	1600.0

Table 9. Correlation matrix between Meloidogyne hapla density in September and soil nutrient level, two phytoparasitic nematode genera and vesicular-arbuscular mycorrhizal spores.

	Final M. hapla density	pH	P	K	Ca	Mg	Lesion[a] nematode	Stunt[b] nematode
pH	.6233							
P	-.4104	-.6714						
K	.2468	.0215	.3989					
Ca	.4667	.5621	-.3565	.2448				
Mg	-.0604	.0320	.3301	.7384	.3343			
Lesion nematode	.0533	.2847	-.3023	-.3655	.0478	-.1725		
Stunt nematode	-.2486	-.2911	.3365	.0210	-.0003	.3663	-.0899	
VAM[c] spores	.2010	.5499	-.6183	-.5682	.1268	-.4273	.0947	-.1632

[a] Pratylenchus spp.

[b] Tylenchorhynchus spp.

[c] vesicular-arbuscular mycorrhizal

DISCUSSION

Pratylenchus spp. were the most common phytoparasitic nematodes inhabiting onion acreage in Michigan in 1980. Pratylenchus penetrans is reportedly pathogenic to A. cepa (Ferris, 1970), although losses incurred by Michigan growers due to this pest have not been estimated. Levels of nematodes detected in soil and root samples increased over the growing season. Since Pratylenchus spp. overwinter as juveniles, the observed increase in nematode levels was most likely due to reproduction by those nematodes present at the beginning of the season, indicating that A. cepa is a suitable host for Pratylenchus spp. Relative to other crops such as corn, however, A. cepa does not seen to support a large or rapid increase in Pratylenchus population growth.

Tylenchorhynchus, Trichodorus, and Paratlyenchus spp. were also detected by this survey. None of these nematodes, with the possible exception of Trichodorus, are believed to be highly pathogenic to A. cepa. The four genera were generally present in low numbers and probably do not increase on A. cepa to levels economically damaging to most agricultural crops.

Nematodes of the genus Heterodera were found only in the Grant swamp area of Newaygo County. Cysts and juveniles were observed in soil samples but were never found within plant roots. Although the nematodes were not identified to the species level, it is unlikely that they are a parasite of A. cepa. The species was tentatively identified as H. carotae and will be examined in more detail in future studies.

Ditylenchus spp. were not found in any site. This was unexpected, since Chitwood identified Ditylenchus dipsaci as a major pest of A. cepa production in Michigan in 1953. It is possible that crops tolerant or of non-host status to

Ditylenchus are now grown in place of A. cepa in areas where this nematode was a problem.

Soil densities of all phytoparasitic nematodes detected in May were below the minimum level for the recommendation of a nematode management tactic, and remained low throughout the growing season. The inconsistent detection of nematodes within the same site during the growing season was probably due to low nematode levels, since an effort was made to reduce sampling error due to nematode aggregation within a sample site by careful mapping and the collection of a large number of soil cores per site.

Pratylenchus spp., a migratory endoparasite, and Heterodera spp., a sedentary endoparasite, reached maximum levels in July, as has been observed for other crops (Norton, 1978). The ectoparasitic genera Tylenchorhynchus spp. and Trichodorus spp., and the endoparasitic genus Meloidogyne spp. reached peak levels in September. The high level of Heterodera spp. in July was probably due to the stimulation of hatch of overwintering eggs by warm soil temperatures. Cooler climatic conditions in September may have inhibited egg hatch. No reproduction by Heterodera was thought to occur during the growing season, since these nematodes were never observed within onion root tissue. Population densities of Pratylenchus may have declined in September due to the natural senescence of onion roots. These nematodes live primarily within root tissue and may have died by the time the samples were collected or migrated to a soil depth of greater than ten cm, escaping detection.

The five counties surveyed were similar in terms of the nematode genera present in September, as measured by Sorensen's Index of Similarity. The counties were less similar when nematode densities were included in the Index of

Similarity, as described by Bray and Curtis. Even so, there were few significant differences in the densities of phytoparasitic nematodes between counties (Table 3). Similarity in the incidence and abundance of nematode genera between distinct geographic regions in Michigan is not surprising, since the habitats sampled in the survey were similar in terms of soil type and general cropping history. In addition, all nematodes observed have a fairly wide host range including A. cepa and many cosmopolitan annual and perennial weeds. Ottawa and Newaygo Counties were least similar to the other counties surveyed. Only three different nematode genera were found in Ottawa County, as compared to five genera in Newaygo County, and four genera in each of the remaining counties (Table 4). Almost all of the sites in Ottawa County had been planted with onion in the three years preceding the survey, which may have been a factor contributing to this county's lack of diversity in phytoparasitic nematode genera.

M. hapla, the target of this survey, was detected in 28.30% of the sites examined. The increasing number of M. hapla found in soil and root samples as the season progressed, suggests that M. hapla populations may be maintained on A. cepa. This contention is supported by the presence of this nematode in sites where A. cepa was grown the previous year.

The incidence and abundance of M. hapla seemed to be related to edaphic factors rather than to the preceding crop or A. cepa cultivar. There was a positive correlation between final M. hapla densities in September and pH, or levels of potassium and calcium in the soil. Contrastingly, a negative correlation between soil phosphorus levels and M. hapla density was observed. These data must be considered cautiously, however, since M. hapla occurred primarily within one county included in the survey. It is possible that some attribute(s) of this

geographic area not measured contributed more to the presence of M. hapla than the quality of the soil environment.

Of the many organisms that influence the population dynamics of M. hapla and other phytoparasitic nematodes, only predatory nematodes and mycorrhizal fungi were detected by this survey. Predatory nematodes were observed in only 20% of the sites, none of which contained M. hapla. Mycorrhizal fungi, on the other hand, were present in all the sites sampled. Concentrations of VAM were higher in nutrient-deficient areas and were mildly correlated with M. hapla density.

The absence of predatory nematodes may be a more important factor contributing to the abundance of M. hapla than the presence of mycorrhizal fungi. The highest counts of M. hapla in soil and root samples was obtained from the Grant swamp area in Newaygo County, where growers generally rotate onion and carrot crops. D. carota is very intolerant of M. hapla (Slinger, 1976) and sites for carrot production are commonly treated with a soil fumigant for nematode control. The high level of M. hapla in the Grant area may be due to the build-up of populations when carrots were grown. However, it is also possible that fumigation indirectly contributes to the increase of M. hapla by eliminating soil biota, such as predatory nematodes, which normally keep M. hapla levels in check.

Reduced plant growth in sites containing M. hapla was not evident by visual inspection, and no attempt as made to quantitatively assess the pathogenicity of this nematode to A. cepa during the survey. Studies to investigate the impact of M. hapla infection on the growth and yield of A. cepa were conducted in the greenhouse and in small experimental plots, and will be discussed in the next section.

Pathogenicity and Increase of *Meloidogyne hapla* Infecting *Allium cepa* as Influenced by Initial Inoculum Level

INTRODUCTION

Meloidogyne hapla is commonly found within the muck soils used to grow many vegetable crops in Michigan and is widely recognized as a serious pest of *Daucus carota* L., *Apium graveolens* L., and *Lactuca sativa* L. These crops are often grown in rotation with *Allium cepa*. The importance of *M. hapla* in *A. cepa* production has not been adequately assessed, nor has the ability of *A. cepa* to support *M. hapla* populations been characterized.

Two studies were conducted in the greenhouse to assess the pathogenicity of *M. hapla* to *A. cepa*. A wide range of nematode inoculum levels was used to determine the relationship between initial *M. hapla* density and parameters of plant growth and yield. These experiments were also used to assess the infuence of nematode density on the increase of *M. hapla* populations.

Processes of natality, mortality, and fecundity influence *M. hapla* population growth. The impact of environmental factors, such as temperature and moisture, on the population dynamics of nematodes has been examined in several studies (Ferris and Van Gundy, 1979; Wong and Mai, 1973a). The influence of the environment is generally assumed to operate independent of nematode density and is considered by some ecologists (Bodenheimer, 1928; Uvarov, 1931) to be the most important determinant of population growth. Others (Nicholson, 1954; Lack, 1954) argue that density-dependent interactions among organisms, such as competition or predation, are of equal or greater importance in regulating population change.

The impact of nematode density on the development of nematode populations has rarely been examined. The experiments described here provided an opportunity to assess the influence of nematode numbers on M. hapla levels in roots and oviposition by M. hapla females.

MATERIALS AND METHODS

Experiment I. Impact of low (500-15,000) initial M. hapla egg density on A. cepa.

Ten seeds of two onion cultivars, Downing Yellow Globe and Krummery Special, were planted in clay pots containing 800 cm^3 (ca 868 g dry wt) of pasteurized muck soil with a pH of 6.6 and a soil phosphorus level of 57 ppm. One of five initial inoculum densities of M. hapla were added to 10 pots of each cultivar: (1) 100 eggs, (2) 1,000 eggs, (3) 5,000 eggs, (4) 10,000 eggs, (5) 15,000 eggs, or (6) water (check). Ten ml of water and the appropriate egg inoculum were placed around the onion seed at planting on 15 September 1979. The plants were thinned to one seedling per pot at emergence. All plants were maintained in the M.S.U. Nematology Greenhouse and watered daily. The experiment was harvested on 3 January 1980, after ca 34,838 degree hours (base 9 C) and the following measures of plant growth were recorded: (1) fresh root weight, (2) root area, (3) fresh bulb area, (4) bulb volume, (5) fresh leaf weight, (6) leaf area, and (7) dry leaf weight. The volume of each bulb was determined by measuring the volume (cm^3) of water displaced when the bulbs were immersed in a water-filled graduated cylinder. Root and leaf areas (cm^2) were measured with a Li-Cor area meter. No attempt was made to compensate for the surface area not measured due to the cylindrical shape of leaves and roots. The measurements, therefore,

should be considered as relative rather than absolute indicators of surface area. One gram of roots from each plant was stained in a solution of lactophenol and 0.01% acid fuchsin, and examined for nematodes under a dissecting microscope. In addition, 100 cm^3 of soil from each pot was assayed for nematodes by a modified sugar flotation-centrifugation techique (Jenkins, 1964). My preliminary experiments demonstrated that ca 35% of the M. hapla present within 100 cm^3 of soil are detected using this extraction method.

Experiment II. Impact of high (15,000-40,000) initial M. hapla egg density on A. cepa.

An experiment similar to the preceding study was conducted in the greenhouse in the winter of 1981. Forty-eight clay pots containing 1400 cm^3 of pasteurized muck soil were assigned to each of six M. hapla density treatments: 1) 15,000 eggs, 2) 20,000 eggs, 3) 25,000 eggs, 4) 30,000 eggs, 5) 40,000 eggs, or 6) 0 eggs (control). Inoculation was as follows: 1300 cm^3 of soil from each pot was placed in a plastic bag: nematode inoculum in 10 ml of water was added to the bag and mixed thoroughly; the inoculated soil was then placed back into the clay pot from which it was removed and covered with 100 cm^3 of non-inoculated soil; a pregerminated onion seedling, cv Krummery Special, was planted in each pot.

Soil temperature and moisture were monitored during the experiment by a Campbell CR-21 weather-monitoring microcomputer. Six plants from each treatment were destructively sampled ca 2300 degree hours (base 9 C). The exact sample schedule is presented in Table 10. Root length, root weight, and shoot weight of each plant were recorded at every sampling date. The entire

Table 10. Sampling schedule for Experiment 2: Impact of high initial inoculum levels of *Meloidogyne hapla* on *Allium cepa*.

Sample	Days after planting	Degree hours* after planting
1	8	2276
2	16	4604
3	26	7531
4	34	9907
5	43	12370
6	50	14766
7	58	17000
8	73	21741

* base = 9 C

root system of all plants was stained in lactophenol with 0.01% acid fuchsin and examined for galls and nematodes. Two 100 cm^3 soil samples were removed from each pot on the first, second, third, fourth, and seventh sampling dates and assayed for eggs, juveniles, and males by a sugar flotation-centrifugation technique (Jenkins, 1964). Counts of nematodes from the two samples were averaged. One 100 cm^3 soil sample was examined for M. hapla on the fifth, sixth, and eighth sampling sates. Eggs, released from the gelatinous matrix surrounding the eggs by gentle agitation in a solution of 0.5% sodium hypochlorite, were enumerated from the first occurence of oviposition until the final sample date.

RESULTS

There was no significant linear relation between the mean fresh weight of either KS or DYG A. cepa and the initial density of M. hapla for inoculum densities ranging from 100 - 15,000 (Figure 11). There was, however, a significant (P=0.05) linear relationship (Figure 12) between the fresh weight of KS plants and the actual number of M. hapla recovered per gram of root at harvest, described by the equation:

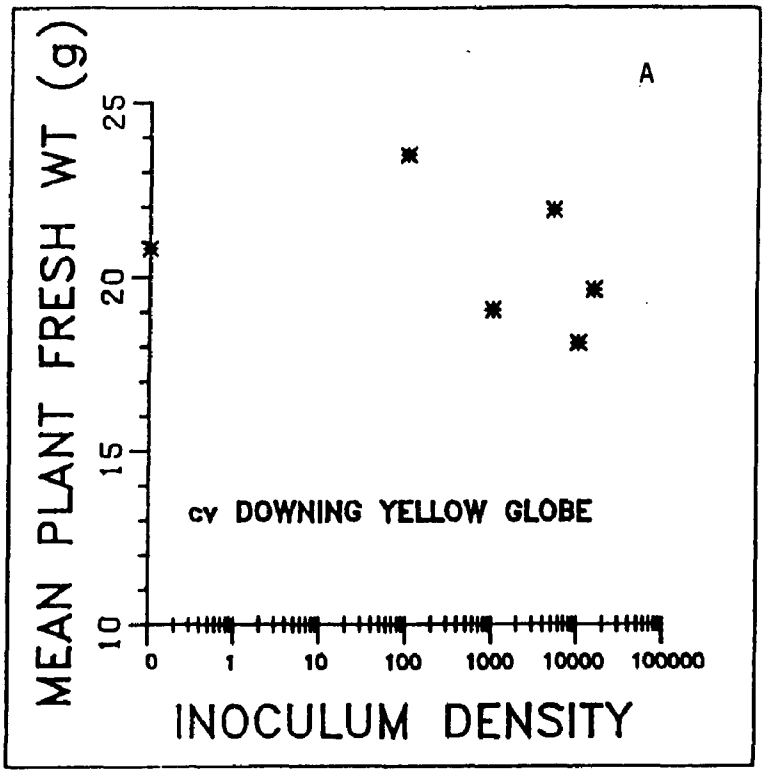
$$Y = 18.80 - 0.0196X$$

where:

Y = total plant fresh weight in grams

X = number of M. hapla/1.0 g root

The relation between final nematode density in roots and plant fresh weight was non-significant for the DYG cultivar. The mean plant fresh weight of KS onions was also not significantly related to initial nematode density when inoculum levels of 15,000 - 40,000 were administered (Figure 13A). Without data



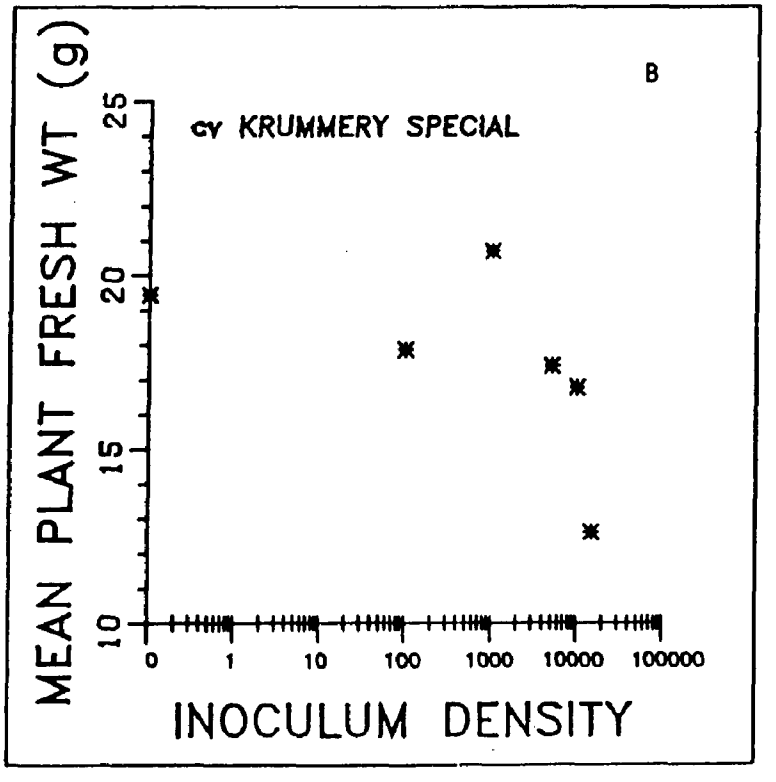


Figure 11. Relationship between inoculum density of Meloidogyne hapla eggs and fresh weight of Downing Yellow Globe (A) and Krummery Special (B) Allium cepa.

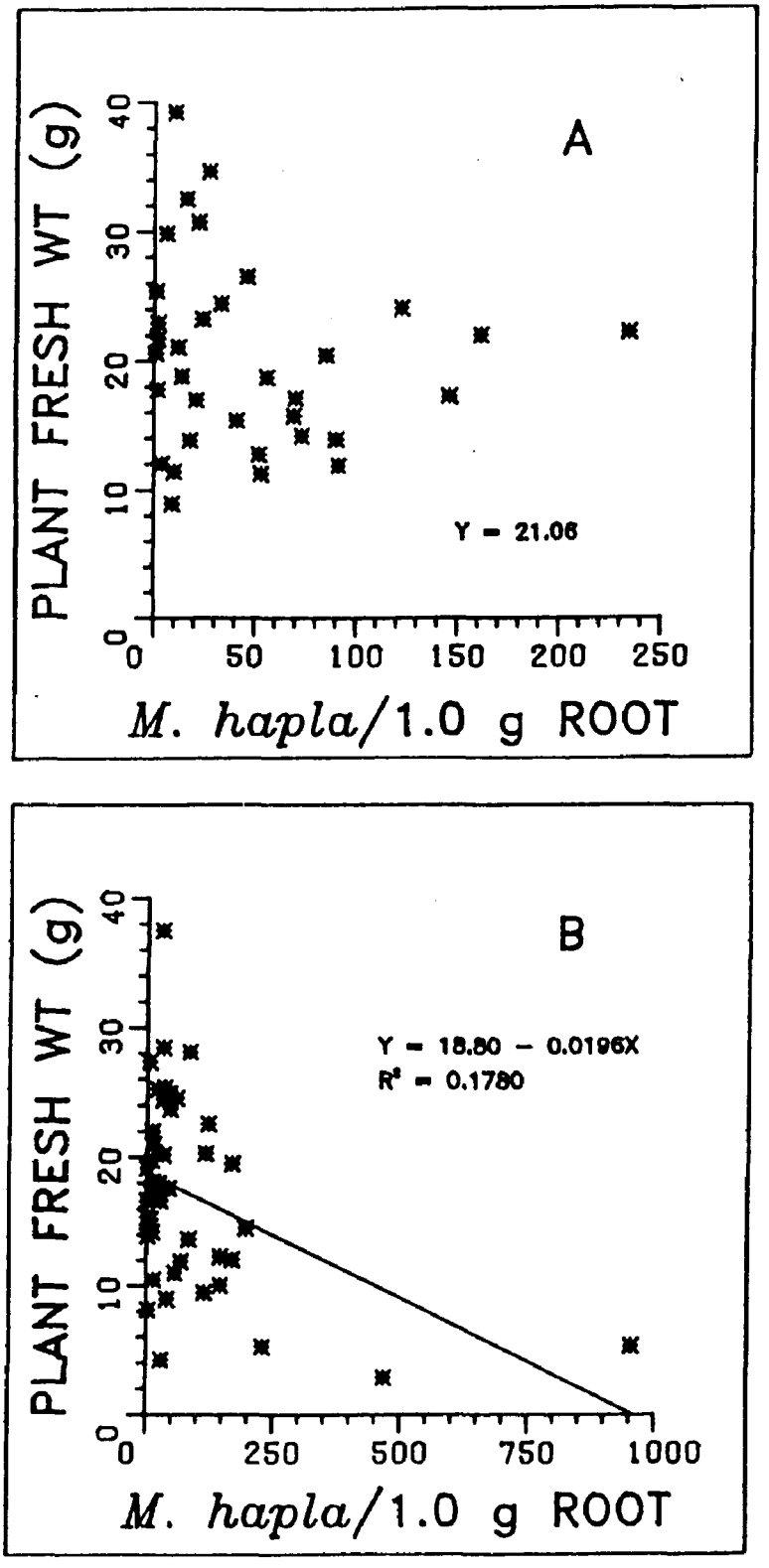
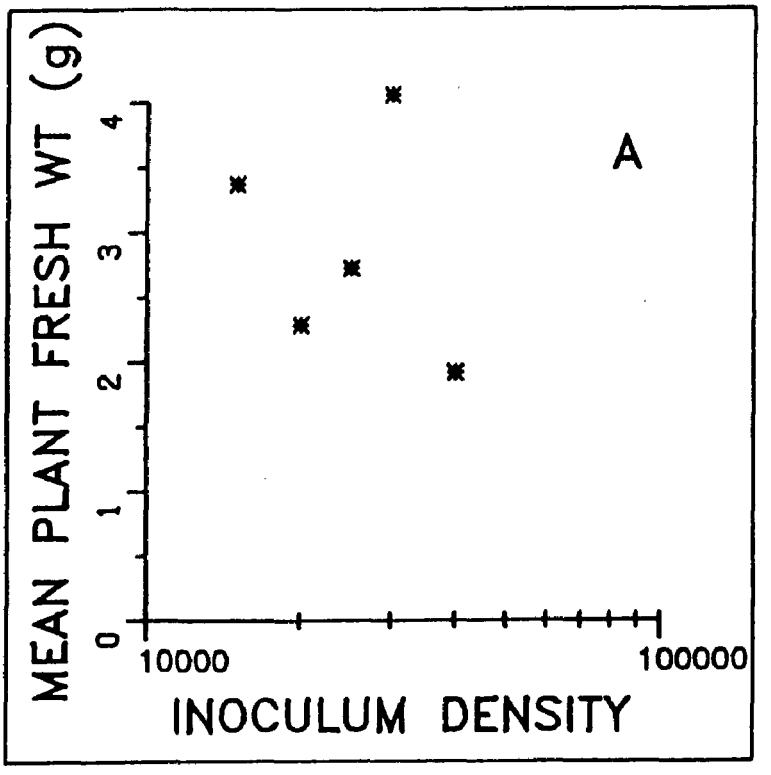


Figure 12. Relationship between the number of Meloidogyne hapla recovered per 1.0 gram root tissue and fresh weight of Downing Yellow Globe (A) and Krummery Special (B) Allium cepa.



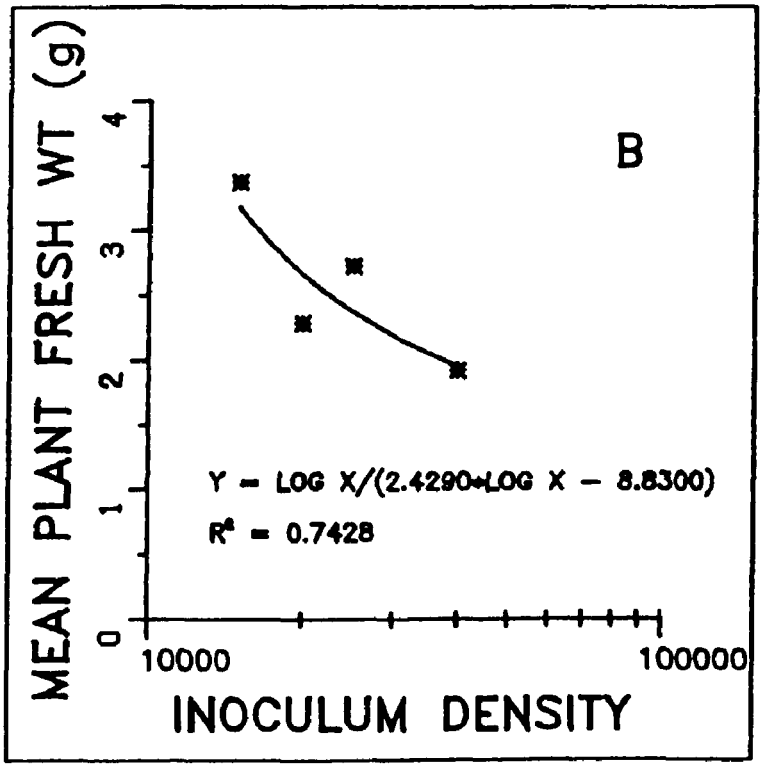


Figure 13. Relationship between fresh weight of Krummery Special Allium cepa and inoculum density of Meloidogyne hapla including (A) and excluding (B) and inoculum level of 30,000 eggs per plant.

from the inoculum treatment of 30,000 M. hapla per plant, however, the relation between inoculum level and mean plant fresh weight at harvest (Figure 13B) was described by a negative exponential equation:

$$Y - \log X / (2.4290 \log X - 8.83)$$

where:

Y = mean total plant fresh weight in grams

X = initial number of M. hapla eggs inoculated/plant

Root fresh weight of KS plants inoculated with 15,000 M. hapla eggs was significantly (P=0.05) reduced as compared to plants inoculated with 1000 nematode eggs or non-inoculated control plants, but there was no difference in root area between any treatment (Table 11). Root fresh weight was not different between DYG plants inoculated with 100 to 15,000 M. hapla eggs, although root area was significantly (P=0.05) less for plants inoculated with 10,000 eggs as compared to plants receiving 100 eggs (Table 11). An examination of fresh KS root weights over time for plants inoculated with 15,000 to 40,000 M. hapla eggs shows that root weight was increasingly reduced with increasing inoculum levels, except for the treatment level of 30,000 M. hapla eggs per plant (Figure 14). Similar but more pronounced trends were noted for the relationship between mean length of the KS root system, inoculum level, and time (Figure 15).

Fresh shoot weight of KS plants inoculated with 15,000 M. hapla eggs was significantly (P=0.05) less than that of plants inoculated with 1000 eggs (Table 12). There were no differences in shoot dry weight or area of the KS cultivar for inoculum levels ranging from 100 to 15,000 M. hapla eggs per plant. Similarly, shoot fresh weight, dry weight, or area of the DYG cultivar was not different for

Table 11. Root weight and area of two onion cultivars inoculated with one to five levels of *Meloidogyne hapla* or non-inoculated.

Cultivar inoculum level	Root	
	Fresh wt (g)	Area (cm^2)
Downing Yellow Globe		
Check	4.92 a	52.60 ab
100	5.89 a	56.80 a
1000	4.74 a	47.34 ab
5000	5.16 a	44.77 ab
10000	4.12 a	41.12 b
15000	4.39 a	44.41 ab
Krummery Special		
Check	4.70 a	46.48 a
100	4.17 ab	41.50 a
1000	5.12 a	45.86 a
5000	3.64 ab	36.34 a
10000	3.49 ab	35.02 a
15000	2.68 b	34.95 a

Values within a column followed by the same letter are not significantly ($P = 0.05$) different according to Duncan's Multiple Range Test.

Figure 14. Fresh root weight of Krummery Special *Allium cepa* inoculated with one of five levels of *Meloidogyne hapla* or noninoculated.

The relationship between root fresh weight and accumulated degree hours at base 9 C was described by the equation:

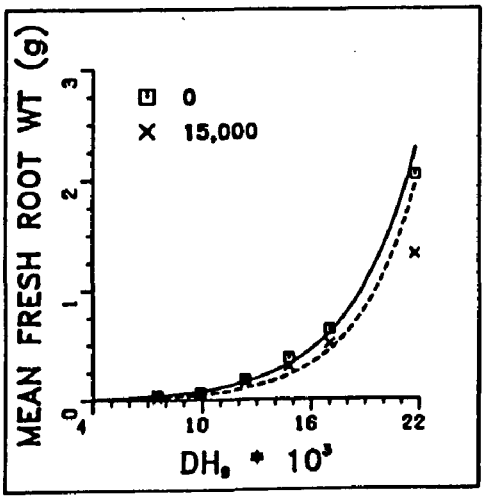
$$Y = ae^{bX}$$

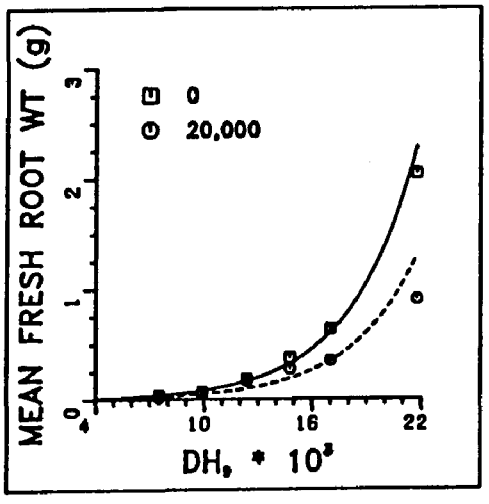
where:

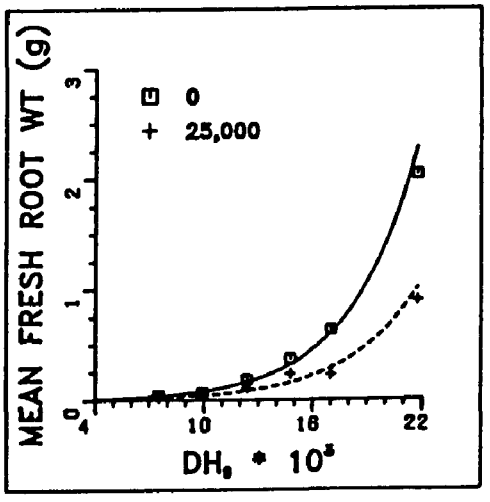
Y = fresh root weight in grams

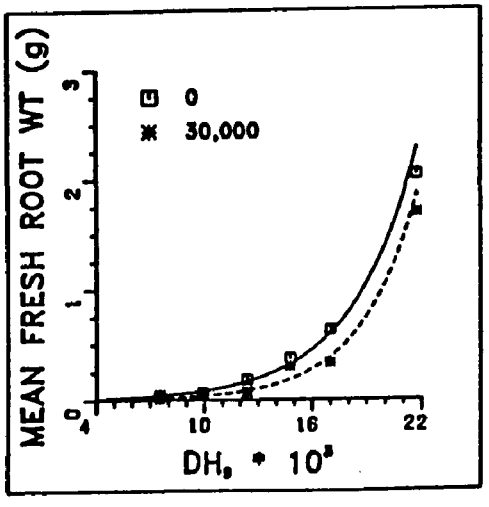
X = accumulated degree hours after seeding

Inoculum density	a	b	R^2
0	0.005126	0.000281	0.9783
15,000	0.002246	0.000312	0.6424
20,000	0.003082	0.000278	0.6816
25,000	0.003231	0.000264	0.9571
30,000	0.001400	0.000331	0.9609
40,000	0.001689	0.000293	0.6337


MEAN FRESH ROOT WT (g)
0
15,000
0
1
2
3
4
10
16
22
DH$_g$ * 10^3


MEAN FRESH ROOT WT (g)
0
20,000
0
1
2
3
4
10
16
22
DH$_g$ * 10^3


MEAN FRESH ROOT WT (g)
0
25,000
0
1
2
3
4
10
16
22
DH$_g$ * 10^3


MEAN FRESH ROOT WT (g)
0
30,000
0
1
2
3
4
10
16
22
DH$_g$ * 10^3

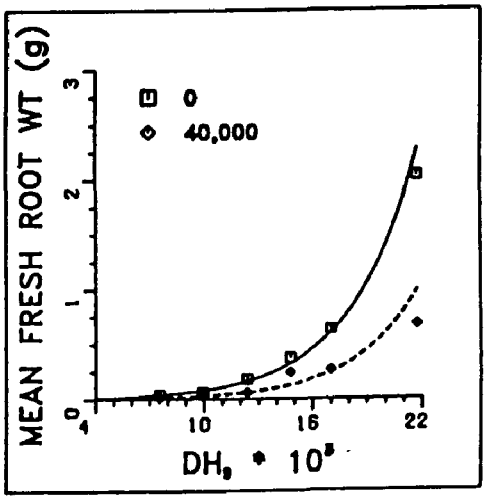

MEAN FRESH ROOT WT (g)
0
40,000
0
1
2
3
4
10
16
22
DH$_g$ * 10^3

Figure 14.

Figure 15. Root length (cm) of Krummery Special *Allium cepa* inoculated with one of five levels of *Meloidogyne hapla* or noninoculated.

The relationship between root length and accumulated degree hours at base 9 C was described by the equation:

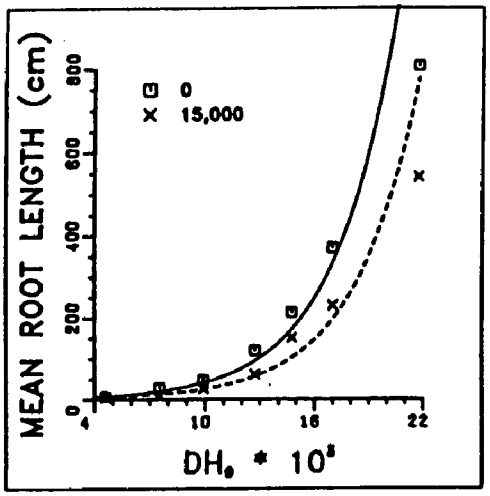
$$Y = ae^{bX}$$

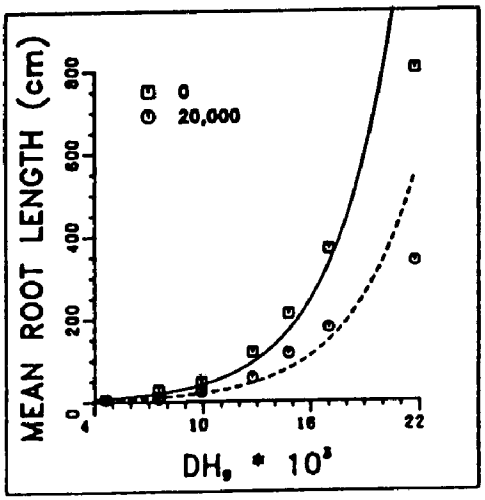
where:

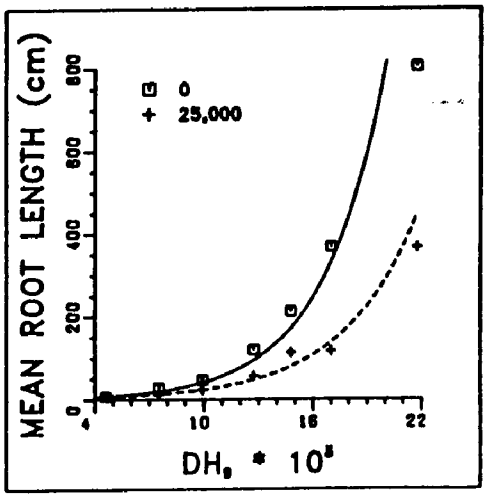
Y = root length in centimeters

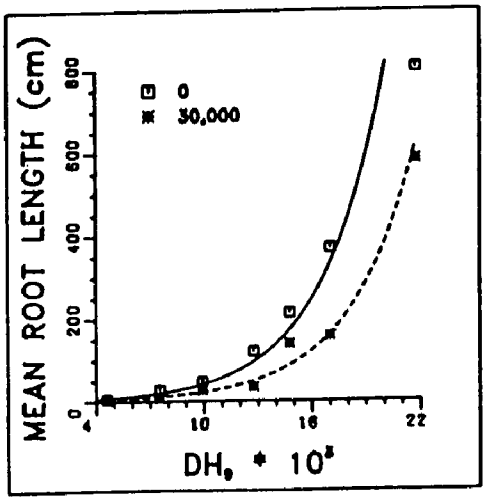
X = accumulated degree hours after seeding

Inoculum density	a	b	R^2
0	2.223876	0.000295	0.4443
15,000	1.141120	0.000290	0.7539
20,000	1.295380	0.000279	0.5115
25,000	2.017317	0.000248	0.9224
30,000	1.306399	0.000284	0.9831
40,000	1.035552	0.000272	0.8013


MEAN ROOT LENGTH (cm)
0
15,000
0
200
400
600
800
4
10
16
22
DH$_0$ * 10^5


MEAN ROOT LENGTH (cm)
0
20,000
0
200
400
600
800
4
10
16
22
DH$_0$ * 10^5


MEAN ROOT LENGTH (cm)
0
25,000
0
200
400
600
800
4
10
16
22
DH$_0$ * 10^5


MEAN ROOT LENGTH (cm)
0
30,000
0
200
400
600
800
4
10
16
22
DH$_0$ * 10^5

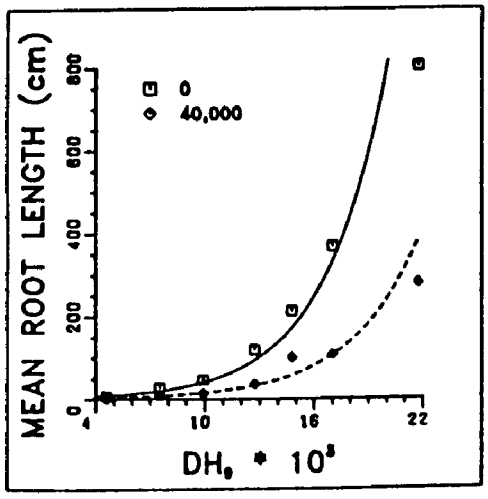

MEAN ROOT LENGTH (cm)
0
40,000
0
200
400
600
800
4
10
16
22
DH$_0$ * 10^5

Figure 15.

Table 12. Fresh and dry weights and areas of leaves of two onion cultivars inoculated with one of five levels of *Meloidogyne hapla* or noninoculated.

Cultivar and inoculum level	Leaf		
	Fresh wt (g)	Dry wt (g)	Area (cm^2)
Downing Yellow Globe			
Check	14.45 a	0.77 a	115.65 a
100	16.36 a	0.69 a	128.33 a
1000	13.18 a	0.64 a	99.16 a
5000	15.49 a	0.74 a	137.22 a
10000	12.60 a	0.75 a	106.09 a
15000	14.18 a	0.61 a	118.00 a
Krummery Special			
Check	13.59 ab	0.68 a	102.47 a
100	12.64 ab	0.66 a	99.43 a
1000	14.49 a	0.79 a	107.34 a
5000	12.79 ab	0.68 a	100.62 a
10000	12.25 ab	0.71 a	98.69 a
15000	9.25 b	0.52 a	73.99 a

Values within a column followed by the same letter are not significantly ($P = 0.05$) different according to Duncan's Multiple Range Test.

inoculum densities of 100 to 15,000 M. hapla per plant. Differences in fresh shoot weight with increasing inoculum levels of 15,000 to 40,000 nematode eggs per plant were not as great as the differences in fresh root weight or length (Figure 16). As with root weight and length, an inoculum level of 30,000 eggs per plant had less impact on plant growth than did inoculum levels of 20,000 or 25,000 eggs per plant.

The mean fresh bulb weight and volume of KS onions inoculated with 15,000 M. hapla eggs per plant was significantly (P=0.05) less than that of non-inoculated control plants, but was not different than that of plants inoculated with from 100 to 10,000 eggs per plant (Table 13). Plants inoculated with 15,000 to 40,000 nematode eggs per plant did not form bulbs, due to the length and time of the experiment. There was no difference in the fresh weight or volume of DYG onions inoculated with 100 to 15,000 eggs per plant.

For the experiment using inoculum densities ranging from 15,000 to 40,000 M. hapla, levels of eggs detected in soil samples decreased for all treatments until the fifth sampling date (ca 12730 DH), after which time an increase was observed (Figure 17). A positive correlation between egg inoculum level and egg density/100 cm^3 soil was noted throughout the experiment.

Levels of M. hapla juveniles in the soil were greatest 16 days after planting (ca 4604 DH) for all treatments and steadily declined thereafter (Figure 17). Again, a positive correlation between egg inoculum level and the density of juveniles was apparent. A significant (P=0.05) linear relationship between inoculum density and the number of juveniles/100 cm^3 soil was observed at the second sampling date (ca 4604 DH), indicating that the proportion of eggs that hatched was independent of inoculum density (Figure 18).

Figure 16. Fresh shoot weight of Krummery Special *Allium cepa* inoculated with one of five levels of *Meloidogyne hapla* or noninoculated.

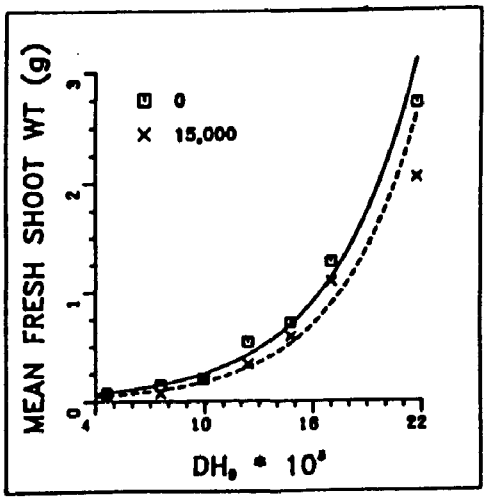
The relationship between shoot fresh weight and accumulated degree hours at base 9 was described by the equation:

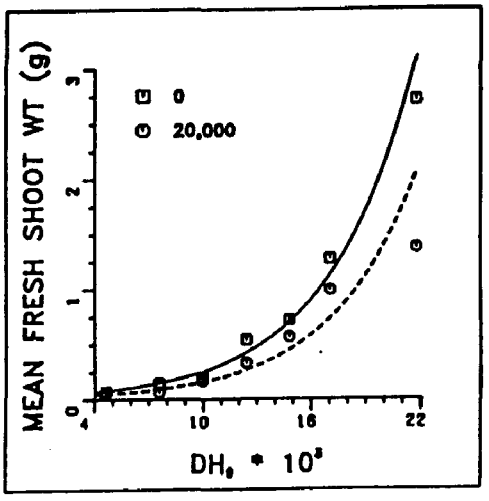
$$Y = ae^{bX}$$

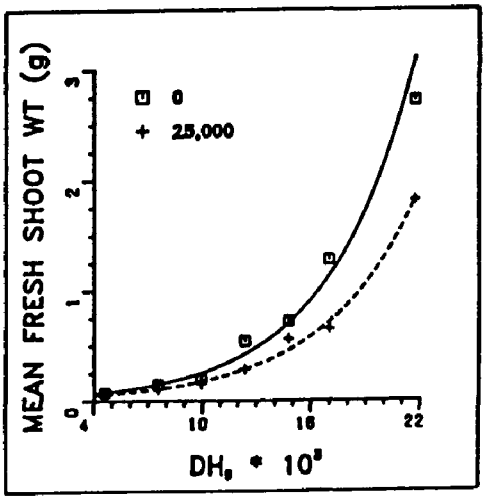
where: Y = fresh shoot weight in grams

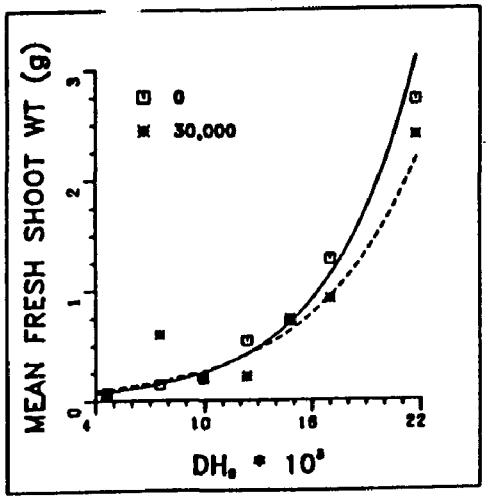
X = accumulated degree hours after seeding

Inoculum density	a	b	R^2
0	0.030426	0.000213	0.9639
15,000	0.018398	0.000228	0.8746
20,000	0.018088	0.000218	0.6401
25,000	0.024257	0.000199	0.9938
30,000	0.045851	0.000178	0.9246
40,000	0.012702	0.000231	0.7757


MEAN FRESH SHOOT WT (g)
0
15,000
0
1
2
3
4
10
16
22
DH$_0$ * 10^3


MEAN FRESH SHOOT WT (g)
0
20,000
0
1
2
3
4
10
16
22
DH$_0$ * 10^3


MEAN FRESH SHOOT WT (g)
0
25,000
0
1
2
3
4
10
16
22
DH$_0$ * 10^3


MEAN FRESH SHOOT WT (g)
0
30,000
0
1
2
3
4
10
16
22
DH$_0$ * 10^3

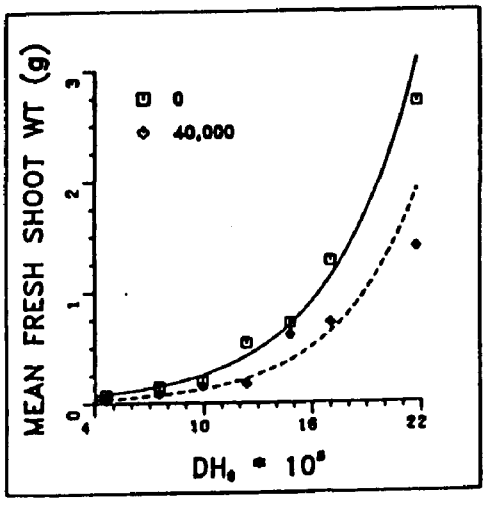

MEAN FRESH SHOOT WT (g)
0
40,000
0
1
2
3
4
10
16
22
DH$_0$ * 10^3

Table 13. Bulb weight and volume of two onion cultivars inoculated with one of five levels of *Meloidogyne hapla* or non-inoculated.

Cultivar and inoculum level	Bulb	
	Fresh wt (g)	Volume (cm^3)
Downing Yellow Globe		
Check	1.45 a	1.81 a
100	1.26 a	1.42 a
1000	1.14 a	1.25 a
5000	1.26 a	1.50 a
10000	1.39 a	1.66 a
15000	1.07 a	1.06 a
Krummery Special		
Check	1.16 a	1.47 a
100	1.07 ab	1.22 ab
1000	1.10 ab	1.28 ab
5000	0.98 ab	1.19 ab
10000	1.04 ab	1.08 ab
15000	0.68 b	0.80 b

Values within a column followed by the same letter are not significantly ($P = 0.05$) different according to Duncan's Multiple Range Test.

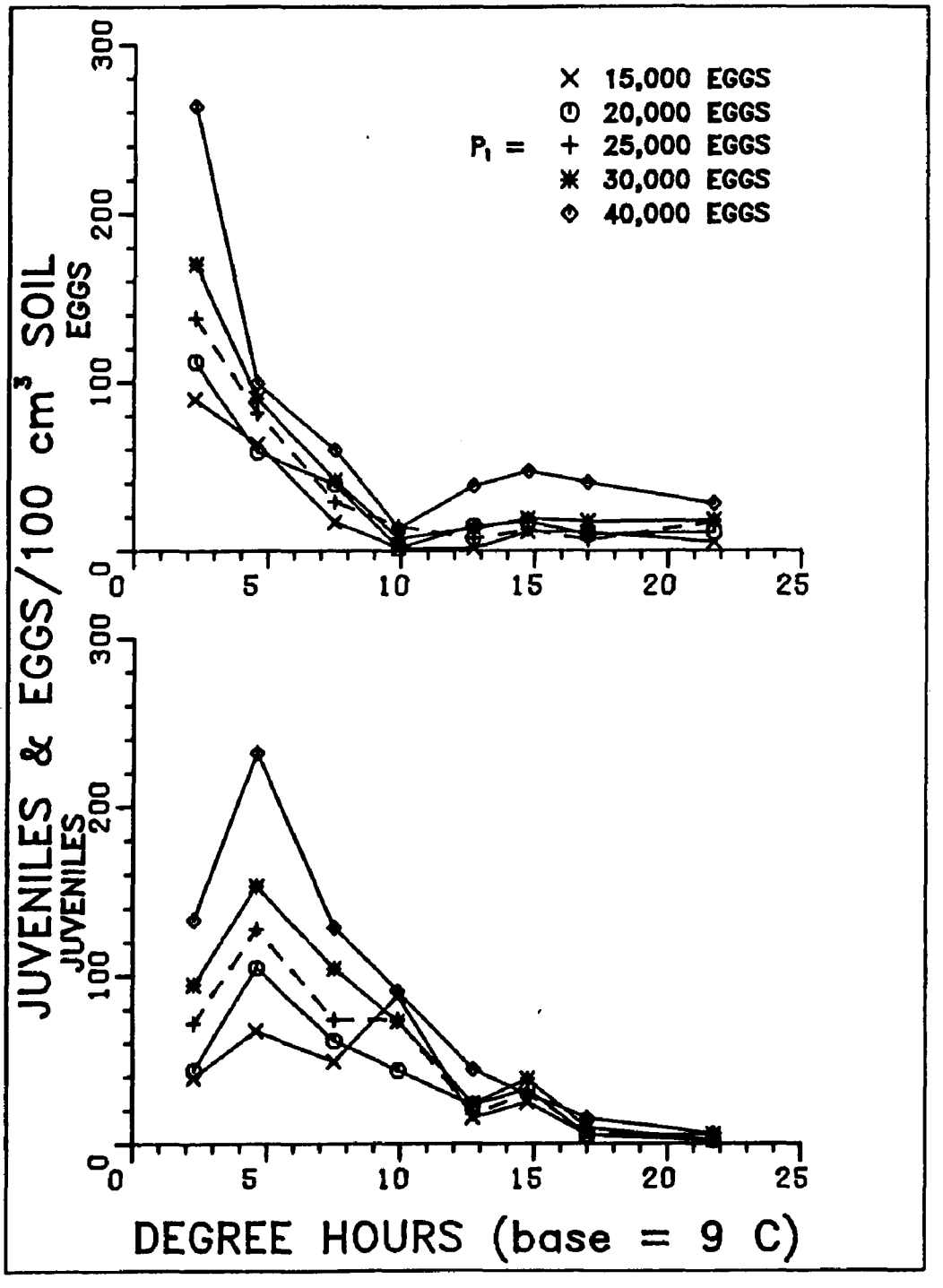


Figure 17. Levels of Meloidogyne hapla eggs and juveniles in 100 cm^3 of soil surrounding Allium cepa inoculated with one of five levels of nematodes or noninoculated.

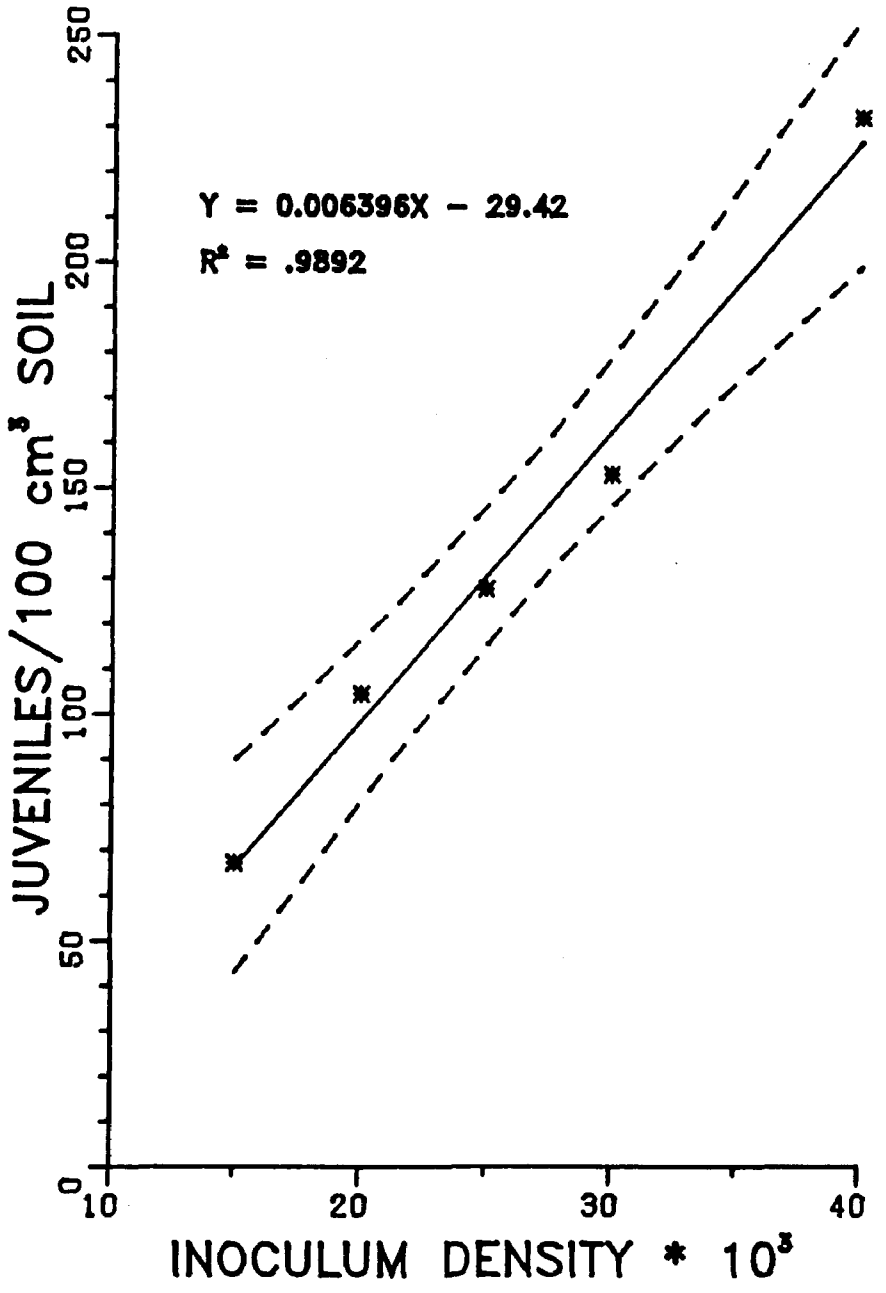
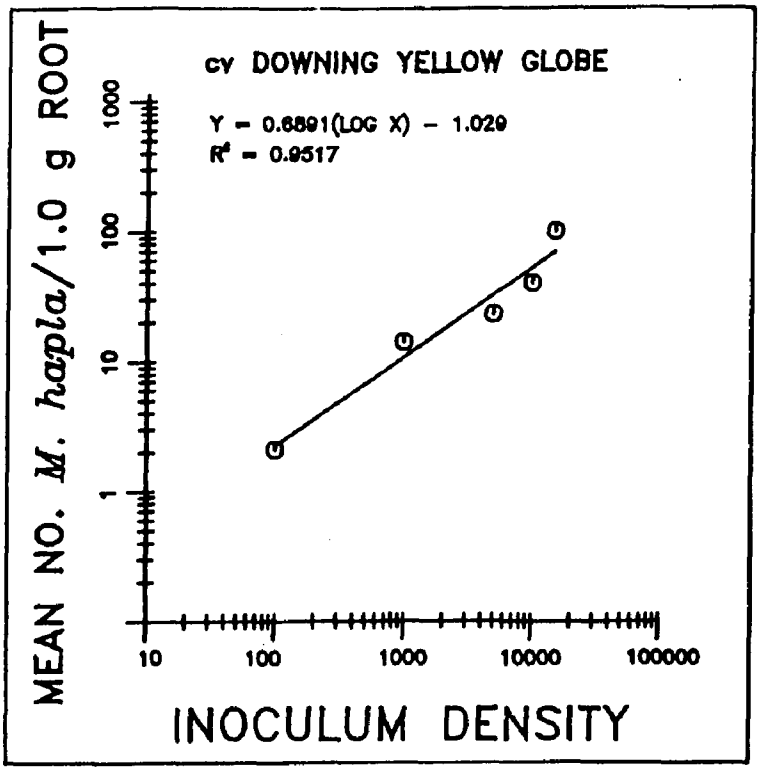


Figure 18. Levels of Meloidogyne hapla juveniles in 100 cm^3 of soil 4604 degree hours after inoculation and planting of Allium cepa.

There was a significant (P=0.05) linear relationship between the initial inoculum density and the final observed density of M. hapla per 1.0 g of root tissue for DYG plants inoculated with 100 - 15,000 eggs per plant (Figure 19A). The relation between initial inoculum density and final root levels of nematodes for the KS cultivar was described by a curvilinear function, since inoculum densities from 1000 - 15,000 eggs resulted in similar final nematode densities for this cultivar (Figure 19B). The mean number of M. hapla per 0.1 g of root tissue for KS onions inoculated with nematode egg densities ranging from 15,000 - 40,000 per plant was greatest 34 days after planting (ca 4406 DH) and decreased until the experiment was terminated 73 days (ca 21741 DH) following planting (Figure 20). The density of M. hapla per 0.1 g of root tissue was not different between treatments (inoculum levels) on any sampling date.

Similarly, the numbers of juvenile and adult M. hapla per root system were not significantly different between treatments, although plants inoculated with high levels of eggs tended to support high numbers of nematodes (Figure 21). The ranking of the treatments in terms of nematode density/plant, however, was not consistent throughout the experiment. Plants receiving the highest inoculum level supported the lowest nematode population levels 16 and 50 days after planting. Plants receiving the lowest inoculum level did not support the least number of nematodes on any sampling date.

In the experiment based on nematode inoculum densities of 15,000 to 40,000 eggs per plant, there was very little correlation between the final density of nematodes per root system at 21741 DH after planting and the initial inoculum density, nematode density 4604 or 9907 DH after planting, or length of the root system 4604, 9907, 14766, or 21741 DH after planting (Table 14). Initial



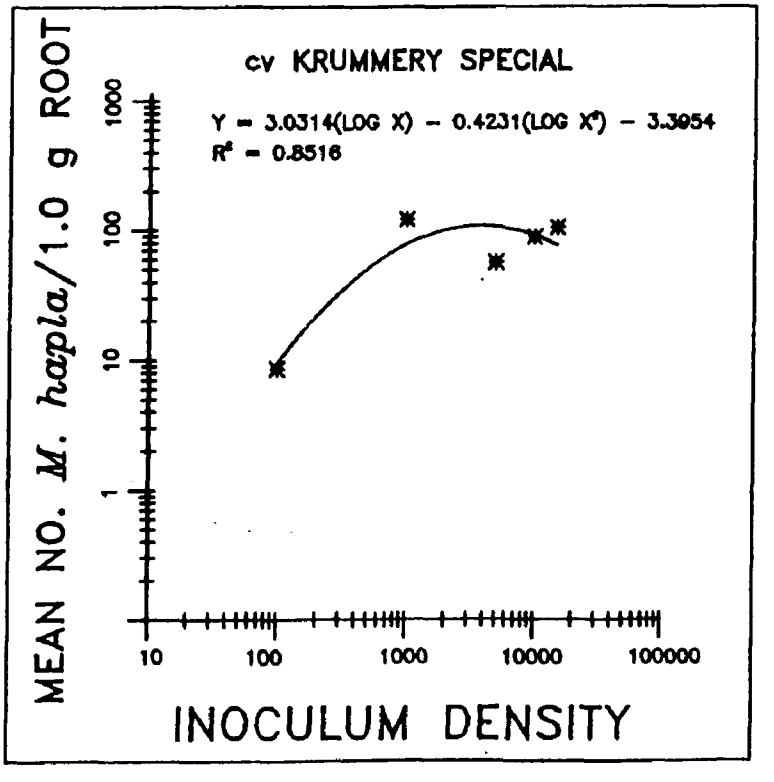


Figure 19. Relationship between the inoculum density of Meloidogyne hapla eggs and the number of nematodes recovered per gram root tissue from Downing Yellow Globe (A) or Krummery Special (B) Allium cepa.

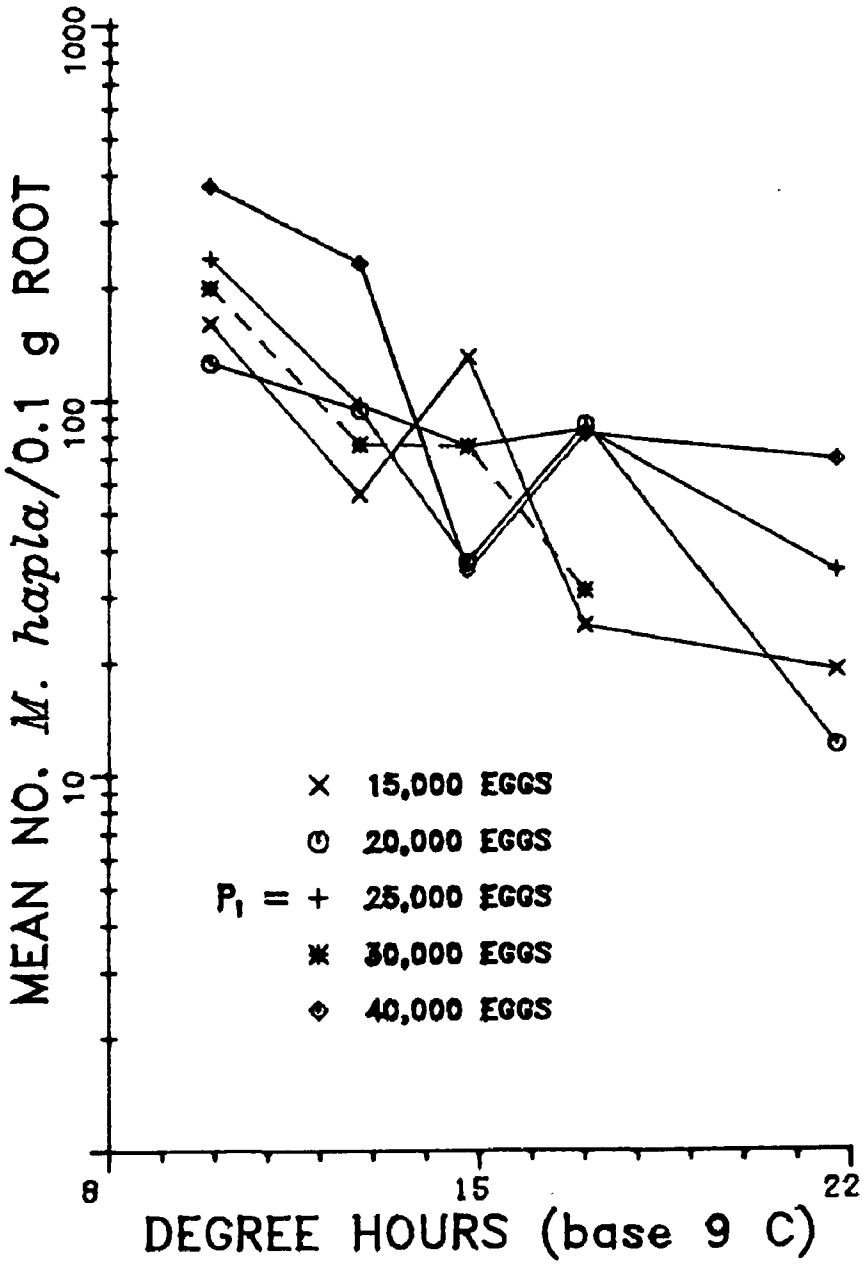


Figure 20. Number of Meloidogyne hapla in 0.1 gram of Allium cepa roots inoculated with one of five levels of nematodes or noninoculated.

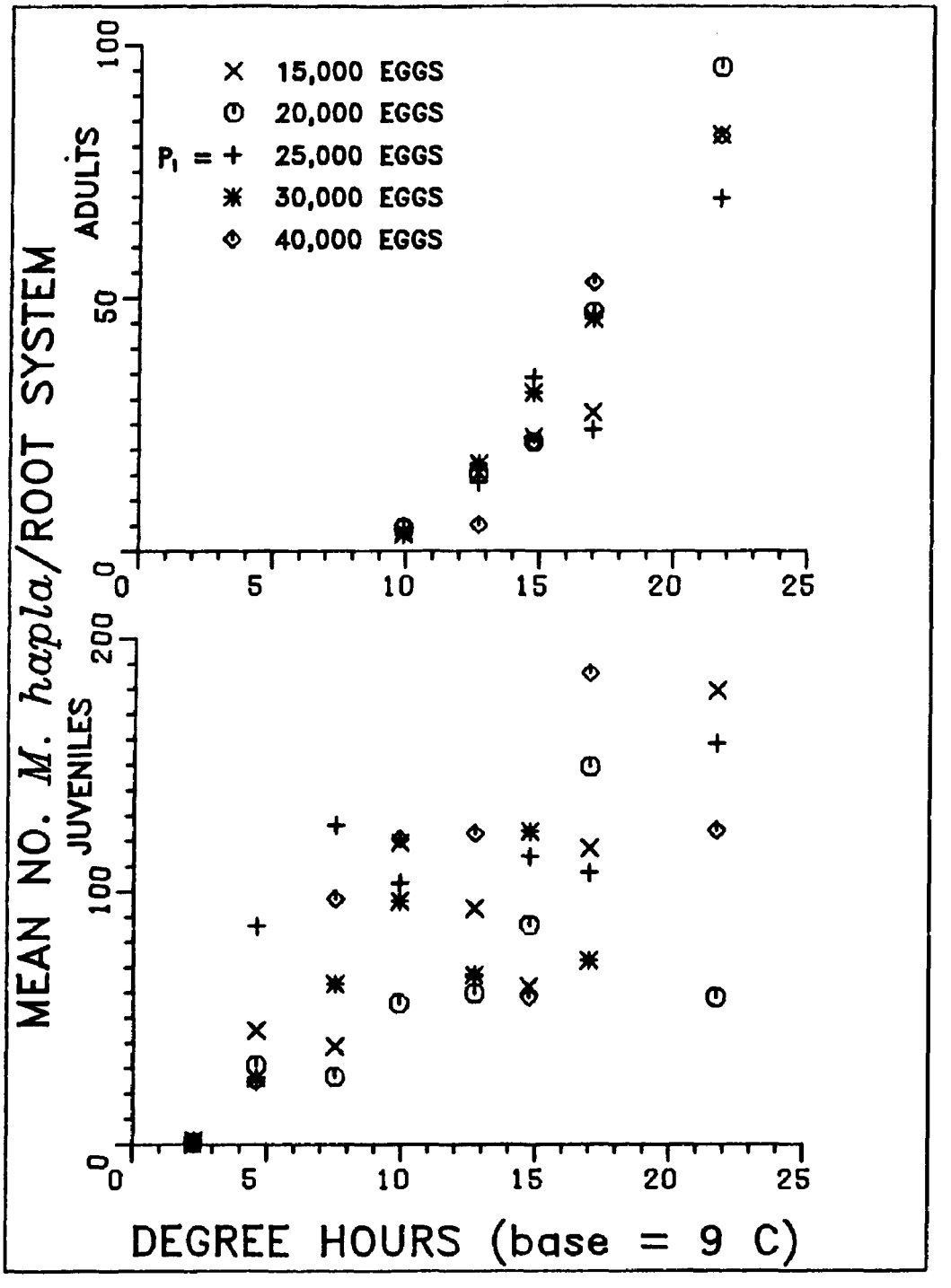


Figure 21. Levels of Meloidogyne hapla juveniles and adults in Allium cepa inoculated with one of five levels of nematodes or noninoculated.

Table 14. Correlation matrix between final density of Meloidogyne hapla per Allium cepa root system and nematode density and length of root systems throughout the experiment.

	Final density	Initial density	Root length 4604 *	Nema density 4604	Root length 9907	Nema density 9907	Root length 14766	Nema density 14766
Initial density	-.0887							
Root length 4604	.0723	-.6548						
Nema density 4604	.0927	-.3101	.9046					
Root length 9907	.0777	-.9681	.8186	.5158				
Nema density 9907	.0851	.7511	-.5354	-.1512	-.7772			
Root length 14766	.1638	-.8680	.3439	.0752	.7460	-.3732		
Nema density 14766	-.0152	-.1982	.8520	.9035	.4373	-.3476	-.1987	
Root Length 21741	.2345	-.3013	.1191	-.0197	.2709	-.2750	.2592	-.0210

* Degree hours base 9 C accumulated since planting.

nematode inoculum density was inversely correlated with root length for all sampling dates tested. The root length on a particular sampling date and the nematode density per root system on the same date were positively correlated 4604 and 21741 DH after planting, and negatively correlated 9907 and 14766 DH after planting. The nematode density at sampling date t was inversely correlated with nematode density on sampling date t+2 for those dates tested. Root length at sample date t was positively correlated with root length at sample date t+2, as would be expected for any growing plant.

On the final sampling date there were an average of 1.106, 1.027, 3.199, and 24.389 females which had commenced oviposition per 0.1 g of root for plants inoculated with 15,000, 20,000, 25,000, and 40,000 eggs, respectively. Seventy-three days after planting (ca 12741 DH), females reared on plants receiving the lowest inoculum level produced an average of 392.21 eggs each, as compared to 329.44 eggs for females reared on plants receiving the highest inoculum level. Because the initial level of eggs inoculated unto a plant did not adequately reflect final population levels of nematodes in roots (Figure 21), the data from the eight sampling dates were pooled, so that inoculum treatments were ignored. The mean number of eggs counted per egg sac for an entire root system was regressed on the total number of nematodes counted within that root system. No significant relationship between nematode density/plant and egg production was obtained (Figure 22).

Counts of galls did not accurately reflect M. hapla levels within A. cepa roots. Gall counts were not significantly correlated with the total number of nematodes or the number of adult females within a single root system (treatment ignored) on any sampling date.

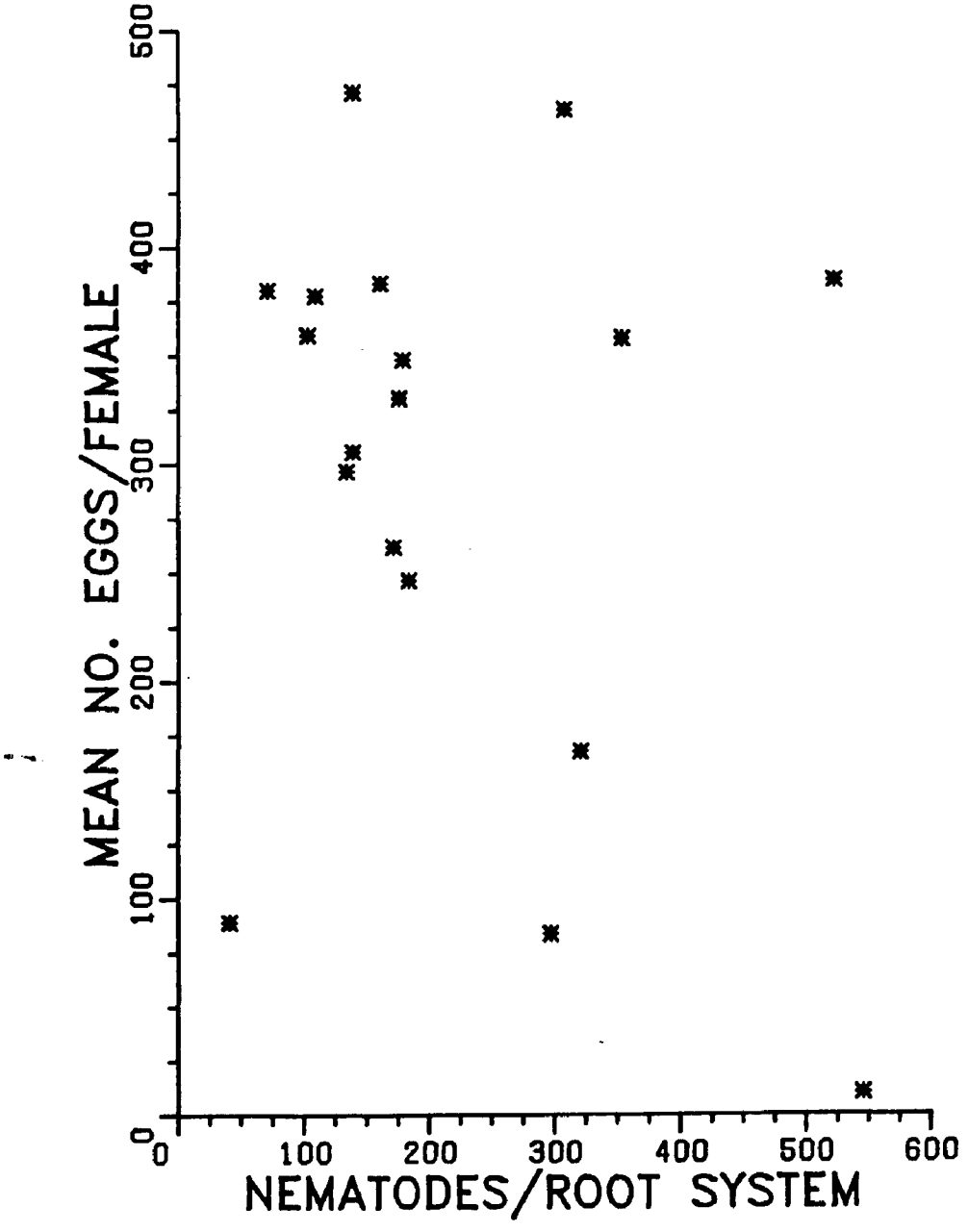


Figure 22. Relationship between egg production by Meloidogyne hapla females and total nematode density per Allium cepa root system.

DISCUSSION

Krummery Special and Downing Yellow Globe A. cepa varied in response to M. hapla infection. The KS cultivar was less tolerant of M. hapla parasitism, as measured by total plant, root, shoot, and bulb fresh weight. Although the final density of M. hapla per root system was not different between the cultivars for plants inoculated with 15,000 nematode eggs, more nematodes were produced on the KS cultivar than on the DYG cultivar when the initial inoculum level was less than 15,000, indicating that KS onions are more susceptible (i.e. support more reproduction). Similar results were obtained by Kotcon (1979) when he compared the increase of M. hapla on KS, DYG, and three other A. cepa cultivars.

There was no apparent relation between the initial inoculum level of M. hapla and KS plant fresh weight at harvest for nematode densities of 15,000 or less, although there was a significant relationship between observed final nematode densities and plant weight. It is possible that a very low number of nematodes survived on some plants in this experiment. Since the effect of each individual nematode decreases as nematode density increases (Seinhorst, 1970), the high variability between pots with differing rates of nematode survival was probably sufficient to mask any relation between initial nematode densities and plant growth. For nematode densities greater than 15,000, however, evidently a sufficient number of nematodes survived that the variation in actual nematode density between pots (plants) did not significantly affect the analysis. Even so, results of the experiment using nematode densities of 15,000 to 40,000 were confounded by the growth of plants inoculated with 30,000 eggs. The inconsistent results associated with this treatment, in regards to the trends in plant growth observed for the other treatments, suggests that some error in inocula-

tion occurred. I feel confident that the data from this treatment does not reflect the true relationship between M. hapla density and the growth of A. cepa, but nevertheless have not excluded it from any analyses.

The general relationship observed between plant weight and initial inoculum levels ranging from 100 to 40,000 M. hapla eggs in the two experiments (Figures 11, 13) is similar to the relation between plant yield and initial nematode density described by Seinhorst (1970) (Figure 23). Plant growth is not affected by nematode densities less than some tolerance threshold. Nematode densities above this threshold have an increasingly negative impact on plant growth, until some level is reached beyond which no further damage occurs. Values of plant growth parameters should not be directly compared between the two experiments described here, due to differences in the duration and the time of year in which they were conducted. Trends showed by the data, however, indicate that the growth of A. cepa cv KS is not severly affected by less than 15,000 M. hapla eggs per plant. An M. hapla egg density of 15,000/plant is roughly equivalent to 3000 juveniles/plant, as calculated by the percentage of egg hatch (Figure 18) and nematode extraction efficiency (35%). It is doubtful if initial soil populations of M. hapla approach this level in Michigan. Considering that DYG A. cepa were even more tolerant of M. hapla than the KS cultivar, the data from these greenhouse experiments support earlier contentions that M. hapla is not usually an economically important pest of A. cepa (Chitwood, 1951; Tyler, 1933).

It is possible that an onion crop could be affected if an M. hapla density of 15,000 eggs per plant or greater was present at planting, since the root, shoot, and bulb weight of KS A. cepa were reduced by initial M. hapla levels of this

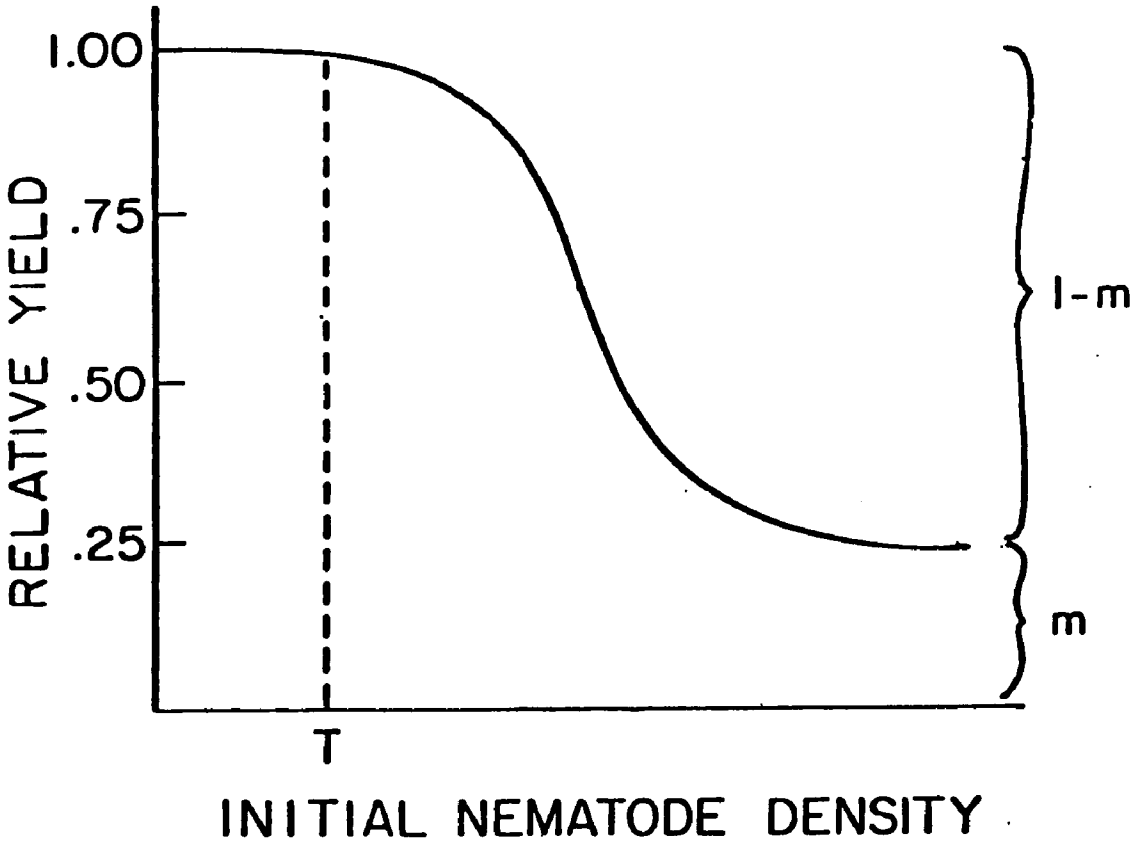


Figure 23. Parameters descriptive of the relationship between plant growth and preplant nematode density. Tolerance threshold (T), minimum yield (m). (from Duncan and Ferris, 1982)

magnitude under greenhouse conditions. Root length was inversely correlated with initial M. hapla egg density and with observed M. hapla density after nematodes entered root tissue (Table 14). The reduction in root length and other growth parameters observed for plants maintained under optimal soil moisture conditions in these experiments might be more pronounced when soil moisture is limiting, as is often the case under field conditions.

The relationship between the initial inoculum level of M. hapla and final population level was difficult to characterize, primarily because of the continuous recruitment of nematodes into A. cepa roots. This problem could have been avoided if M. hapla juveniles rather than eggs had been used as inoculum, since the period of survival in the soil for juveniles is less than the time required for the development and eclosion of eggs. Generally, it seemed that nematode density did affect the number of M. hapla capable of inhabiting A. cepa. There was a negative correlation between nematode density in root tissue at sample t and sample t+2 (Table 14). Initially, high inoculum levels tended to be associated with high root levels of M. hapla (Figure 21). By the end of the experiment, however, plants initially supporting the fewest M. hapla contained the greatest number of nematodes, due probably to an increased availability of root substrate for nematode invasion.

Counts of eggs and empty egg shells within eggs masses provided an estimate of the total number of eggs produced and indicated that there was no relation between the density of nematodes per root system and the reproduction of M. hapla females, although such a relationship might have been evident had the experiment been continued. It is also very likely that egg production would be limited by higher nematode densities than that observed in this experiment.

No estimate of the relation between nematode density and egg viability or hatch was attempted because densities of juveniles in the soil indicated that very few eggs had hatched when the experiment was terminated.

Counts of galls were a very poor indicator of nematode levels within A. cepa roots. The highest correlation between the number of galls and nematode density was 0.7942, obtained 17000 DH after planting. My findings support those of Kinloch and Allen (1972) who showed a poor relationship between the number of M. hapla and galls on tomato roots. The data demonstrate that gall indices, often used as a measure of Meloidogyne population development, should be supported by other measures of nematode abundance.

Pathogenicity and Increase of *Meloidogyne hapla* on Two Onion Cultivars, Two Carrot Cultivars, and Sudax

INTRODUCTION

Meloidogyne hapla is often associated with *Allium cepa* grown in Michigan. Some reports indicate that economic losses of *A. cepa* may result from *M. hapla* infection (Sherf and Stone, 1956). My studies showed that *A. cepa* is generally tolerant to parasitism by *M. hapla* under greenhouse conditions, even when subjected to as many as 40,000 *M. hapla* eggs per plant. The ability of *M. hapla* to reproduce on *A. cepa* was disputed in the past (Chitwood, 1951; Tyler, 1933), although recent studies conducted in Michigan (Kotcon, 1979; Van Arkel, 1982; MacGuidwin, unpublished) demonstrated that *M. hapla* infecting *A. cepa* do develop and produce eggs. According to my experiments and work by Kotcon (1979), different *A. cepa* cultivars vary in their response to *M. hapla*, which may account for the discrepant reports appearing in nematological literature.

In 1980, one range (*ca* 15.4 x 30.8 m) at the M.S.U. Muck Research Farm was found to be partially infested with *M. hapla*. This nematode occurred primarily in only half of the range, due to the cropping history of the site. *Pratylenchus* spp. was detected throughout the range.

An experiment was designed to utilize the pattern of nematode distribution in this range to investigate the tolerance and susceptibility of *A. cepa* to *M. hapla*. The objectives of this study were 1) to assess the influence of *M. hapla* on the yield of two *A. cepa* cultivars, and 2) to determine the suitabilty of *A. cepa* for the increase of *M. hapla*.

To accomplish the latter objective, the increase of *M. hapla* on two *A. cepa* cultivars was compared to that on two *Daucus carota* cultivars and sudax, a

sorghum x sudangrass hybrid. The carrot and sudax crops were included in the experimental design so that the increase of M. hapla on A. cepa could be judged relative to hosts with varying degrees of susceptibility for M. hapla reproduction. One cultivar of D. carota was determined experimentally to be susceptible to M. hapla and the other was characterized as resistant to M. hapla (L. Baker, personal comm.). Sudax was a poor host for M. hapla and other nematodes in past nematological experiments conducted in Michigan (H. C. Olsen, pers. comm.). The two A. cepa cultivars selected for this study were determined to be differentially suitable for the development of M. hapla by Kotcon (1979).

MATERIALS AND METHODS

Equal areas of range C-17 at the M.S.U. Muck Research Farm, planted to carrot and potato in 1979, were used in this study. Pre-plant soil samples showed that there were 7.32 M. hapla/100 cm^3 soil in the area previously planted to carrot, and 0.80 M. hapla/100 cm^3 soil in the area planted to potato the preceding year. The two areas were both divided into four equal-sized blocks, which were planted with one three-row replicate of each crop, 6.15 m in length. A 4.0 m alley, unplanted and fallow the previous year, was established between the areas of high and low M. hapla infestation, so that an area of low M. hapla density would not be included in the area designated "high M. hapla density". The crops used in this study were (1) A. cepa cv Krummery Special (KS), (2) A. cepa cv Downing Yellow Globe (DYG), (3) D. carota experimental cv 9555B (susceptible), (4) D. carota experimental cv M872B (resistant), and (5) sudax (sorghum x sudangrass).

Soil and root samples were collected on 5/20/80, 6/19/80, 7/19/80, 8/16/80 (soil only), and 9/4/80. Spores of vesicular-arbuscular mycorrhizal (VAM) fungi

and phytoparasitic nematodes were enumerated in a 100 cm^3 subsample from the soil collected at each sampling date. Similarly, a root subsample of 0.25 g from each replicate was stained in a solution of a lactophenol with 0.01% acid fuchsin and examined for nematodes and mycorrhizal colonization.

When the onion and carrot crops were harvested on 9/4/84, data on the number and weight of carrots in three categories was collected from the center row of each replicate: (1) healthy (no disfiguration), (2) stubby and/or forked with galls, and (3) stubby and/or forked without galls. The number and weight of onions harvested from the center row of each replicate was also recorded on this date. The sudax plants were cut once to a height of ca 26 cm on 7/17/80 and were not harvested.

RESULTS

Soil and root levels of M. hapla were greater on the susceptible D. carota cultivar than on the other crops grown in the experiment (Table 15). The number of nematodes in the soil surrounding this crop at harvest was over twofold greater than the nematode density in soil surrounding the resistant D. carota cultivar and more that four times greater that the levels of nematodes observed from A. cepa soil samples. Similarly, more M. hapla inhabited the roots of the susceptible D. carota than in the other crops combined (Table 16). There was no difference in the increase of M. hapla on the two A. cepa cultivars tested. Levels of M. hapla in both soil and roots of A. cepa remained low throughout the season, and were not different from the levels of nematodes supported by sudax. The differential population density of M. hapla between the two halves of range C-17, which was observed before any crops were planted, was maintained throughout the experiment.

Table 15. Number of *Meloidogyne hapla* detected in 100 cm^3 soil from five crops on five sampling dates.

Nematode level and crop[a]	Mean no. nematodes				
	Plant	Julian day			Harvest
	140	170	210	238	257
P_i = High *M. hapla*					
KS *A. cepa*	3.5 ab	2.0 a	0.0	2.0 a	2.5 a
DYG *A. cepa*	12.0 b	2.5 a	0.5 a	1.5 a	2.0 a
SUS *D. carota*	12.0 b	1.5 a	0.0	24.5 b	14.5 b
RES *D. carota*	0.5 a	1.5 a	3.0 a	4.0 a	6.0 a
Sudax	8.5 ab	0.0	1.0 a	2.0 a	1.0 a
P_i = Low *M. hapla*					
KS *A. cepa*	1.5 a	0.5 a	0.0	3.5 a	0.0
DYG *A. cepa*	1.0 a	0.0	0.0	0.0	0.5 a
SUS *D. carota*	0.0	0.0	3.5 a	2.0 a	1.5 a
RES *D. carota*	0.5 a	0.0	0.0	0.5 a	4.5 a
Sudax	2.0 a	0.0	0.0	0.5 a	1.0 a

Values within a column followed by the same letter are not significantly (P = 0.05) different according to Duncan's Multiple Range Test.

[a] KS = Krummery Special / DYG = Downing Yellow Globe
SUS = *M. hapla* susceptible / RES = *M. hapla* resistant

Table 16. Number of Meloidogyne hapla juveniles and adults in 0.25 g of roots from five crops on three sampling dates.

Nematode level and crop[a]	Julian day 170 Juve.	170 Adult	210 Juve.	210 Adult	257 Juve.	257 Adult
P_i = High M. hapla						
KS A. cepa	4.38 a	0.00	1.75 a	0.00	0.00	0.00
DYG A. cepa	1.56 a	0.00	0.25 a	0.25 a	0.00	0.00
SUS D. carota	6.56 a	0.00	18.50 b	15.75 b	11.75 a	10.25 a
RES D. carota	3.75 a	0.00	10.25 a	3.50 a	0.25 b	3.50 ab
Sudax	0.00	0.00	1.50 a	0.00	0.00	0.00
P_i = Low M. hapla						
KS A. cepa	0.00	0.00	0.25 a	0.00	1.25 ab	0.25 b
DYG A. cepa	0.00	0.00	0.25 a	0.50 a	0.00	0.00
SUS D. carota	0.31 a	0.00	1.50 a	4.00 a	10.87 ab	3.00 ab
RES D. carota	4.06 a	0.00	2.50 a	1.00 a	0.25 b	3.50 ab
Sudax	0.00	0.00	0.00	0.00	0.00	0.00

Values within a column followed by the same letter are not significantly (P = 0.05) different according to Duncan's Multiple Range Test.

[a] KS = Krummery Special / DYG = Downing Yellow Globe
SUS = M. hapla susceptible / RES = M. hapla resistant

A multiple regression of final M. hapla density and the following variables was performed for both onion and carrot crops: (1) initial M. hapla density, (2) initial VAM spore density, (3) final VAM spore density, (4) initial Pratylenchus spp. density, (5) final Pratylenchus spp. density, and (6) final density of predaceous nematodes. There was a significant (P=0.05) linear relationship between these variables and the final M. hapla density associated with carrot (Table 17). Individually, there was a significant (P=0.05) relationship between M. hapla density and final VAM spore density. The relationship between M. hapla density and initial VAM spore levels and final Pratylenchus spp. levels was less significant statistically (P=0.10). The variables tested were not individually or jointly, linearly related with M. hapla densities on onion (Table 18).

Correlation coefficients for the two analyses are presented in Tables 19 and 20. Final M. hapla density was positively correlated with initial population levels on both A. cepa and D. carota hosts. Initial and final VAM spore density was not consistently related to M. hapla density. Levels of Pratylenchus spp. at harvest were negatively correlated with M. hapla density on both crops. Predatory nematode density was positively correlated with M. hapla associated with onion and negatively correlated with M. hapla associated with carrot.

Both cultivars of A. cepa tended to support more Pratylenchus spp. than the D. carota cultivars or sudax (Table 21). There was no apparent difference in the host status of the two carrot cultivars for Pratylenchus spp. Levels of this nematode were similar on the same crop grown in different halves of the field (corresponding to low and high initial densities of M. hapla) for all sampling dates.

Paratylenchus spp., predaceous nematodes (family Mononchidae), and fungal-feeding nematodes (superfamily Aphelenchoidea) were present in low num-

Table 17. Coefficients for the multiple linear regression of final Meloidogyne hapla density associated with Daucus carota and six biotic variables.

Coefficients	Standard error	T	P
12.1301398525			
0.4077104181	0.3535971529	1.153	0.40
-0.0394171610	0.1682699180	-0.234	0.50
-0.0374288585	0.0209659620	-1.785	0.10
0.3115788979	0.1431692828	2.176	0.05
-0.4997634179	0.2510027254	-1.991	0.10
-1.0781269963	1.0135338577	-1.064	0.40

$$Y = 12.1301 + 0.4077X_1 - 0.0394X_2 - 0.0374X_3 + 0.3116X_4 - 0.4998X_5 - 1.0781X_6$$

where:

X_1 = Initial M. hapla density

X_2 = Initial mycorrhizal spore density

X_3 = Final mycorrhizal spore density

X_4 = Initial Pratylenchus spp. density

X_5 = Final Pratylenchus spp. density

X_6 = Final density of predaceous nematodes

$R^2 = 0.7756$

$P = 0.05$

Table 18. Coefficients for the multiple linear regression of final Meloidogyne hapla density associated with Allium cepa and six biotic variables.

Coefficients	Standard error	T	P
1.41740552677			
0.08725455107	0.0938694023	0.930	0.40
-0.01427585718	0.0341870186	-0.418	0.50
0.017330147147	0.0761320951	0.228	0.50
-0.05133029312	0.0652800643	-0.786	0.50
0.00758324926	0.1112443527	0.068	0.50
0.57834855221	0.6755953970	0.856	0.40

$$Y = 1.4174 + 0.0873X_1 - 0.0143X_2 + 0.0173X_3 - 0.0513X_4 + 0.0076X_5 + 0.5783X_6$$

where:

$R^2 = 0.2938$

X_1 = Initial M. hapla density

X_2 = Initial mycorrhizal spore density

X_3 = Final mycorrhizal spore density

X_4 = Initial Pratylenchus spp. density

X_5 = Final Pratylenchus spp. density

X_6 = Final density of predaceous nematodes

Table 19. Correlation matrix for final *Meloidogyne hapla* density associated with *Daucus carota* and six biotic variables.

	P_f *M. hapla*	P_i *M. hapla*	P_i Mycorrhizae	P_f Mycorrhizae	P_i *Pratylenchus*	P_f *Pratylenchus*
P_i *M. hapla*	0.7329	1.0000				
P_i Mycorrhizae	0.5779	0.8194	1.0000			
P_f Mycorrhizae	-0.3675	-0.3123	-0.2461	1.0000		
P_i *Pratylenchus*	0.4031	0.3405	0.5080	-0.1495	1.0000	
P_f *Pratylenchus*	-0.3276	-0.2231	-0.0449	-0.2433	0.4342	1.0000
P_f Predaceous nematodes	-0.4111	-0.2543	0.0047	-0.0917	0.1176	0.1176

Table 20. Correlation matrix for final *Meloidogyne hapla* density associated with *Allium cepa* and six biotic variables.

	P_f *M. hapla*	P_i *M. hapla*	P_i Mycorrhizae	P_f Mycorrhizae	P_i *Pratylenchus*	P_f *Pratylenchus*
P_i *M. hapla*	0.3112	1.0000				
P_i Mycorrhizae	-0.0192	0.5343	1.0000			
P_f Mycorrhizae	0.1911	0.6356	0.5359	1.0000		
P_i *Pratylenchus*	-0.3343	-0.1955	-0.1426	-0.0842	1.0000	
P_f *Pratylenchus*	-0.3463	-0.4262	-0.1308	-0.2555	0.6204	1.0000
P_f Predaceous nematodes	0.2308	-0.3112	-0.5126	-0.2522	-0.0137	-0.1015

Table 21. Number of *Pratylenchus* spp. detected in 100 cm^3 soil from five crops on five sampling dates.

Nematode level and crop[a]	Mean no. nematodes				
	Plant	Julian day			Harvest
	140	170	210	238	257
P_i = High *M. hapla*					
KS *A. cepa*	9.5 a	5.0 ab	7.5 ab	20.0 a	9.0 a
DYG *A. cepa*	10.5 a	6.0 abc	10.0 ab	23.5 a	6.5 a
SUS *D. carota*	15.5 a	5.0 ab	4.5 a	8.5 ab	7.5 a
RES *D. carota*	3.5 a	2.0 a	5.5 a	2.0 b	4.0 a
Sudax	8.0 a	0.5 a	10.0 ab	11.5 ab	8.0 a
P_i = Low *M. hapla*					
KS *A. cepa*	10.0 a	10.5 c	10.5 ab	21.0 a	10.5 a
DYG *A. cepa*	19.0 a	8.5 bc	7.0 ab	19.5 a	13.5 a
SUS *D. carota*	5.3 a	4.0 ab	6.5 ab	10.5 ab	13.0 a
RES *D. carota*	16.5 a	4.0 ab	16.5 b	16.0 ab	14.0 a
Sudax	9.5 a	1.0 a	2.5 a	9.5 ab	12.0 a

Values within a column followed by the same letter are not significantly (P = 0.05) different according to Duncan's Multiple Range Test.

[a] KS = Krummery Special / DYG = Downing Yellow Globe

SUS = *M. hapla* susceptible / RES = *M. hapla* resistant

bers on all crops (Tables 22, 23, 24). There was no difference in the population increase of these nematodes on different crops or in the areas with high and low initial M. hapla population density.

All soil samples were monitored for spores of the vesicular-arbuscular mycorrhizal (VAM) fungi Glomus and Gigaspora spp. In general, spore levels decreased significantly (P=0.05) on A. cepa, sudax, and the M. hapla-susceptible D. carota cultivar (Table 25). Spore levels associated with the two A. cepa cultivars were initially different and became more similar with time. In contrast, spore levels were initially similar on the two D. carota cultivars and became more different as the season progressed. Spore densities in the soil were generally higher on D. carota than on A. cepa. The levels of spores associated with sudax were intermediate to those associated with carrot and onion. Colonization of roots by VAM was noted in all samples, but was not quantified because of difficulties with the staining procedure.

There was no difference in bulb weight or number between the KS and DYG cultivars (Figure 24). Final yields were not different in the areas with low and high initial M. hapla density, although both cultivars tended to yield better in the area with low initial M. hapla density. Neither the yield of the KS or DYG cultivars was significantly related to initial M. hapla density on a per sample basis (Table 26). However, there was a significant (P=0.05) positive linear relationship between the harvested weight of the KS cultivar and the initial density of Pratylenchus spp. A multiple linear regression model including both M. hapla and Pratylenchus nematodes was also significant (P=0.05) for the KS cultivar.

Although there was no difference in the total yield between the two D. carota cultivars in the area with high initial M. hapla density, there were fewer

Table 22. Number of Paratylenchus spp. detected in 100 cm^3 soil from five crops on five sampling dates.

Nematode level and crop[a]	Mean no. nematodes				
	Plant	Julian day			Harvest
	140	170	210	238	257
P_i = High *M. hapla*					
KS *A. cepa*	1.5 a	0.5 a	0.0 a	1.0 a	0.5 a
DYG *A. cepa*	1.0 a	2.5 a	0.5 a	0.0	0.5 a
SUS *D. carota*	3.0 a	0.0	0.0	1.0 a	0.0
RES *D. carota*	4.0 a	2.5 a	1.5 a	0.0	0.5 a
Sudax	0.0	1.0 a	1.5 a	2.0 a	0.5 a
P_i = Low *M. hapla*					
KS *A. cepa*	0.0	0.0	0.0	1.5 a	0.0
DYG *A. cepa*	0.0	1.0 a	0.5 a	0.0	0.5 a
SUS *D. carota*	0.0	0.5 a	0.5 a	0.5 a	1.5 a
RES *D. carota*	0.0	0.0	0.0	1.0 a	0.5 a
Sudax	0.0	2.0 a	1.5 a	1.0 a	0.5 a

Values within a column followed by the same letter are not significantly (P = 0.05) different according to Duncan's Multiple Range Test.

[a] KS = Krummery Special / DYG = Downing Yellow Globe
SUS = *M. hapla* susceptible / RES = *M. hapla* resistant

Table 23. Number of predaceous nematodes (F. Mononchidae) in 100 cm^3 soil samples from five crops on five sampling dates.

Nematode[a] level and crop	Mean no. nematodes				
	Plant	Julian day			Harvest
	140	170	210	238	257
P_i = High *M. hapla*					
KS *A. cepa*	0.5 a	0.5 a	0.0	1.0 a	1.0 a
DYG *A. cepa*	0.5 a	1.5 a	2.0 a	0.0	0.0
SUS *D. carota*	1.5 ab	0.5 a	0.0	0.5 a	0.0
RES *D. carota*	3.0 b	0.0	1.5 a	0.0	1.0 a
Sudax	0.5 a	1.0 a	1.0 a	0.5 a	0.5 a
P_i = Low *M. hapla*					
KS *A. cepa*	1.0 ab	0.5 a	0.0	0.0	0.0
DYG *A. cepa*	1.5 ab	0.5 a	0.0	0.0	0.5 a
SUS *D. carota*	0.5 a	0.5 a	0.5 a	0.0	1.0 a
RES *D. carota*	1.5 ab	0.5 a	0.0	0.0	1.0 a
Sudax	0.0	0.5 a	0.0	0.0	1.5 a

Values within a column followed by the same letter are not significantly (P = 0.05) different according to Duncan's Multiple Range Test.

[a] KS = Krummery Special / DYG = Downing Yellow Globe
SUS = *M. hapla* susceptible / RES = *M. hapla* resistant

Table 24. Number of fungivorous nematodes (SF. Aphelenchoidea) in 100 cm^3 soil from five crops on five sampling dates.

Nematode level and crop[a]	Mean no. nematodes				
	Plant	Julian day			Harvest
	140	170	210	238	257
P_i = High *M. hapla*					
KS *A. cepa*	4.5 a	4.0 a	3.5 ab	2.5 a	3.0 ab
DYG *A. cepa*	4.5 a	3.0 a	3.0 ab	2.5 a	1.0 ab
SUS *D. carota*	0.5 b	0.0	6.5 b	3.0 a	1.0 ab
RES *D. carota*	0.5 b	3.0 a	6.0 b	4.0 a	1.0 ab
Sudax	2.0 ab	2.0 a	3.5 ab	4.5 a	1.0 ab
P_i = Low *M. hapla*					
KS *A. cepa*	3.0 ab	1.5 a	4.5 ab	2.0 a	1.0 ab
DYG *A. cepa*	8.5 c	0.5 a	5.5 ab	0.5 a	2.0 ab
SUS *D. carota*	1.5 ab	1.5 a	2.5 ab	5.5 a	4.5 b
RES *D. carota*	0.0 b	0.0	2.5 ab	3.5 a	2.0 ab
Sudax	2.0 ab	0.5 a	0.5 a	1.0 a	0.5 a

Values within a column followed by the same letter are not significantly (P = 0.05) different according to Duncan's Multiple Range Test.

[a] KS = Krummery Special / DYG = Downing Yellow Globe
SUS = *M. hapla* susceptible / RES = *M. hapla* resistant

Table 25. Number of VAM spores (*Glomus* and *Gigaspora* spp.) in 100 cm^3 soil from five crops on five sampling dates.

Nematode[a] level and crop	Mean no. spores				
	Plant	Julian day			Harvest
	140	170	210	238	257
P_i = High *M. hapla*					
KS *A. cepa*	25.5 a	38.0 ab	20.0 ab	18.5 a	15.5 a
DYG *A. cepa*	56.5 b	58.5 c	25.5 bc	30.5 abc	28.0 a
SUS *D. carota*	53.5 b	78.0 d	20.0 ab	26.0 abc	19.0 a
RES *D. carota*	39.5 ab	31.5 a	45.0 d	38.5 bc	89.0 bc
Sudax	50.0 ab	42.5 abc	38.0 cd	27.0 abc	39.5 ab
P_i = Low *M. hapla*					
KS *A. cepa*	32.0 ab	42.0 abc	15.0 a	23.0 ab	15.0 a
DYG *A. cepa*	36.5 ab	31.0 a	17.0 ab	26.0 abc	14.0 a
SUS *D. carota*	36.0 ab	50.5 bc	30.0 bc	33.5 abc	56.5 ab
RES *D. carota*	34.5 ab	30.5 a	29.0 bc	44.5 c	132.0 c
Sudax	39.0 ab	28.0 a	21.5 ab	29.0 abc	28.0 a

Values within a column followed by the same letter are not significantly (P = 0.05) different according to Duncan's Multiple Range Test.

[a] KS = Krummery Special / DYG = Downing Yellow Globe
SUS = *M. hapla* susceptible / RES = *M. hapla* resistant

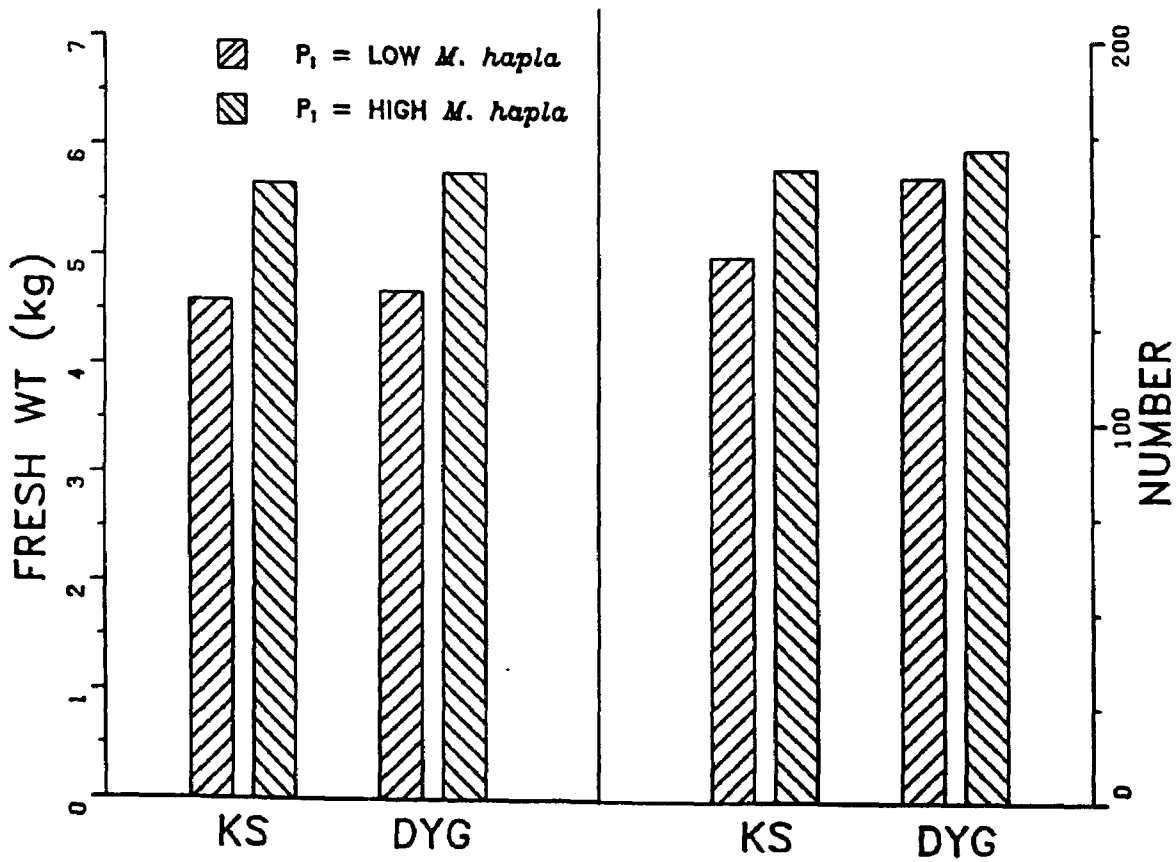


Figure 24. Number and fresh weight of Krummery Special (KS) and Downing Yellow Globe (DYG) Allium cepa produced per 6.15 m row.

Table 26. Regression statistics for the relationship between *Meloidogyne* and/or *Pratylenchus* spp. and the weight of *Allium cepa*.

(Y)[a]	(X)[b]	Regression equation [c]	R^2	P [d]
KS yield	*Meloidogyne*	$Y = 12.80 - 5.198X_1$	0.2640	.19
KS yield	*Pratylenchus*	$Y = 2.72 + 9.531X_2$	0.5979	.02*
KS yield	*Meloidogyne* + *Pratylenchus*	$Y = 4.67 - 1.623X_1 + 3.729X_2$	0.7285	.05*
DYG yield	*Meloidogyne*	$Y = 12.44 - 1.803X_1$	0.1010	.44
DYG yield	*Pratylenchus*	$Y = 5.96 - 4.864X_2$	0.1241	.39
DYG yield	*Meloidogyne* + *Pratyelnchus*	$Y = 8.03 - 0.434X_1 + 1.523X_2$	0.1454	

[a] Total weight (lb) of onions harvested in a 6.15 m row.

[b] Log_{10}(Initial nematode density + 1) / 100 cm^3 soil.

[c] Y = yield / X_1 = *Meloidogyne* density / X_2 = *Pratylenchus* density

[d] significant at P = 0.05 level.

resistant carrots damaged by M. hapla. Fifty-five percent of the M. hapla-susceptible cultivar were disfigured, as compared to 24% of the resistant cultivar. In the area with low initial M. hapla density the M. hapla-resistant cultivar yielded better than the susceptible cultivar, but both cultivars produced similar numbers of galled carrots (Table 27).

Yields of the resistant D. carota cultivar were significantly (P=0.05) reduced in the area infested with M. hapla, whereas similar yields of the susceptible cultivar were harvested from each area. The proportion of susceptible carrots which were disfigured and galled, however, was much reduced in the area with low initial M. hapla levels.

DISCUSSION

This experiment was designed to evaluate the growth of five crops and M. hapla populations without the use of pesticides or the inoculation of nematodes. Initial densities of M. hapla were discrepant between the two areas used for this experiment, but were not different enough to produce results comparable to tests where nematode levels were regulated by inoculation (see previous section). The trends noted in this experiment do deserve attention, however, since the experimental design approximated natural conditions better than could be achieved by the artificial manipulation of the field environment.

The similarity in yield between cultivars and the absence of a significant linear relationship between initial M. hapla density and onion yield may have been due to the low numbers of nematodes present in the experimental site. Previous studies in the greenhouse showed that neither the KS or DYG A. cepa cultivars are affected by M. hapla densities of 100 eggs per plant. It is possible

Table 27. Number and fresh weight (kg) of two carrot cultivars per 6.15 meter row.

Nematode level and crop[a]	Healthy		Stubby and forked with galls		Stubby and forked without galls		Total	
	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.
P_i = High *M. hapla*								
SUS *D. carota*	33.0 a	3.48 a	48.8 a	4.20 a	7.75 a	0.73 a	89.5 a	8.55 a
RES *D. carota*	66.0 a	6.48 a	23.0 b	1.70 b	7.75 a	0.58 a	96.8 a	8.40 a
P_i = Low *M. hapla*								
SUS *D. carota*	54.5 a	6.45 a	22.5 b	1.95 b	14.00 a	0.90 a	91.0 a	9.30 a
RES *D. carota*	103.3 b	11.48 b	24.5 b	1.83 b	15.00 a	1.08 a	175.8 b	14.38 b

Values within a column followed by the same letter are not significantly (P = 0.05) different according to Duncan's Multiple Range Test.

[a] SUS = *M. hapla* susceptible / RES = *M. hapla* resistant

that densities of M. hapla were not much greater than this in the experimental area, or that the distribution of nematodes was such that relatively few plants were subjected to densities greater than 100. The two cultivars did respond differentially to M. hapla (Table 26), supporting my earlier findings that the KS cultivar is less tolerant of M. hapla infection than the DYG cultivar.

Total yields of the M. hapla-susceptible D. carota cultivar were comparable in the areas with low and high M. hapla levels, but more than twice as many nematode-damaged carrots were produced in the area with high M. hapla levels (Table 27). In contrast, carrot yields of the M. hapla-resistant cultivar were significantly (P=0.05) reduced in the area with high initial M. hapla density, despite the fact that relatively few nematodes were observed within soil or root samples from this crop (Tables 15, 16). This result suggests that despite the reduced ability of M. hapla to infect or reproduce on this cultivar, those nematodes which do invade roots affect plant growth more severely than do nematodes infecting the M. hapla-susceptible cultivar. The reduced number of carrots produced by the M. hapla-resistant cultivar in the area with high initial M. hapla density supports the possibility that young carrots of this cultivar can be killed by lower nematode densities than the suceptible cultivar. Like the M. hapla-resistant D. carota, many crops differ in their ablilty to support nematode infection and to sustain nematode injury, justifying the distinction between the susceptibility/resistance and the tolerance/intolerance of a plant to nematode parasitism (Nusbaum and Barker, 1971).

M. hapla levels decreased on A. cepa and increased on D. carota, confirming the contention of some authors that A. cepa is not as good a host for M. hapla as other vegetables commonly grown in muck soil (Bird, 1981; Kotcon,

1979; Van Arkel, 1982). The reason for this is not clear, since final densities of M. hapla were not significantly related to the density of VAM spores, Pratylenchus spp., or predaceous nematodes. The significant relationship between these factors and M. hapla density on carrot suggests that the failure of M. hapla populations to increase on onion may have been due solely to attributes, or lack of attributes, of this host plant. Despite the fact the some juvenile nematodes entered roots of A. cepa, few if any matured to the adult stage (Table 16). It is possible that parasitism by Pratylenchus spp. was sufficient to reduce the suitability of A. cepa as a substrate for M. hapla development, particularly in light of the significant relationship bewteen the yield, and presumably the growth, of the KS cultivar and Pratylenchus density (Table 26).

A differential number of VAM spores was detected in soil samples from A. cepa and D. carota (Table 25). Significantly (P=0.05) more spores were associated with the M. hapla-resistant D. carota cultivar. The reason for this is not clear, but may have been a factor contributing to the reduced number of nematodes supported by this cultivar (See Chapter II). Sudax did not promote the increase of VAM, which is surprising since sorghum is often used to culture VAM in the greenhouse.

Stage-specific Survival of *Meloidogyne hapla* Associated with *Allium cepa*.

INTRODUCTION

Estimates of the survival rates of *M. hapla* can be useful in understanding and predicting the population dynamics of this nematode. The increase of a *M. hapla* population is determined by: (1) the number of nematodes recruited into the population by birth or immigration, (2) the rate at which nematodes develop and reproduce, and (3) the number of nematodes lost due to mortality or emigration. The decline of nematode populations from the beginning to the end of a single season, or over multiple seasons, has been addressed in several studies (for further discussion see Norton, 1978), but the data obtained provides only a gross estimate of change occurring within a population and does little to elucidate the biology of the nematodes studied. The expected survival of each life stage present in populations of *M. hapla*, or other plant-parasitic nematodes, is not known. For some stages, survival rates can be estimated from data collected for different purposes. For example, examination of data from studies by Dropkin (1963), Griffin and Elgin (1977), Wong and Mai (1973b), and this author show that *ca* 20 - 38% of *M. hapla* eggs successfully advance to root-inhabiting stages. In contrast, survival rates for the four life stages of *M. hapla* inhabiting root tissue are not available.

The major objective of this study was to determine the survival rates for those stages of *M. hapla* inhabiting *A. cepa* roots. Field census data of *M. hapla* from the M.S.U. Muck Research Farm was collected during the summer of 1981 to accomplish this objective. In addition, the pathogenicity of *M. hapla* to *A.*

cepa was assessed by examining the relationship between nematode density and onion yield.

MATERIALS AND METHODS

A. cepa (cv Krummery Special) was planted in 3-row beds in range C-17 of the M.S.U. Muck Research Farm on 6/1/81. After planting, nine consecutive beds of onions were divided into five 30.77 cm sections. The sections were marked by stakes and numbered from 1 - 45. A soil sample consisting of ca 150 cm^3 of soil from a depth of 1 - 15 cm was removed from each section six times from planting until harvest: 6/30/81, 7/14/81, 8/12/81, 8/25/81, and 9/22/81. On the last sampling date two soil samples were removed from each section; one from a depth of 1 - 15 cm and another from a depth of 15 - 30 cm. A 100 cm^3 subsample was removed from each sample for nematode analysis. The root fragments contained in the subsample were removed, weighed, stained in a solution of lactophenol with 0.01% acid fuchsin, and examined for nematodes using a dissecting microscope. Nematodes were extracted from the soil of each subsample by a modified sugar flotation-centrifugation technique (Jenkins, 1964). The onions were harvested on 9/22/81 and the fresh weights recorded.

Nematodes within roots were enumerated according to the following scheme: (1) early second-stage juveniles (slender body shape), (2) intermediate second-stage juveniles (broadened body shape), (3) late second-stage juveniles (broadened body shape with rounded tail terminus), (4) third/fourth-stage juveniles (as late second-stage juveniles with multiple cuticles present), (5) preovipositing females (globose body shape), and (6) ovipositing females (globose body shape with eggs visible). This scheme was selected because of the ease

with which these stages could be distinguished morphologically, and because biologically, each stage represents phases in the life cycle of M. hapla that might be expected to have unique rates of survival. The latter point is important, since most methods for calculating stage-specific survival assume that mortality rates are constant within individual stages.

The mortality of each M. hapla life stage was estimated using a method developed by Southwood (1966). The counts for each stage were plotted against time. The area under the resulting stage-frequency curves was integrated to give the total incidence of each stage. Since nematodes required more than one day to complete any stage, the total incidence was divided by the time required to complete development of that stage. The values obtained were estimates of the total number of M. hapla entering each life stage. The daily survival for each stage was computed by comparing the total number of nematodes that entered each stage:

$$S_{II} = \frac{\text{No. in stage II + 1}}{\text{No. in stage II}} \quad (S_{II} = \text{daily survival of Stage II})$$

Developmental times for 20 C, the average soil temperature from the sample site at a 15 cm depth, were estimated from the data presented by Tyler (1933), Vrain et al., (1978), and Wong and Mai (1973a). Separate estimates of developmental times for the early second-, intermediate second-, and late second-stages could not be obtained from these studies, so the data for the stages were combined. The estimated developmental times were: 16 days for the second juvenile stage, 2 days for the combined third and fourth stages, and 10 days from the molt to adulthood until oviposition.

RESULTS

Root levels of M. hapla were maximal on 7/29/81 (Julian day 210) and very low at harvest on 9/22/81 (Julian day 265). On 7/29/81 there were an average of 3.40 nematodes in the root fragments contained within 100 cm^3 of soil, or ca 4.277 nematodes per gram of root tissue. M. hapla levels in the soil decreased from planting until the first sampling date on 6/30/81 (Julian day 181), and then increased until the last sampling at harvest (Figure 25). Both root and soil nematode levels were lower at a depth of 15 - 20 cm than at a depth of 1 - 15 cm on 9/22/81. Comparing final soil + root densities (P_f) to initial soil densities of M. hapla (P_i) yields a seasonal rate of increase of 4.904 nematodes.

All root-inhabiting stages of M. hapla occurred simultaneously on and after Julian day 210 (7/39/81) (Table 28). Levels of second-stage juveniles peaked at the second sampling date on Julian day 195 (Figure 26). Third/fourth stage juveniles and pre-ovipositing adults reached maximal levels on Julian day 210. The occurrence of ovipositing females was first noted on the same sampling date, and was greatest on Julian day 224, 73 days after planting. The survival rates for the second, and the combined third and fourth stages were 0.7944 and 1.0763, respectively. The survival of pre-ovipositing adults could not be estimated since the length of the ovipositional period was not known.

There was a slight yet significant (P=0.05) linear relationship between the peak M. hapla density at 7/29/81 and the final fresh weight of onion (Figure 27) The mean fresh weight of onions in each 30 cm bed (3 rows) was 1090.92 ± 41.550 g.

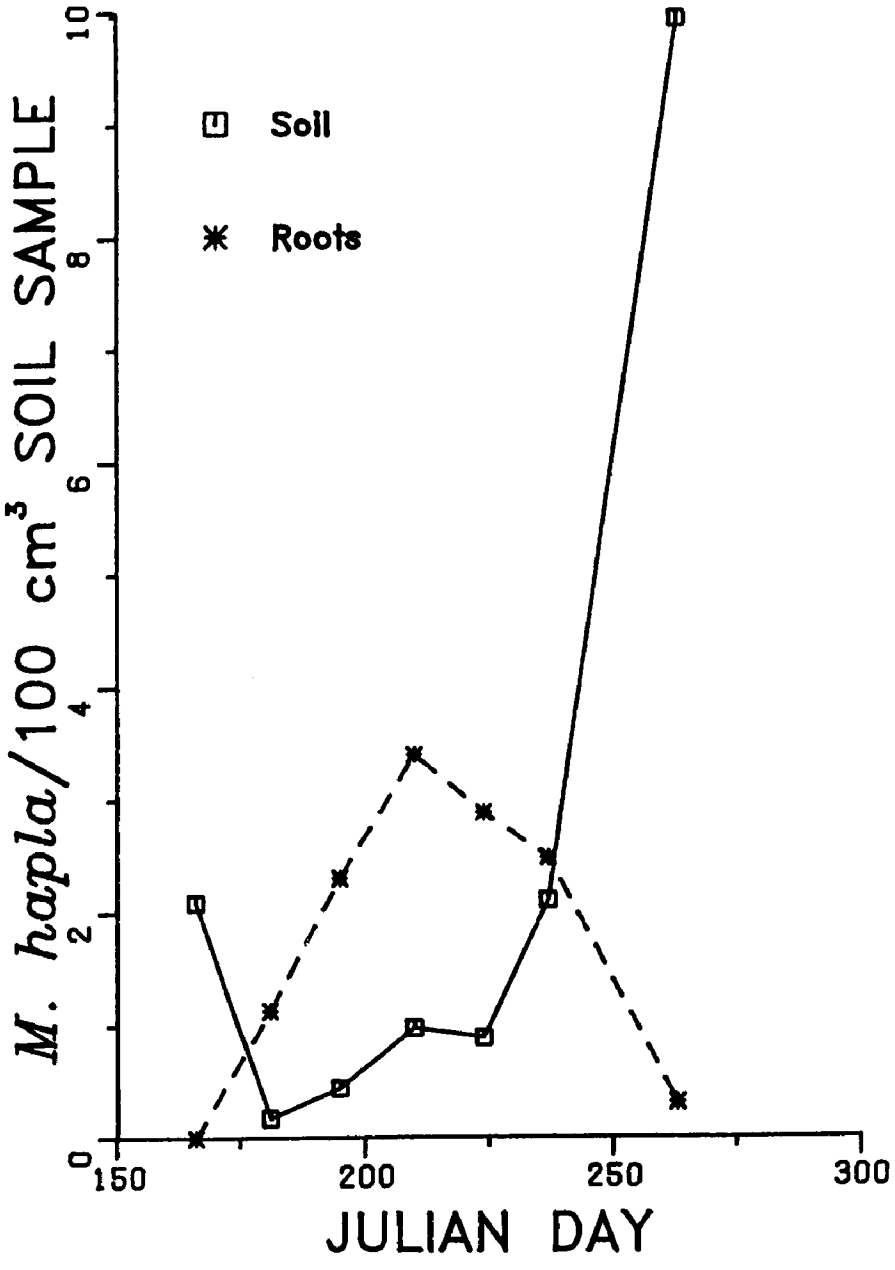


Figure 25. Levels of Meloidogyne hapla in 100 cm^3 soil samples collected from the M.S.U. Muck Research Farm in 1981.

Table 28. Incidence of Meloidogyne hapla in 1981 by life-stage.

SECOND-STAGE JUVENILES:

Date	Mean	Variance	Standard Error	
181	1.1333	3.2711	0.2696	
195	2.2444	7.3847	0.4051	
210	1.2889	2.1165	0.2169	Average standard error of mean = 23%
224	0.5111	0.6943	0.1242	
237	0.9556	1.9995	0.2981	

THIRD/FOURTH-STAGE JUVENILES:

Date	Mean	Variance	Standard Error	
195	0.0444	0.0425	0.0307	
210	0.4000	0.6844	0.1233	Average standard error of mean = 49%
224	0.1778	0.2351	0.0723	
237	0.0667	0.0622	0.0372	

PRE-OVIPOSITING FEMALES:

Date	Mean	Variance	Standard Error	
195	0.0222	0.0217	0.0220	
210	1.5778	4.8217	0.3273	Average standard error of mean = 41%
224	1.2000	2.6930	0.2446	
237	0.7556	1.4625	0.1803	

OVIPOSITING FEMALES:

Date	Mean	Variance	Standard Error	
210	0.1333	0.1156	0.0507	
224	0.9333	2.5500	0.2381	Average standard error of mean = 29%
237	0.6889	1.1810	0.1620	

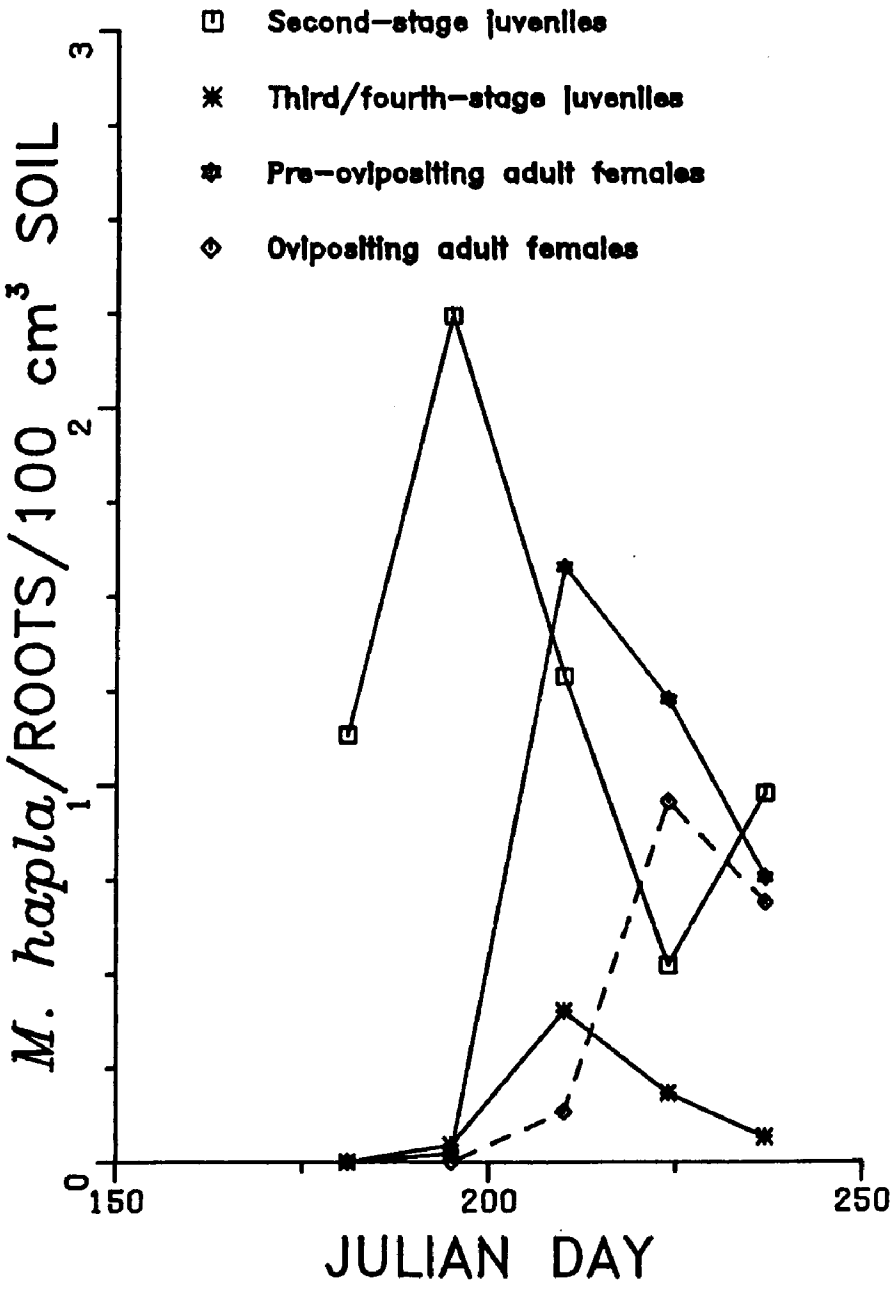


Figure 26. Stage-specific density of Meloidogyne hapla inhabiting roots of Allium cepa in 100 cm^3 of soil from the M.S.U. Muck Research Farm in 1981.

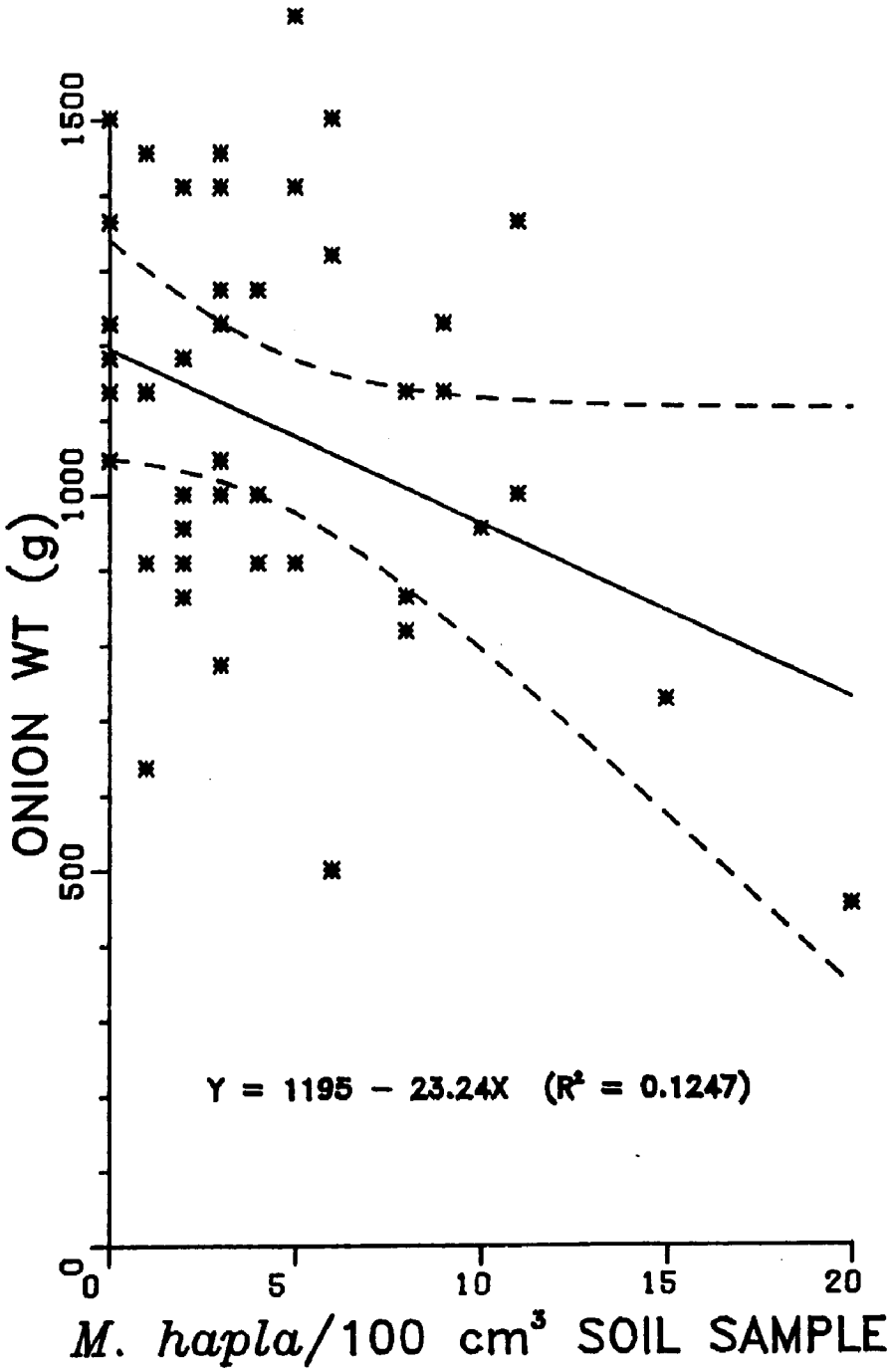


Figure 27. Relationship between Meloidogyne hapla density and the fresh weight of bulbs of Allium cepa harvested per 30 cm row.

DISCUSSION

Eighty percent of the second-stage M. hapla juveniles inhabiting root tissue survived to become third- and fourth-stage juveniles. Survival of the third- and fourth-stage juveniles was ca 100%. These results concur with knowledge on the biology of M. hapla. Second-stage juveniles must locate and establish feeding sites within the root, a process that can be influenced by the number of nematodes already inhabiting that root, and by the nutrient status of the host plant. It is not likely that all nematodes entering a root will be successful in procurring enough space and nutrients to complete development. Third- and fourth-stage juveniles, on the other hand, do not feed and are probably not vulnerable to mortality factors unless the host plant dies.

Second-stage juveniles were subdivided into early, intermediate, and late substages for this study because I felt it likely that survival differed from the beginning to the end of this stage. Unfortunately, the data could not be analyzed according to this classification, since estimates of developmental time for each substage were not available. Southwood's method for calculating age-specific mortality assumes that mortality is distributed equally throughout the stage. If mortality is heavy at the onset of the stage, as may be the case with M. hapla, then this method will under-estimate the total incidence of the stage, and consequently, over-estimate the mortality of the stage. If I am correct in assuming that most mortality occurs in the early portion of the second juvenile stage, then the actual survival of second-stage juveniles may be greater than 80%.

There was a four-fold increase in levels of M. hapla from the beginning until the end of this experiment. The data indicate that only one generation of nematodes occurred (Figure 26). Survival of pre-ovipositing females was not

calculated. Inspection of the data in Figure 26, however, shows that it is unlikely that all females commenced oviposition before the senescence of onion roots at harvest. The phenology of A. cepa, then, exerts the same pressure on the population increase of M. hapla as if mortality in an earlier stage had occurred; in either case, the affected nematode does not replace itself in the population. The early senescence of onion roots may be the major factor limiting the increase of M. hapla populations relative to those crops in which root growth continues until and beyond harvest.

M. hapla was not highly pathogenic to A. cepa in this study, although onion yields were significantly (P=0.05) reduced due to this nematode (Figure 27). The increase and detrimental influence of M. hapla associated with A. cepa indicate that this crop, or at least the cultivar Krummery Special, may be a better host for M. hapla than is generally assumed.

GENERAL DISCUSSION

The preceding studies demonstrated that M. hapla is present in much of the onion acreage in Michigan and that it may be detrimental to onion production. M. hapla was pathogenic to A. cepa under greenhouse conditions, and decreased onion yield in a small field plot (Figure 27). Since the numbers of nematodes necessary to reduce plant growth are not commonplace or uniformly distributed in commercial onion production sites, however, this nematode does not seem to be of major economic significance to A. cepa.

Differences in the reactions of different A. cepa cultivars to M. hapla infection were observed, as was demonstrated in earlier experiments (Franklin, 1959; Kotcon, 1979; Sasser, 1954) Different cultivars of many plant species vary

in their response to nematodes. Generalizations on the nonhost status of a plant species, as have been made for A. cepa, can be misleading and should be avoided.

In general, it seems that A. cepa is not well-suited for the increase of M. populations, particularly as compared to D. carota. However, M. hapla does reproduce on A. cepa and populations can increase from the beginning to the end of a growing season, as evidenced by my greenhouse experiments and the field census data collected to compute M. hapla survival rates. The ability of A. cepa to maintain M. hapla populations should be considered when a muck vegetable rotation is followed. The final levels of nematode present at the harvest of A. cepa may not need to be very great to threaten the production of future crops, especially carrot or celery (Slinger, 1976). Although an onion-carrot rotation is preferable to the monoculture of carrot, A. cepa should not be viewed as a panacea to problems caused by M. hapla.

There was no significant relationship between nematode densities of 1000-15,000 eggs per plant and the increase of M. hapla on the A. cepa cultivar Krummery Special (Figures 19B). For the Downing Yellow Globe cultivar, however, a significant relationship between inoculum levels of 500 - 15,000 eggs and final nematode density was observed (Figure 19A). It may be that the first juveniles entering KS onions sufficiently alter root growth to reduce the number of sites available for the entry of additional nematodes. By the time a flush of new root growth occurs, juveniles in the soil may have depleted their food reserves and be less able to locate and penetrate the new root growth. If so, an A. cepa cultivar more tolerant to M. hapla infection, such as DYG, may have been a more suitable cultivar than KS to test the density-dependence of M. hapla increase. It is also possible that the numbers of M. hapla entering KS roots

increases with increasing inoculum levels, but that the survival of nematodes is reduced when root nematode levels are high. Appropriate data to test this possibility was collected in the greenhouse experiment using high nematode inoculum levels. An analysis of stage-specific survival rates was not performed, however, since the stage frequency counts increased steadily throughout the experiment and a reasonable end point to each stage could not be estimated.

CHAPTER II.

Studies on the Interaction Between *Meloidogyne hapla* and the Vesicular-Arbuscular Mycorrhizal Fungus, *Glomus fasciculatus*, Associated with *Allium cepa*

Colonization of plants by vesicular-arbuscular mycorrhizae (VAM) may have a depressive effect on the increase of phytoparasitic nematode populations. The mechanisms responsible for this influence cannot be identified until the relationship between VAM and nematodes is understood in greater detail. The major objective of the research presented here was to identify which portion of the nematode life cycle is most affected by VAM colonization in the host plant. Five experiments were performed to: (1) establish whether VAM can limit the increase of nematodes, (2) identify the influence of mycorrhizal colonization on the location and penetration of plant roots by nematodes in the soil, and (3) characterize and compare the development and reproduction of nematodes within mycorrhizal and non-mycorrhizal roots. Although the experiments were conducted with the nematode *Meloidogyne hapla*, the VAM *Glomus fasciculatus*, and the host plant *Allium cepa*, they were intended to provide information that might be useful in characterizing other nematode-VAM-plant associations.

Influence of the Vesicular-Arbuscular Mycorrhizal Fungus, *Glomus fasciculatus*, on the Pathogenicity and Increase of *Meloidogyne hapla* Infecting *Allium cepa*

INTRODUCTION

Vesicular-arbuscular mycorrhizae (VAM) and phytoparasitic nematodes are both commonly associated with many plant species. The stimulation and inhibition of plant growth by VAM and nematodes, respectively, has been described in detail (Safir, 1980; Seinhorst, 1965). The concomitant influence of VAM and nematodes on plant growth has also been examined but is not yet thoroughly understood. According to some studies, nematode parasitism is not as deleterious to plants with an established VAM association as to plants inhabited by nematodes alone. For example, Kellam and Schenck (1980) demonstrated that yields of soybean inoculated with both *Meloidogyne incognita* and *Glomus macrocarpus* were significantly greater than plants inoculated with the nematode alone. Similarly, Atilano *et al.* (1981) found that dry shoot weight of grape infected with *M. arenaria* was increased when plants were colonized by *G. fasciculatus*.

It is not clear if the ability of some plants to better withstand nematode infection when colonized by VAM is due to the reduction of nematode population levels, or to the increased tolerance of mycorrhizal plants for nematode parasitism. VAM colonization decreased population levels of *Heterodera solanacearum*, *M. hapla*, and *M. incognita* infecting tobacco, carrot, and tomato, respectively (Fox and Spasoff, 1972; Sikora and Schonbeck, 1975; Kellam and Schenck, 1980), indicating that VAM adversely affects the ontogeny of some

phytoparasitic nematodes. Conversely, mycorrhizal plants supported greater numbers of M. incognita and were larger than non-mycorrhizal plants (Atilano et al., 1981), suggesting that the increased plant nutrition associated with VAM colonization enhanced the ability of plants to tolerate nematode infection.

The interaction between M. hapla and the growth of A. cepa was examined in experiments described previously (see Chapter I). Correspondingly, the influence of VAM on the growth of A. cepa has been reviewed by Carling and Brown (1982) and Gerdemann (1975). The objective of my research was to assess the impact of VAM on the relationship between M. hapla and A. cepa. Two experiments were performed to determine if: (1) VAM enhances plant growth and has no impact on M. hapla, or (2) VAM has a positive influence on A. cepa and a negative impact on M. hapla, or (3) both A. cepa and M. hapla are benefited by VAM colonization.

MATERIALS AND METHODS

Greenhouse experiment. A Houghton muck soil with a pH of 6.6 and a phosphorus (P) level of 60 ppm was obtained from the M.S.U. Muck Research Farm for this experiment. Approximately 1500 cm^3 of pasteurized soil was thoroughly mixed with inoculum prior to placement into each of 120 clay pots. Inoculation treatments, administered in 5 ml of water, were: (1) 10,000 M. hapla eggs, (2) 2000 G. fasciculatus spores, (3) 10,000 M. hapla eggs and 2000 G. fasciculatus spores, or (4) water passed over eggs and spores (control). Presumably, the water filtered over the nematode and mycorrhizae inoculum contained any microbial organisms present in the cultures. One A. cepa seed (cv Krummery Special), pregerminated for 48 hours, was planted into the middle of

each pot. The plants were maintained in the greenhouse for eight weeks and watered daily.

Six replicates of each treatment were harvested 2, 4, 6, and 8 weeks after planting. At each harvest, root, bulb, and leaf fresh weights, and bulb and leaf dry weights were determined. Soil samples from each pot (100 cm^3) were assayed for nematodes and fungal spores by a sugar flotation-(1.37 specific gravity) centrifugation technique (Jenkins, 1964). The entire root system of each plant was stained in a solution of lactophenol and 0.01% acid fuchsin. The colonization of roots by VAM was confirmed, and the nematodes contained within each root system were enumerated using a stereomicroscope. Plants that died during the experiment were included in the analyses of plant growth parameters but were not considered in the analyses of nematode population growth, since M. hapla is an obligate parasite that cannot develop in the absence of a host plant. A preliminary experiment using the same methodology but with a duration of seven weeks was performed six months earlier.

Microplot experiment. A. cepa (cv Krummery Special) plants for this experiment were seeded in the greenhouse and transplanted five weeks later to the M.S.U. Muck Research Farm. Five onion seeds were planted into each of 280 plastic pots containing 217 g (dry wt) of a 1:7 mix of a muck and sand soil with a pH of 6.5 and P level of ca 2 ppm (Bray's P-1 extractable). At planting, one third of the pots were inoculated with 2000 G. fasciculatus spores in 5 ml of water. Another third were fertilized with 5 ml of a 8.60 mg/ml solution of KH_2PO_4 in water, adding ca 48 ppm P to stimulate the growth response obtained using VAM. The final third of the pots were non-inoculated and received no P fertilization. Pots not inoculated with G. fasciculatus received 5 ml of water passed over

spores so that any microbial organisms inhabiting the VAM culture were present in all three treatments. The nutrient levels in all pots were adjusted using KNO_3 (non-P-treated pots) and NH_4NO_3 (P-treated pots) so that only P levels were different between treatments. All pots were thinned to one seedling after one week. The treatments were repeated at transplanting, except that 50 cm^3 of soil from a greenhouse culture of G. fasciculatus containing ca 1200 spores and 14 g of superphosphate replaced the original inoculum preparations.

In June, ten plants from the same treatment were planted in a circular pattern into eight aluminum microplots ca 40 cm in diameter. In addition, four microplots were each planted with eight G. fasciculatus-treated plants and fertilized with superphosphate. One half of the microplots containing control, P-treated, or VAM-inoculated plants, and all of the microplots containing VAM + P-treated plants were inoculated with 2000 M. hapla second-stage juveniles. The nematode inoculum was delivered in 5 ml of water around the roots of each plant. Four replications of the resulting seven treatments were arranged in a randomized block design. The microplots were fumigated with Vorlex (143 l/ha) in May of the previous year and contained no phytoparasitic nematodes at the time of transplanting.

One plant was removed from each microplot every two weeks for 12 weeks. The plant selected for each destructive sample was predetermined by its position in the microplot, in order to eliminate bias in the selection of a single plant to represent each microplot. Bulb and leaf fresh and dry weights were determined. One gram of roots and 100 cm^3 of soil surrounding each plant were assayed for M. hapla and G. fasciculatus as described previously. All plants remaining at the final harvest were removed and included in the last data analysis.

The change in M. hapla density over the growing season was computed by comparing the final and initial density of nematodes in a 100 cm^3 soil sample:

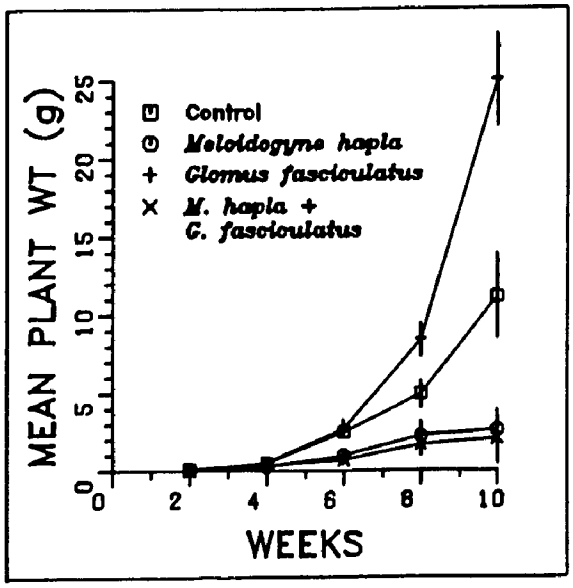
$$I_{M.\ hapla} = \text{final density } (P_f) \text{ / initial density } (P_i)$$

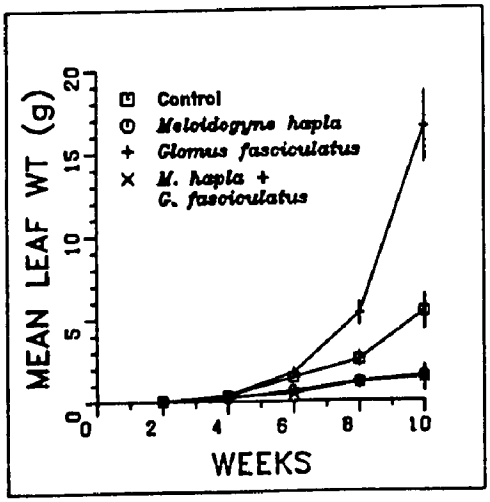
RESULTS

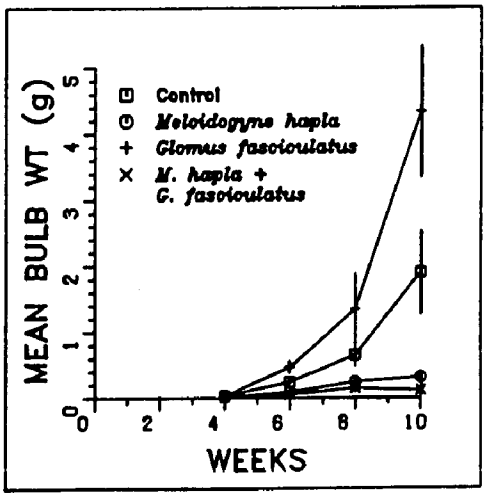
Greenhouse experiment. Plants inoculated with G. fasciculatus alone were significantly (P=0.05) larger after eight weeks than control plants or plants inoculated with M. hapla (Figure 28). Plants infected with M. hapla were consistently smaller than non-infected plants throughout the experiment, regardless of the presence of VAM. By the end of the experiment, ten weeks after planting, leaf fresh weights of plants inoculated with the VAM alone were more than twice as great as control plants and over ten times larger than plants inoculated with M. hapla alone or with M. hapla and G. fasciculatus. These differences are also reflected in a comparison of leaf dry weights. The fresh bulb weight of mycorrhizal plants was also greater than non-mycorrhizal plants when the experiment was terminated. The root systems of VAM-treated plants were significantly larger after ten weeks than those of plants inoculated with nematodes, but were not different from non-inoculated control plants.

Two, four, and six weeks after planting, there was no difference in the levels of M. hapla associated with either mycorrhizal or non-mycorrhizal plants (Figure 29). After the first appearance of ovipositing females eight weeks after planting, however, M. hapla density per root system increased both in the presence and absence of G. fasciculatus. To test whether the increase in nematode numbers was due to an influx of second generation nematodes, an analysis of variance showed that the density ($\log_{10}$) of juveniles during this time

Figure 28. Mean fresh weight (± standard error) of *Allium cepa* inoculated with *Meloidogyne hapla*, *Glomus fasciculatus*, *Meloidogyne hapla* and *Glomus fasciculatus*, and non-inoculated control.


MEAN PLANT WT (g)
0
5
10
15
20
25
0
2
4
6
8
10
WEEKS
Control
Meloidogyne hapla
Glomus fasciculatus
M. hapla +
G. fasciculatus


MEAN LEAF WT (g)
0
5
10
15
20
0
2
4
6
8
10
WEEKS
Control
Meloidogyne hapla
Glomus fasciculatus
M. hapla +
G. fasciculatus


MEAN BULB WT (g)
0
1
2
3
4
5
0
2
4
6
8
10
WEEKS
Control
Meloidogyne hapla
Glomus fasciculatus
M. hapla +
G. fasciculatus

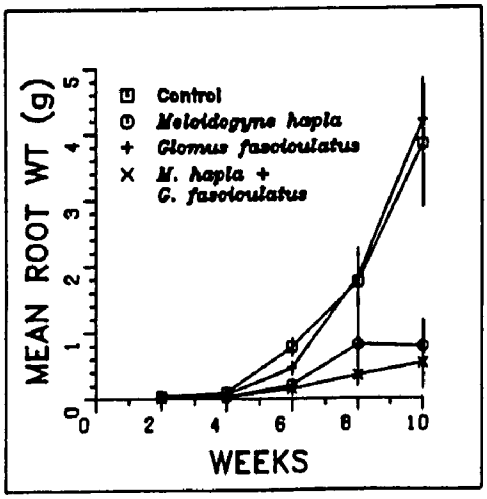

MEAN ROOT WT (g)
0
1
2
3
4
5
0
2
4
6
8
10
WEEKS
Control
Meloidogyne hapla
Glomus fasciculatus
M. hapla +
G. fasciculatus

Figure 28.

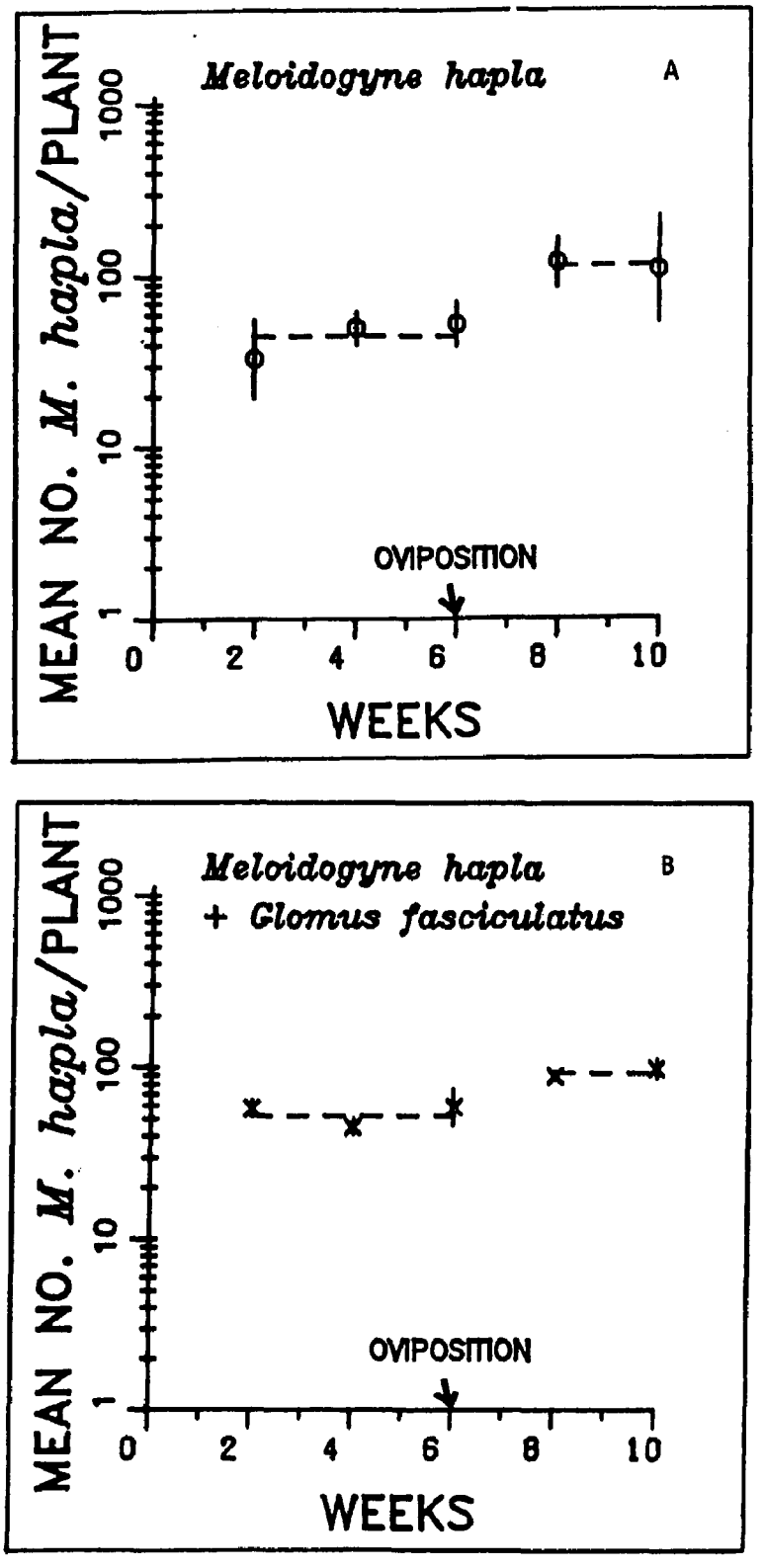


Figure 29. Mean number of Meloidogyne hapla (± standard error) inhabiting root systems of Allium cepa inoculated with Meloidogyne hapla alone (A) or with Meloidogyne hapla and Glomus fasciculatus (B).

was unchanged in the plants inoculated with M. hapla alone, and decreased significantly (P=0.05) in the plants inoculated with both nematodes and mycorrhizae (Table 29).

There was no difference between the number of M. hapla observed within mycorrhizal and non-mycorrhizal plants during this experiment. There was evidence, however, that differences in the rate of nematode development occurred in the presence and absence of VAM. Six weeks after planting, at the time ovipositing females were first observed, twice as many nematodes (P=0.08) had matured to the adult stage on plants inoculated with the nematode alone (Figure 30). Levels of M. hapla juveniles in the soil were not different between treatments except for week two, when significantly (P=0.05) more nematodes were observed in pots containing M. hapla with no VAM (Figure 31).

Levels of VAM spores were not different in pots inoculated with spores alone or with spores and nematodes until ten weeks after planting (Figure 32). Spore densities associated with both treatments decreased six weeks after planting and then increased by the next sampling date. From week eight until week ten, the density of spores in the soil surrounding nematode-infected plants was unchanged, but decreased in the soil surrounding nematode-free plants.

Microplot experiment. There was no difference in the fresh bulb or shoot weights of control, VAM-inoculated, or P-fertilized plants, although control plants tended to be the smallest of the three treatments (Figures 33, 34). M. hapla infection was not detrimental to control or treated plants. Similar trends in plant growth were reflected in bulb and leaf dry weights.

Significantly (P=0.05) more nematodes entered and were maintained in plants inoculated with VAM alone than in plants both inoculated with VAM and

Table 29. Density of second-stage Meloidogyne hapla (log_{10}) in Allium cepa inoculated with nematodes alone or with nematodes and Glomus fasciculatus six and eight weeks following inoculation.

Treatment	Mean	Standard deviation	T[a]	P[b]
Meloidogyne hapla				
Week 6	1.3240	0.235	-1.201	0.2836
Week 8	1.6740	0.608		
Meloidogyne hapla + Glomus fasciculatus				
Week 6	1.6200	0.246	2.879	0.0450
Week 8	1.1000	0.229		

[a] Value obtained from a two sample T test.

[b] Probability that the mean values are different.

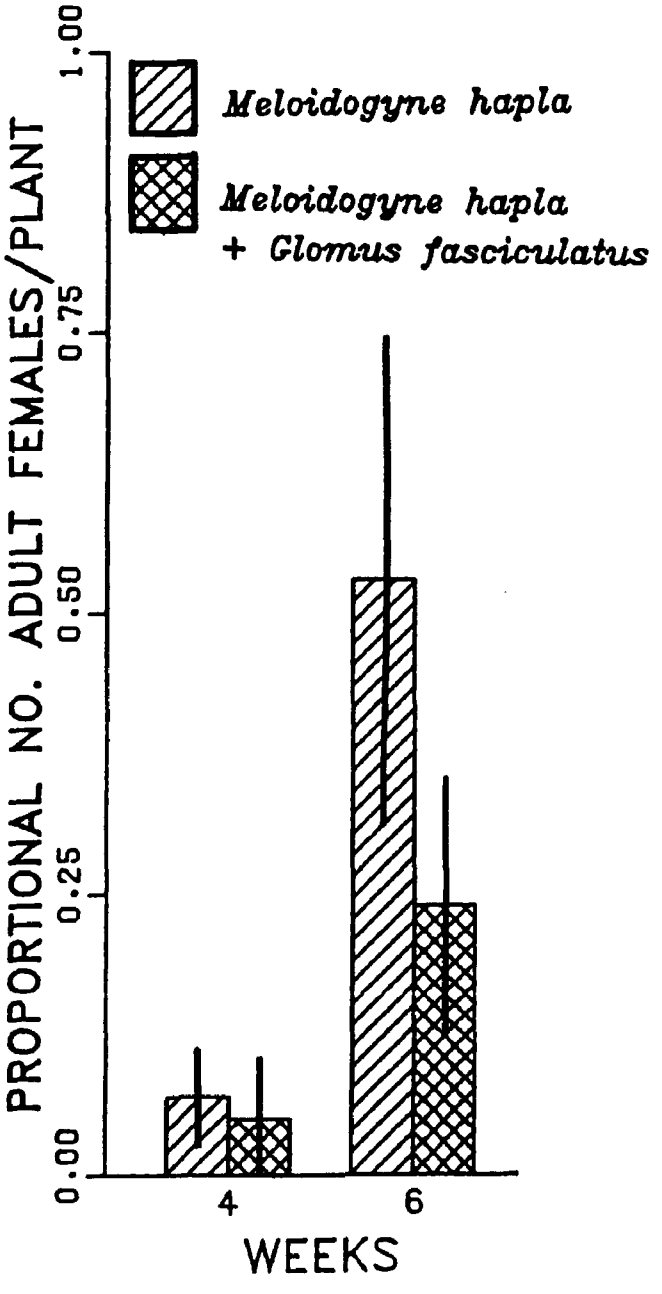


Figure 30. Proportion of the total number of Meloidogyne hapla reaching the adult stage in Allium cepa inoculated with Meloidogyne hapla alone or with Meloidogyne hapla and Glomus fasciculatus (mean values + $CI_{0.05}$).

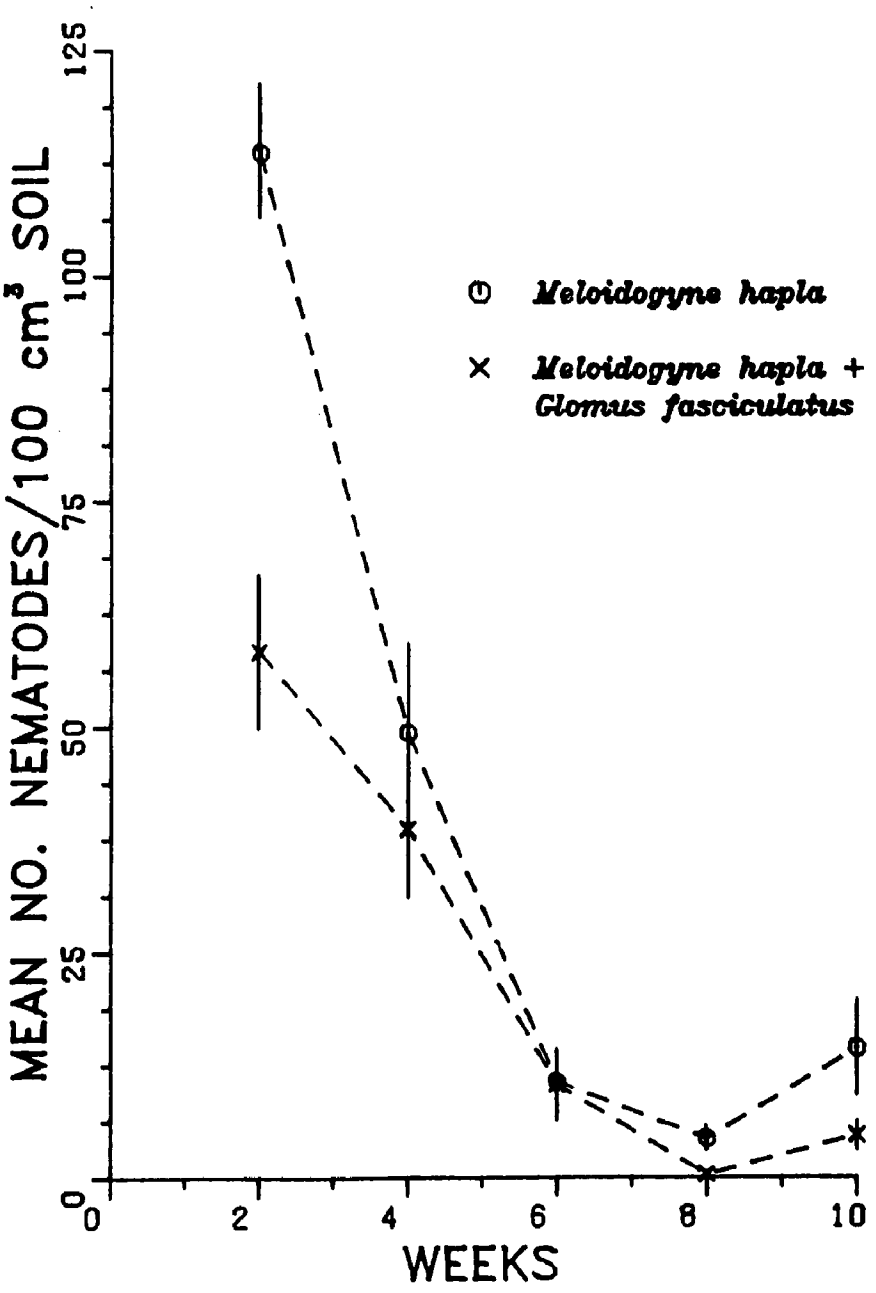


Figure 31. Number of Meloidogyne hapla second-stage juveniles in 100 cm^3 soil surrounding Allium cepa inoculated with Meloidogyne hapla alone or with Meloidogyne hapla and Glomus fasciculatus.

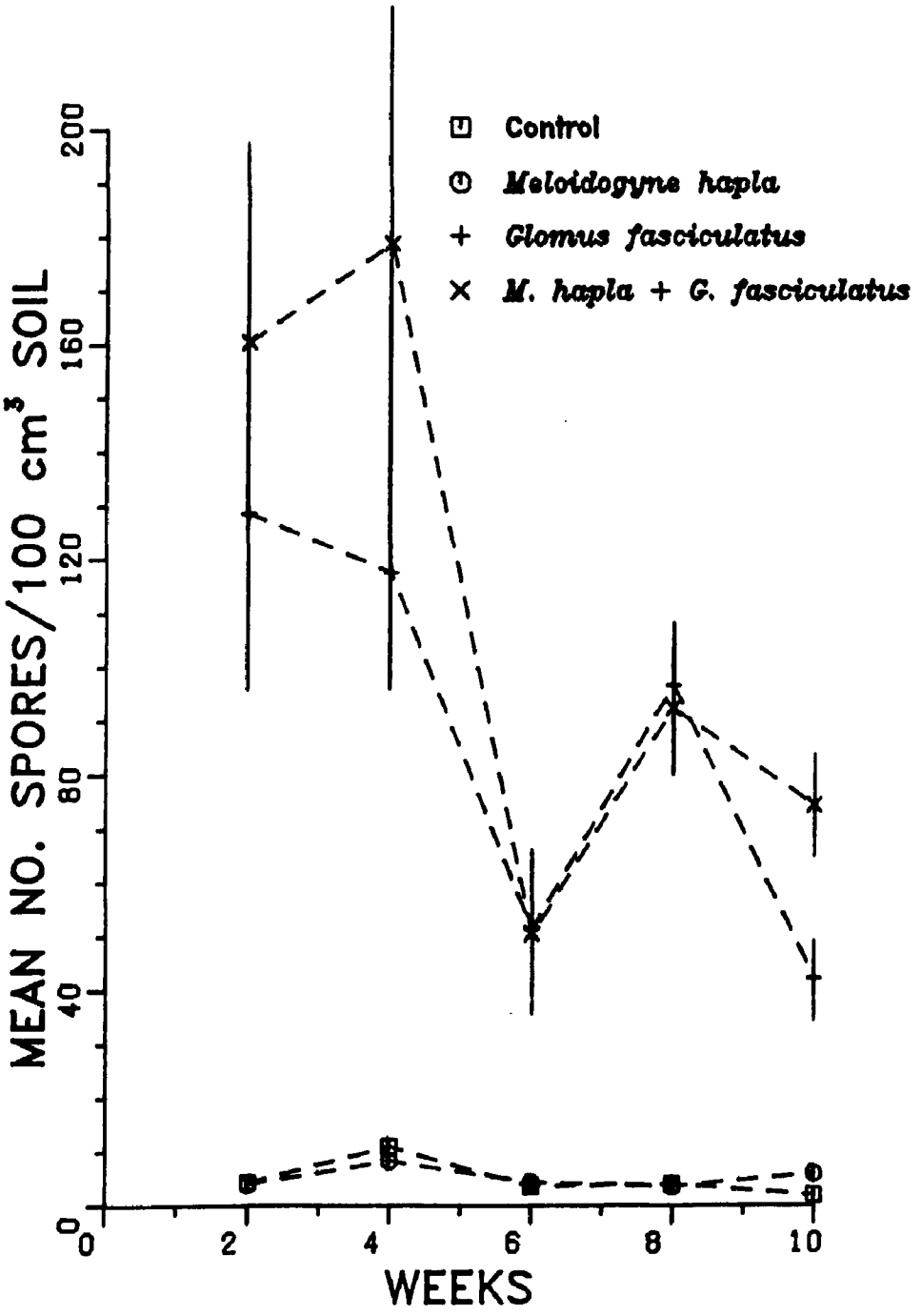


Figure 32. Mean number (± standard error) of Glomus fasciculatus in 100 cm^3 soil surrounding Allium cepa inoculated with Meloidogyne hapla alone or with Meloidogyne hapla and Glomus fasciculatus.

Figure 33. Fresh bulb weight of *Allium cepa* supplemented with phosphorus, inoculated with *Glomus fasciculatus* (myco), amended with both *Glomus fasciculatus* and phosphorus, and unamended control, with and without the nematode *Meloidogyne hapla*.

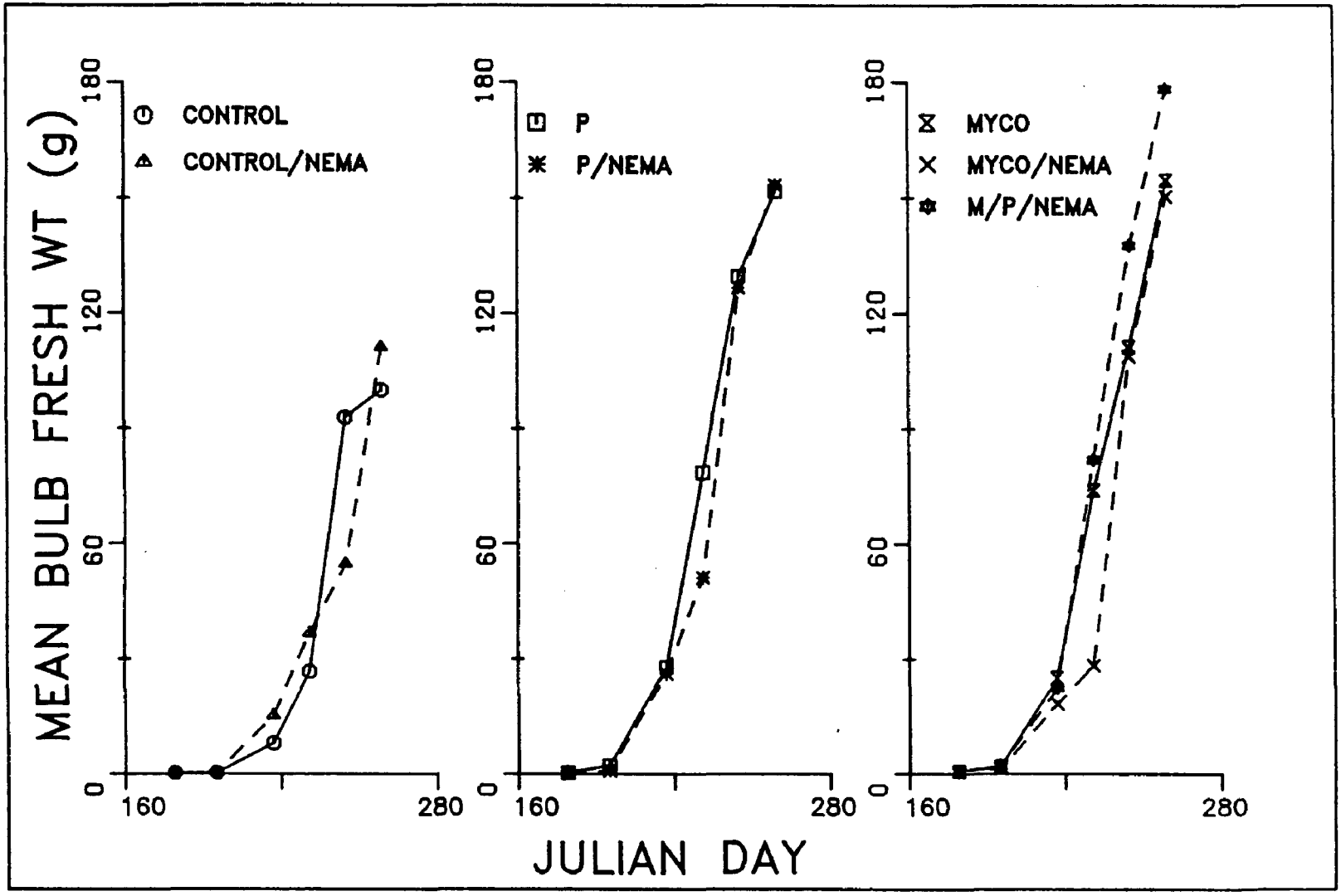

MEAN BULB FRESH WT (g)
180
120
60
0
CONTROL
CONTROL/NEMA
P
P/NEMA
MYCO
MYCO/NEMA
M/P/NEMA
160
280
JULIAN DAY

Figure 33.

Figure 34. Fresh shoot weight of Allium cepa supplemented with phosphorus, inoculated with Glomus fasciculatus (myco), amended with both Glomus fasciculatus and phosphorus, and unamended control, with and without the nematode Meloidogyne hapla.

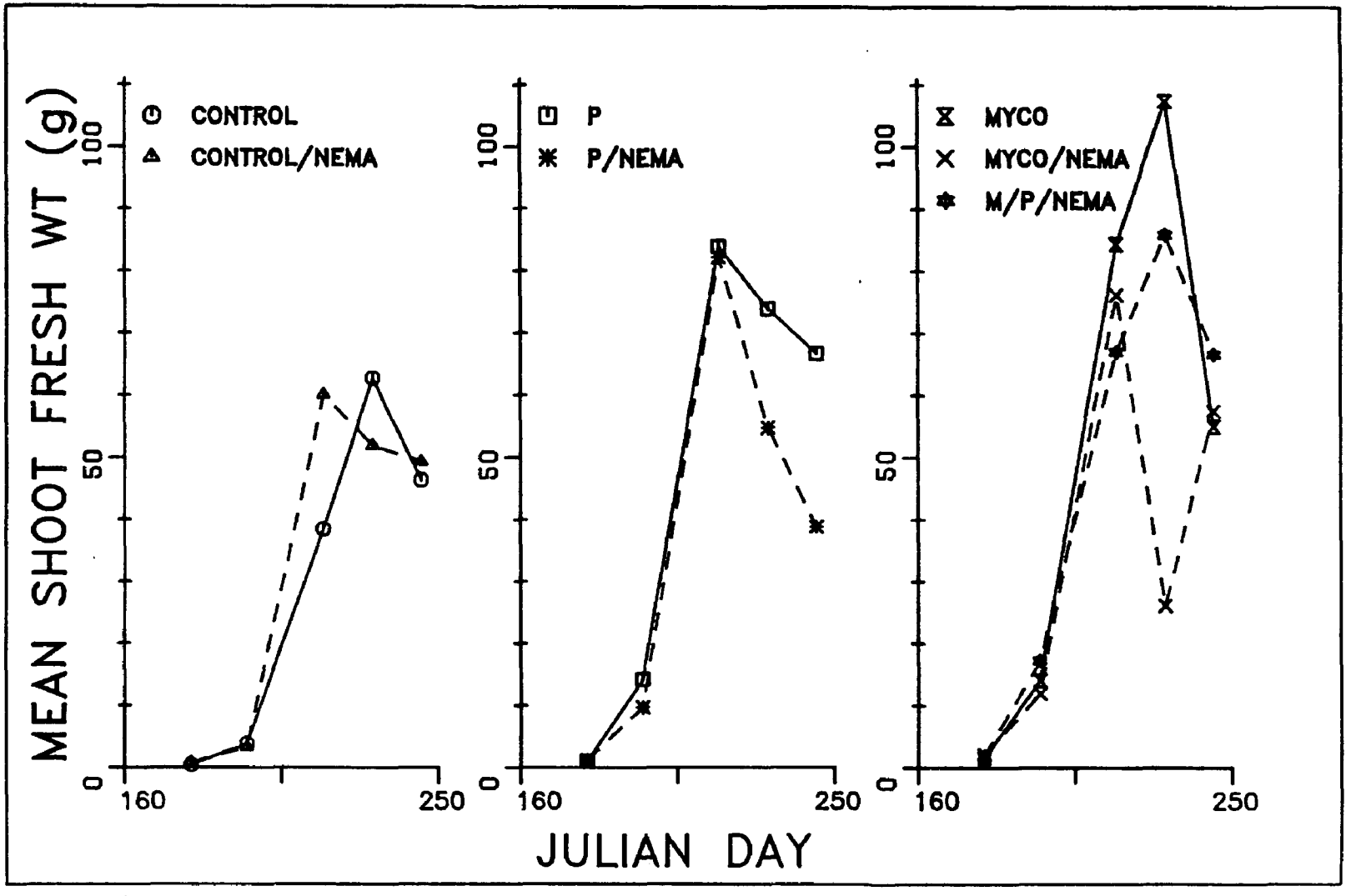

MEAN SHOOT FRESH WT (g)
CONTROL
CONTROL/NEMA
P
P/NEMA
MYCO
MYCO/NEMA
M/P/NEMA
0
50
100
160
250
JULIAN DAY

Figure 34.

fertilized with P at transplant (Figure 35). Plants fertilized with P tended to support more nematodes than control plants and fewer nematodes than plants receiving VAM. Oviposition by M. hapla females was first observed on 8/5/82, ca two months after transplanting. There was no significant difference in nematode levels between sampling dates for any treatment, indicating that only one nematode generation invaded root tissue during the experiment.

Levels of M. hapla juveniles in the soil decreased on the first three sample dates, 6/28-8/5/82, and then increased significantly (P=0.05) from the third until the final sample date, 8/5-9/16/82 (Figure 36). Nematode density was greatest in microplots inoculated with VAM alone and lowest in microplots containing control plants. The index of change in M. hapla density (P_f/P_i) for the VAM, VAM + P, P, and control treatments was 18.8, 3.0, 0.7, and 0.1, respectively.

Levels of G. fasciculatus spores in the soil significantly (P=0.05) decreased, increased, and decreased again over the course of the growing season (Figure 37). There were no significant differences in spore levels for those treatments inoculated with G. fasciculatus. Some indigenous spores were present in microplots not inoculated with G. fasciculatus. The density of spores in non-inoculated microplots was less than that of inoculated microplots, but followed the same general trends over the course of the growing season.

DISCUSSION

Data from the field and greenhouse experiments suggest that mycorrhizal colonization may benefit A. cepa infected by M. hapla. Fresh bulb weight was similar for mycorrhizal plants grown in microplots and fertilized at transplant with P and non-mycorrhizal plants supplemented with P (Figure 33) even

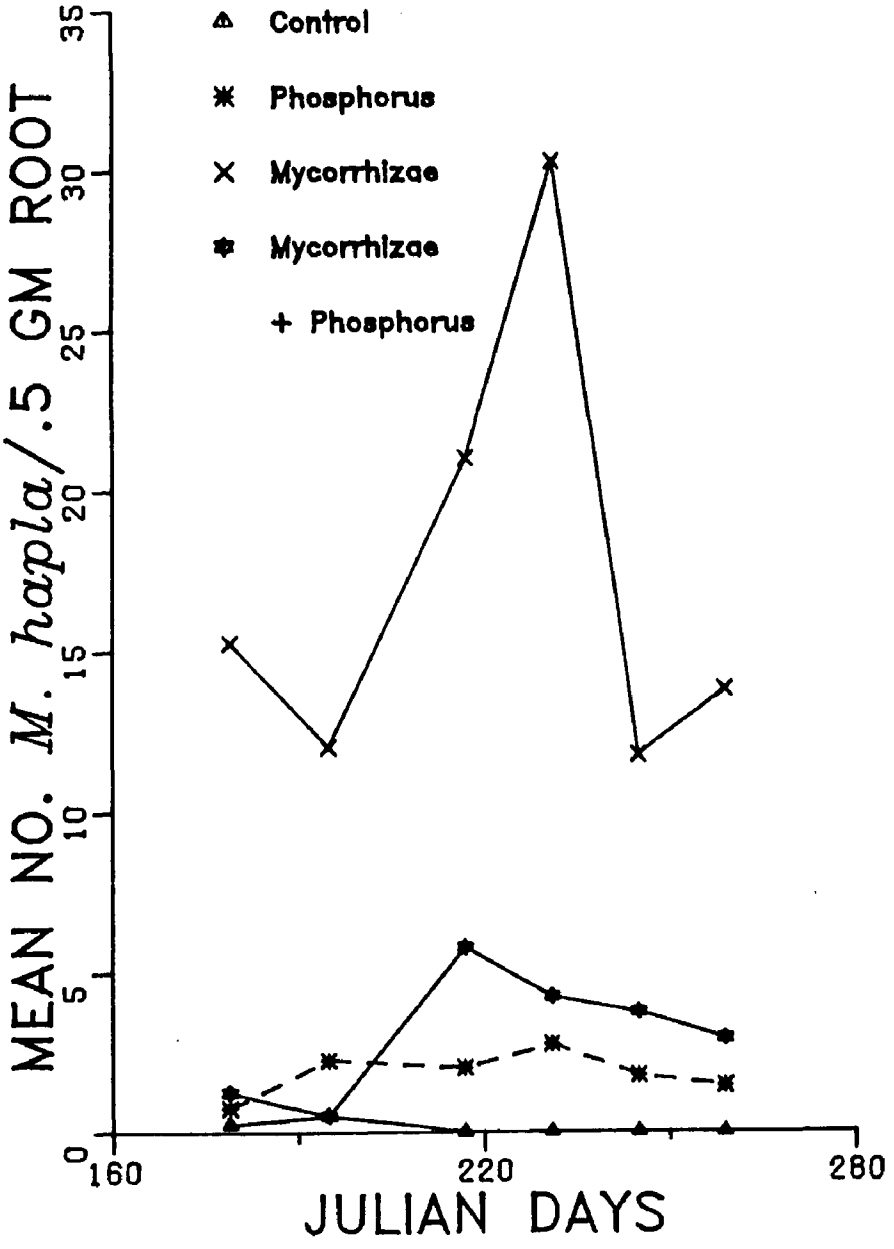


Figure 35. Number of Meloidogyne hapla in 0.5 gram of roots of Allium cepa supplemented with phosphorus, inoculated with Glomus fasciculatus, or amended with both Glomus fasciculatus and phosphorus, and unamended control.

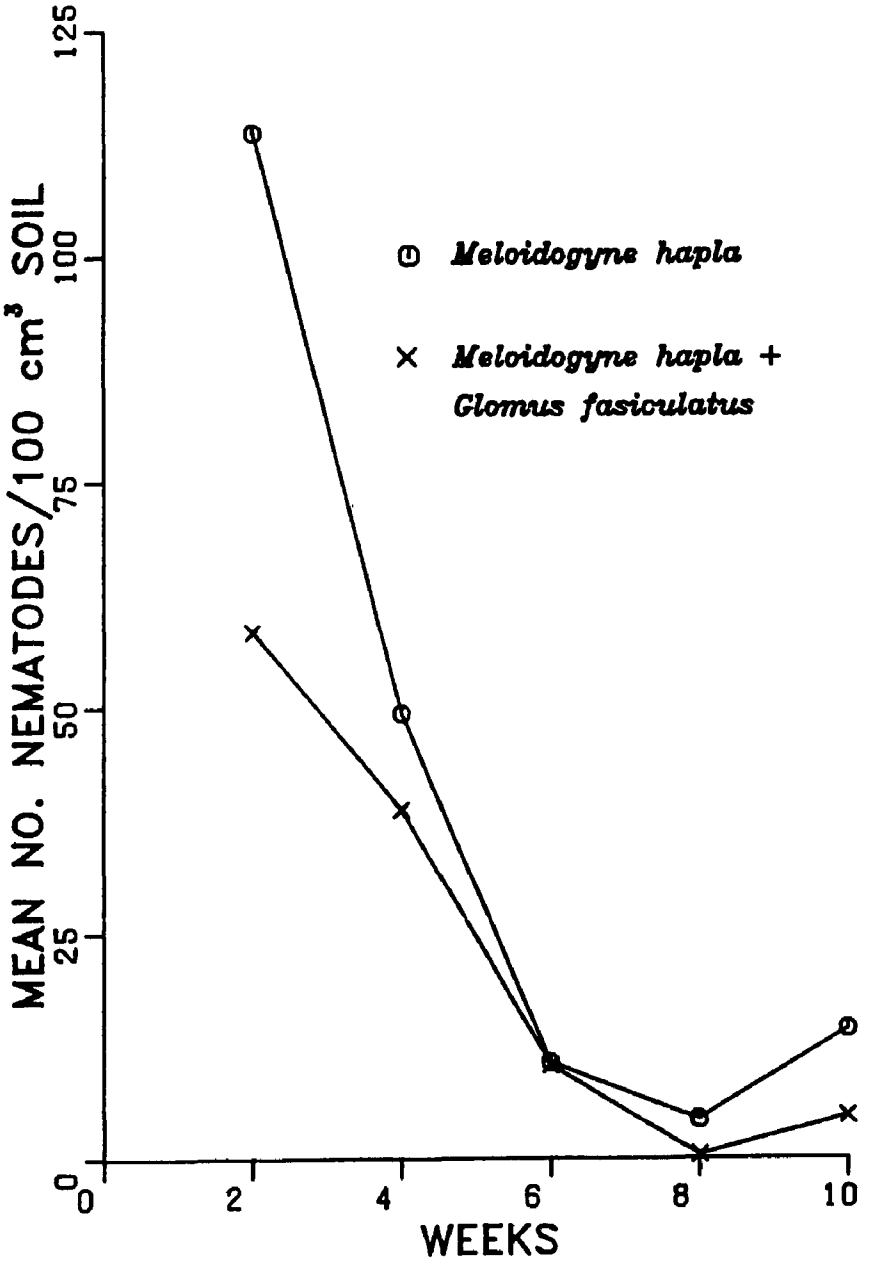


Figure 36. Number of Meloidogyne hapla juveniles in 100 cm^3 of soil surrounding Allium cepa supplemented with phosphorus, inoculated with Glomus fasciculatus, amended with both Glomus fasciculatus and phosphorus, and unamended control.

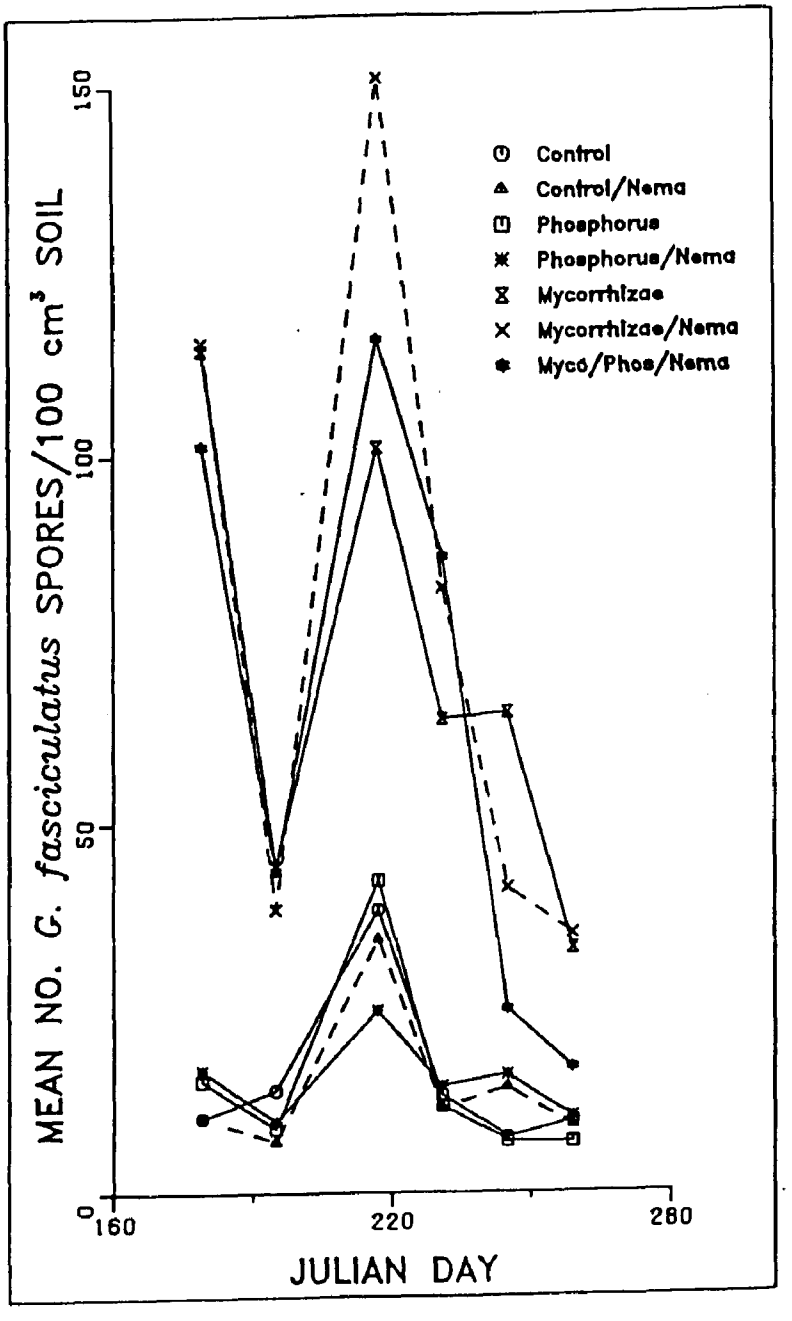


Figure 37. Number of Glomus fasciculatus spores in 100 cm^3 soil surrounding Allium cepa supplemented with phosphorus, inoculated with Glomus fasciculatus (mycorrhizae), amended with both Glomus fasciculatus and phosphorus, and unamended control, with and without the nematode Meloidogyne hapla.

though nematode densities were twofold greater in the mycorrhizal plants (Figure 35). These results indicate that A. cepa with an established VAM association was more tolerant to M. hapla parasitism. Conversely, VAM did not benefit A. cepa infected by M. hapla under greenhouse conditions. In this experiment, nematodes were established in plants before a VAM association was initiated. Nematode density averaged ca 83 M. hapla/root system for the VAM-inoculated plants and was perhaps sufficient to prevent a recovery in growth due to colonization by the fungus.

The presence of M. hapla within A. cepa roots did not seem to influence the production of VAM spores, as indicated by the similarity in spore densities in soil surrounding nematode-infected and non-infected plants both in the greenhouse and microplot experiments (Figures 32, 37). Data from the greenhouse experiment suggests, however, that the infectivity of spores may have been decreased by the presence of nematodes, since more spores remained in the soil surrounding nematode-infected plants at the end of the experiment (Figure 32).

Results from the greenhouse experiment indicate that VAM colonization influences the ontogeny of M. hapla. The number of nematodes within roots was very similar in plants inoculated with the nematode alone or with the nematode and fungus for the first six weeks of the experiment. This is not surprising, since a VAM association was not established when these nematodes entered root tissue. Eight weeks after planting, there was an increase in nematode density on both VAM-treated and non-treated plants which, due to the appearance of eggs in week six, would appear to be due to an influx of second generation nematodes. The decline in second-stage juveniles within the roots of mycorrhizal plants from week six to week eight and the low percentage of adult females observed,

however, suggest that the nematode increase associated with this treatment was due to sampling error rather than to a true change in nematode population levels. Conversely, the data indicate that nematode levels were increased in non-mycorrhizal plants due to reproduction. More than half the nematodes observed in non-mycorrhizal plants six weeks after planting had matured to the adult female stage. Although the difference in the numbers of second-stage juveniles observed in those plants was not different between weeks six and eight, it is highly likely that some influx of nematodes occurred, since the average nematode in the second-juvenile stage at week six would have progressed to the next developmental stage in ca two weeks (Tyler, 1933).

In the microplot experiment, significantly (P=0.05) fewer nematodes penetrated mycorrhizal plants fertilized at transplant with P than mycorrhizal plants not fertilized (Figure 35). Graham et al. (1981) reported that P nutrition associated with VAM decreased root permeability and the exudation of root metabolites into the rhizosphere. The attraction of nematodes to root exudates may thus be mediated by P levels in root tissue. It is possible that the P benefits normally gained by a VAM association were negated by damage to the roots and fungal network in the transplanting process. Supplementing mycorrhizal plants with superphosphate may have restored root P levels so that the host-finding behavior or the penetration of roots by M. hapla was altered. From these data, the failure of nematodes to enter or become established in control plants cannot be attributed to any particular factor. It is possible that control plants were stressed to the point that nematodes entered roots and exited immediately. The time-frame of the experiment, however, was not adequate to confirm this possibility.

In the greenhouse experiment and in preliminary studies, VAM colonization seemed to affect the population growth but not the pathogenicity of M. hapla. In the microplot experiment, VAM colonization alone did not limit M. hapla density, but conferred a tolerance to plants for M. hapla parasitism. The differential ability of mycorrhizal plants to support and alter the pathogenicity of M. hapla in the greenhouse and microplot environments may have been due to differences in the sequence of root colonization by nematodes and fungi or to the disruption of the root system during transplanting.

Soil populations of M. hapla increased only threefold over the course of the growing season in microplots inoculated with VAM + P, as compared to a 19x increase for microplots inoculated with VAM alone. The magnitude of the difference in M. hapla density between these treatments suggests that some unidentified factor or factors influenced the population dynamics of M. hapla in this study and illustrate the complexity of the association. Conflicting reports in the literature concerning the influence of VAM on nematodes may not be due to the specificity of plant-nematode-VAM systems as commonly reported but rather to differences in experimental methodology or environmental conditions. Additional information on the variables mediating the response of plants and nematodes to VAM is required before a true understanding of the interactions between these organisms can be realized.

Influence of Glomus fasciculatus on the Location and Penetration of Allium cepa Roots by Meloidogyne hapla

INTRODUCTION

The growth of some phytoparasitic nematode populations is limited by the presence of vesicular-arbuscular mycorrhizae (VAM) in the host plant (Kellam and Schenck, 1980; Sitaramaiah and Sikora, 1980). Both juvenile and adult nematodes are reportedly influenced by VAM. Sikora (1979) proposed that VAM colonization affects the penetration of roots by juveniles and the development and maturation of nematodes within roots.

The objective of this study was to examine the influence of VAM colonization on the ability of second-stage Meloidogyne hapla juveniles to locate and penetrate roots of A. cepa. Several studies support Sikora's proposal that the ability of nematodes to penetrate root tisses is reduced in the presence of mycorrhizae (Cooper, 1981; Sitaramaiah and Sikora, 1980). The hypothesis that the host-finding behavior of nematodes is adversely affected by mycorrhizal colonization has been supported but not examined in detail.

Two experiments are reported here. One experiment was conducted to assess the ability of nematodes to penetrate mycorrhizal roots when only limited host-searching behavior is required. The relative attractiveness of mycorrhizal and non-mycorrhizal root systems to nematodes in search of a host plant was examined in a second experiment.

MATERIALS AND METHODS

Experiment 1. Penetration of A. cepa by M. hapla placed in close proximity to the root system.

Krummery Special onions were sown into pots (d = 6.40 cm) containing 342 g of a pasteurized mineral soil with a pH of 5.9 and a P level of 1.1 ppm. At the time of planting, one of three soil treatments was administered in 5 ml of water to each pot: water alone, 2000 spores of the VAM Glomus fasciculatus, or 9.13 mg KH_2PO_4 to add 7 ppm P to the soil. The level of P fertilizer used had been predetermined to stimulate onion growth comparable to VAM colonization. Pots fertilized and unfertilized were supplemented with NH_4NO_3 and KNO_3, respectively, so that the only nutrient that differed between treatments was P. All pots, thinned to one seedling one week after planting, were immersed in a water bath maintained at 21 C and watered daily. One month after seeding, 500 M. hapla second-stage juveniles in 5 ml of water were inoculated into three small holes around the base of each plant. Two, four, eight, and 12 days later, six pots from each treatment were destructively sampled. The plants were gently removed from the soil, washed, and weighed. The entire root system was stained in lactophenol with 0.01% acid fuchsin and the nematodes within were enumerated under a dissecting microscope. This experiment was repeated once using a muck soil and the same methodology.

Experiment 2. Location and penetration of A. cepa roots by M. hapla.

Krummery Special onions were sown into pots containing 65.10 g of a pasteurized 7:1 sand/muck soil with a pH of 6.6 and P level of 4.8 ppm. One of three treatments were administered in 5 ml of water to each pot as described previously: water alone, 2000 spores of G. fasciculatus, or 12.9 mg KH_2PO_4 to add 45 ppm P to the soil. The pots were maintained in a greenhouse and watered daily. After one month, the seedlings were transplanted to 25.5 cm lengths of

vinyl pipe (d=7.5 cm) split longitudinally and sealed at each end with tape. There were three holes in the bottom of each tube for water drainage. All six possible combinations of plants from the three soil treatments were represented: C-M, C-P, P-M, C-C, P-P, M-M. One week after transplanting, 800 M. hapla second-stage juveniles in 5 ml of water were added to a single depression in the middle of each tube, equidistant to the two plants. Two, four, eight, and 12 days later, six tubes representing each of the six treatments were harvested. The cumulative degree hours (base = 9 C) for each harvest were 534, 1203, 2705, and 4162 degree hours (DH). Five cm of soil in the middle of each tube was discarded. The remaining 10.25 cm of soil at each end of the tubes and the onion plants were then carefully removed. The plants were washed, weighed, and the root systems cut into small pieces and placed into flasks containing 50 ml of a solution of ethoxyethyl mercuric chloride and dihydrostreptomycin sulfate. The flasks were agitated on a gyratory shaker for two days, after which time the solution was poured through a 400 µmmesh screen and collected for nematode observation. One hundred cm^3 of soil surrounding each plant was assayed by a modified sugar flotation-cetrifugation technique (Jenkins, 1964).

The data were analyzed as if each combination of original treatments constituted one treatment. For example, control plants grown in the same tube with mycorrhizal plants were designated C_m and treated separately from control plants grown with P-fertilized plants, C_p. This classification scheme was designed to demonstrate the relative differences in the attractiveness of control, P-fertilized, and VAM-inoculated plants. An analysis of variance was performed on the data using all nine treatment combinations: C_m, M_c, P_c, C_p, M_p, P_m, C_c, P_p, and M_m. The Wilcoxin signed rank test was used to evaluate sets of paired

observations (P_c-C_p, M_p-P_m, C_m-M_c) on the number of M. hapla in soil and root systems.

RESULTS

Experiment 1. Significantly (P=0.05) fewer M. hapla penetrated mycorrhizal plants than plants amended with phosphorus or unamended plants 12 days following inoculation (Figure 38). Nematode density increased (P=0.01) within each treatment during the course of the experiment (Table 30). Similar trends were observed when nematode levels were considered on a per gram, centimeter, or root tip basis (Figure 39). There were fewer M. hapla per 0.1 gram root and root tip in mycorrhizal plants two days following inoculation.

There were no differences in root length or weight between treatments for any sample date, although values for both growth parameters increased within each treatment in the 12 days following the inoculation of plants with M. hapla. An adjustment of nematode density according to root length by an analysis of covariance produced the same results as the analysis of variance in Table 30.

Experiment 2. There were no significant (P=0.05) differences in the number of nematodes observed in the roots and soil surrounding A. cepa treated with P, VAM, or control plants when all nine treatment combinations were considered in an analysis of variance. When the data were paired according to the arrangement of plants within the tubes, however, significantly (P=0.05) more nematodes were found in the soil around P-fertilized plants four, eight, and 12 days following inoculation when forced to choose between the P-fertilized and VAM-treated plants (P_m vs M_p) (Table 31). The difference in nematode density in and surrounding A. cepa were not statistically significant (P=0.05) for any

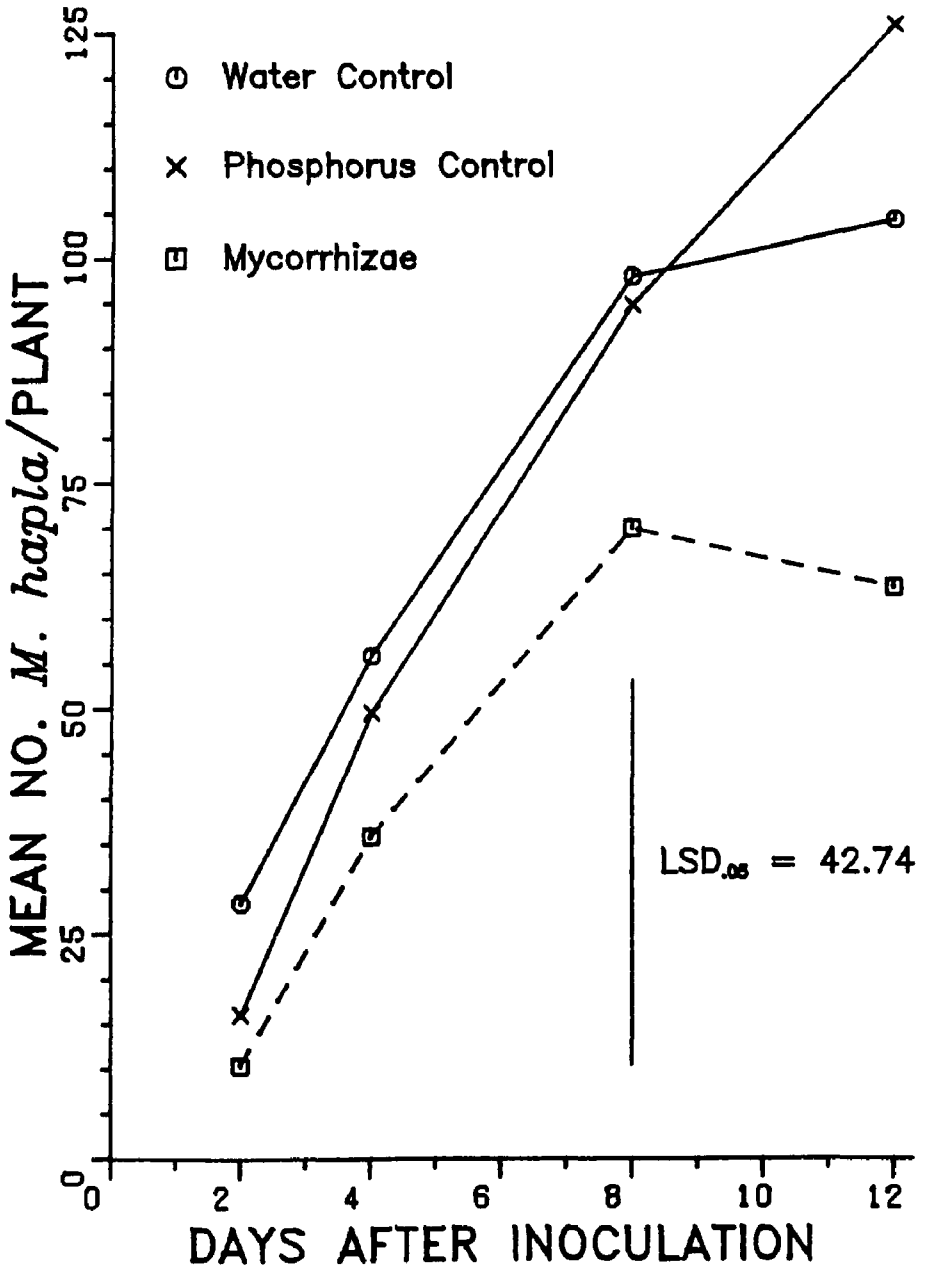


Figure 38. Number of Meloidogyne hapla within root systems of Allium cepa supplemented with phosphorus (phosphorus control), inoculated with Glomus fasciculatus (mycorrhizae), or unamended (water control).

Table 30. Analysis of variance for *Meloidogyne hapla* density within *Allium cepa* treated with *Glomus fasciculatus*, phosphorus, or tap water.

Source	DF	Sum of squares	Mean square	F
Sample date[a]	3	73455.0	24485.0	17.87 **
Treatment[b]	2	11360.0	5680.0	4.15 *
Date x treatment	6	5802.0	967.0	0.71
Error	60	82216.0	1370.0	
Total	71	172830.0		

[a] Plants were destructively sampled 2, 4, 7, and 12 days following inoculation.

[b] Plants were treated with mycorrhizae, phosphorus, or tap water.

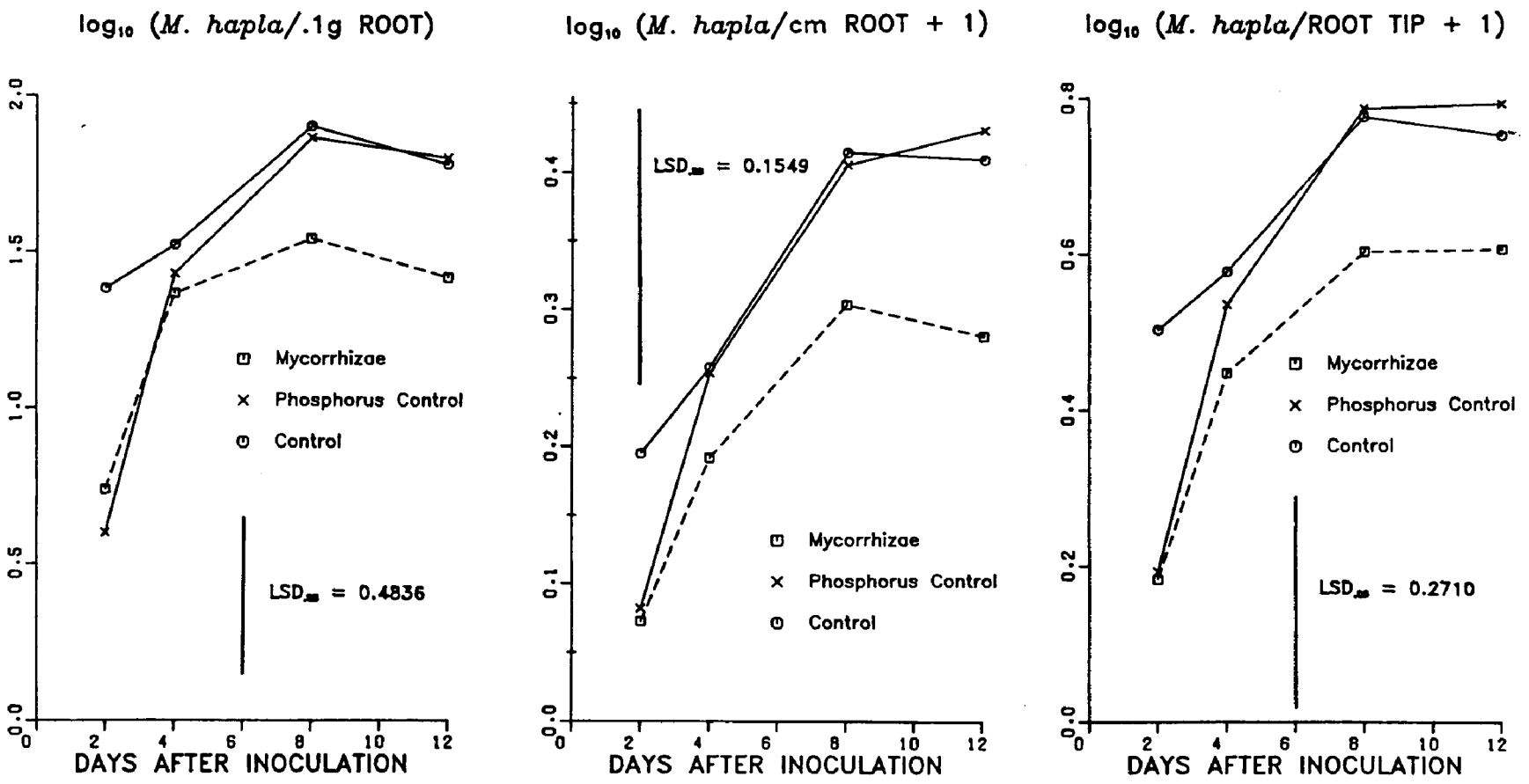


Figure 39. Number of Meloidogyne hapla per centimeter, 0.1 gram, or root tip in the root systems of Allium cepa supplemented with phosphorus (phosphorus control), inoculated with Glomus fasciculatus (mycorrhizae), and unamended control.

Table 31. Summary of the differences in the number of Meloidogyne hapla observed in soil samples from Allium cepa inoculated with Glomus fasciculatus, amended with phosphorus, or noninoculated and unamended.

Days after inoculation			
2 days	4 days	8 days	12 days
$C_m > M_c$ [a]	$C_m < M_c$	$C_m > M_c$	$C_m < M_c$
$C_p \approx P_c$	$C_p < P_c$	$C_p > P_c$	$C_p \approx P_c$
$P_m > M_p$	$P_m > M_p$ *	$P_m > M_p$ *	$P_m > M_p$ *

[a] Capital letter refers to treatment (C = control: M = + Glomus fasciculatus: P = + phosphorus)

Subscript refers to treatment of the other plant grown in the same container.

* Differences between the paired data are significant (P = 0.05) according to Wilcoxin's Signed Rank Test.

other combination of treatments (M_c vs C_m or P_c vs C_p). In tubes containing a control plant and a VAM-inoculated plant, there was a tendency for higher soil levels of M. hapla to be associated with the control plant two and eight days after inoculation and with the mycorrhizal plant four and 12 days after inoculation.

Levels of M. hapla in the roots of A. cepa were low for all treatments. Many nematodes observed in root fragments were evidently not expelled by the rotary shaker method used in this experiment.

Control plants visually appeared to have smaller root and shoot systems than VAM-treated plants, which in turn tended to be smaller than P-fertilized plants (Table 32). The slight discrepancy in root weight between the treatments was consistent but not statistically significant (P=0.05). Shoot weight was significantly (P=0.05) greater in P-fertilized plants grown with VAM-inoculated plants as compared to the other treatment combinations four days after nematode inoculation. Eight days following inoculation, VAM-treated plants grown with control plants had shoot systems significantly (P=0.05) larger than any other treatment.

DISCUSSION

Fewer M. hapla entered roots of A. cepa when a VAM association was present (Figures 38, 39). Preliminary experiments similar to the one described here produced similar results. The detrimental influence of mycorrhizal colonization on the penetration of roots by nematodes was also reported previously by other authors. Cooper (1981) found that M. incognita did not enter roots with greater than 10% levels of mycorrhizal colonization. Similarily,

Table 32. Root and shoot fresh weights (g) of *Allium cepa* inoculated with *Glomus fasciculatus*, amended with phosphorus, and noninoculated and unamended control.

Treatment[a]	Day 2		Day 4		Day 12	
	Root	Shoot	Root	Shoot	Root	Shoot
C_m	0.0950 ab	0.1317 a	0.0633 a	0.1417 ab	0.1017 a	0.1750 ab
M_c	0.0750 ab	0.1550 a	0.0667 a	0.1533 ab	0.1083 a	0.3783 c
C_p	0.0800 ab	0.1367 a	0.0583 a	0.1050 a	0.1050 a	0.1400 a
P_c	0.0730 ab	0.1183 a	0.0800 a	0.1250 ab	0.1200 a	0.1450 ab
P_m	0.0760 ab	0.1300 a	0.0833 a	0.1117 a	0.1217 a	0.2050 ab
M_p	0.0800 ab	0.1150 a	0.0867 ab	0.2167 c	0.1050 a	0.2567 b
C_c	0.0783 ab	0.1150 a	0.0883 ab	0.1233 ab	0.1433 b	0.2117 ab
P_p	0.1150 b	0.1667 a	0.1117 b	0.1850 bc	0.0783 a	0.1600 ab
M_m	0.0600 a	0.1200 a	0.0850 ab	0.1375 ab	0.0650 a	0.2000 ab

Values within a column followed by the same letter are significantly (P = 0.05) different according to Duncan's Multiple Range Test.

[a] Capital letter = treatment (C = control: M = + *Glomus fasciculatus*: P = + phosphorus)
Subscript = treatment of the other plant grown in the same container

Sitaramaiah and Sikora (1980) reported that the penetration of Rotylenchulus reniformis into cotton was impeded in the presence of VAM. The mechanism responsible for this phenomenon has not been identified. Similarities in the numbers of nematodes penetrating P-fertilized and VAM-inoculated A. cepa two and four days after inoculation suggest that some trait shared by these plants, perhaps root P levels, was involved.

Results from this study may provide some insight for findings that VAM fungi inoculated slightly prior to or simultaneously with nematodes have no noticeable influence on nematode population level for several weeks. Kellam and Schenck (1980) found no significant difference in the number of galls produced by M. incognita on soybean until 12 weeks following inoculation of the nematode and fungus. Similarly, Sikora and Schonbeck (1975) reported that differences in M. hapla density in mycorrhizal and non-mycorrhizal carrot roots were greater 12 weeks following inoculation than at six weeks.

In the studies referred to above, mycorrhizal colonization may not have been sufficiently established to inhibit root penetration by inoculated nematodes. The progeny of inoculated nematodes, however, faced with the task of invading root systems with a well-developed VAM association, may have been impeded in their efforts to enter roots. Although the reduced nematode levels in these studies may also be due to the decreased survival or delayed development of nematodes in mycorrhizal roots, it seems unlikely that VAM colonization would not have been sufficiently established to also affect population levels of the initial nematode generation.

Evidence that the ability of nematodes to locate and penetrate roots is reduced in the presence of VAM should be considered when interpreting data

from nematode/VAM interaction studies. For example, Schenck et al. (1975) concluded from levels of M. incognita juveniles in the soil that nematode populations were increased in the presence of mycorrhizae. Results from my study demonstrate that high levels of juveniles in the soil may also be due to the reduced ability of nematodes to enter plant roots. Schenck et al.'s conclusions may have been different had they assessed the total nematode density in soil and roots. Certainly not all reports of increased nematode levels in mycorrhizal plants are due to the erroneous measurement of the nematode population. The total root biomass available for nematode penetration, nematode inoculum levels, and the density of nematodes established within roots may modify the influence of VAM on the penetration process.

The hypothesis that M. hapla is differentially attracted to mycorrhizal and non-mycorrhizal A. cepa was supported but not confirmed (Table 31), since the experiment presented here provided only a gross indication of the direction nematodes moved to locate a host. Unfortunately, the failure of the shaker technique to extract M. hapla from the roots of A. cepa made interpretation of the data difficult. Decreased nematode levels in the soil could not be confidently attributed to the failure of nematodes to migrate towards a plant since the number of nematodes successfully penetrating roots was not known. M. hapla seemingly preferred P-fertilized A. cepa over mycorrhizal plants, as evidenced by the paired difference test. It is possible that mycorrhizal plants possessed (or lacked) some attribute making them less attractive to M. hapla. Size of the root system did not seem to be an important factor since root weights of mycorrhizal plants were not different from the other plants.

Recent studies demonstrated that root membrane permeability is influenced by the P levels within root tissue (Ratnayake et al., 1978; Graham et al.,

1981). It is possible that greater amounts of root exudates are released by roots low in P, which are important stimuli attracting nematodes to a host plant (Prot, 1980). It may be that root P levels differed dramatically between treatments in this experiment and contributed to the differential attractiveness of roots to M. hapla. Since root P levels were not measured, the magnitude of the differences between the treatments are not known, although it seems likely that all P-fertilized plants had higher root P levels than control plants. The inability of nematodes to consistently choose control plants, therefore, suggests that other plant attributes are also involved in the host-finding behavior of M. hapla or that different types of stimuli are involved in the long- and short-range location of roots by nematodes. For example, it is possible that CO_2 gradients proportional to plant size are the initial stimuli attracting nematodes and that root exudates or other stimuli influence nematode movement to a specific location on the root surface. Until more is known concerning the host locating behavior of nematodes, the influence of VAM on the attraction of nematodes to plants cannot be fully determined.

Influence of Glomus fasciculatus on the Stage-Specific Development and Reproduction of Meloidogyne hapla Inhabiting Roots of Allium cepa

INTRODUCTION

The ontogeny of Meloidogyne spp. within plant roots has been described in detail (Bird, 1959; Triantaphyllou and Hirschmann, 1960). Feeding, sexual differentiation, and growth of these nematodes commence in the second juvenile stage. The third- and fourth-juvenile stages are devoted to sexual development in the absence of feeding or increased size, and occur in relatively rapid succession. After the final molt to the adult stage and prior to oviposition, female nematodes enter a period of feeding and growth. Rates of development for the juvenile and pre-ovipositional adult stages are closely related to temperature (Davide and Triantaphyllou, 1967; Wong and Mai, 1973a; Tyler, 1933; Vrain et al., 1978) and are modified by attributes of the host plant (Ferris and Hunt, 1979; Griffin and Hunt, 1972). The importance of food in the developmental process suggests that competition with concomitant organisms for plant resources may also alter the developmental rate of some life stages of Meloidogyne spp. My studies on the interaction between vesicular-arbuscular mycorrhizae (VAM) and M. hapla within roots of Allium cepa support this hypothesis. In one experiment, for example, over twice (P=0.08) as many adult M. hapla were observed in A. cepa inoculated with the VAM, Glomus fasciculatus, even though the total number of nematodes per root system was not different between non-mycorrhizal and mycorrhizal plants.

The objective of this study was to examine the influence of mycorrhizal colonization on the developmental rates of the juvenile and adult stages of M.

hapla infecting A. cepa. Due to the genetic variability in individuals, both the fastest and median developmental rates of nematodes were considered. The slowest developmental time was not calculated for any nematode stage, since dead nematodes could not be accurately distinguished from living nematodes within root tissue.

MATERIALS AND METHODS

Onions (cv Krummery Special) were sown in compartments of plastic nursery trays containing 217 g (dry wt) of a 7:1 mix of sand and a Houghton muck soil with a pH of 6.5 and a soil P level of 1.3 ppm. One third of the trays were inoculated ca 3 cm below the soil surface with 1600 spores of the mycorrhizal fungus Glomus fasciculatus in 5 ml of water. Another third of the trays were fertilized with 43 mg KH_2PO_4 per compartment, adding 48 ppm P to the soil to stimulate onion growth comparable to mycorrhizal colonization. The remaining trays were not fertilized or inoculated. Uniformity between treatments in all regards except the presence of fungal spores or P levels was accomplished by adding water passed over spores to those trays not receiving mycorrhizal inoculum and by adjusting soil nutrient levels with KNO_3 and NH_4NO_3 for trays unamended and amended with KH_2PO_4, respectively. The plants were grown in an environmentally-controlled chamber with a 12/12 hour light cycle, and air temperature ranging from 20-23 C. After one month, each plant was inoculated with 650 M. hapla second-stage juveniles in 2 ml of water. Four days following inoculation, the plants were gently removed from the trays, washed free of soil and nematodes, and transplanted into 10.5 cm pots containing ca 500 g of muck soil with a pH of 6.5 and a soil P level of 16 ppm. This procedure assured that all

nematodes considered in this experiment entered the roots within a four day period. The three soil treatments were repeated at transplanting, except that the spore and P levels added were 1230/pot and 14.8 mg/pot, respectively. Levels of N and K were adjusted as described previously. The pots were maintained in the greenhouse and watered daily. Soil temperature was recorded hourly by a Campbell CR-21 micrologger for degree-hour computation commencing at the time of transplanting.

Seven potted plants from each soil treatment were harvested every 3 days (ca 1000 degree hours (base 9 C)) for 33 days. Fresh root and shoot weights were determined and entire root systems stained in lactophenol with 0.01% acid fuchsin. Nematodes were teased from roots, observed using a dissecting microscope, and enumerated according to the following scheme of classification (Figure 40): (1) Stage 1, Early Second-Stage Juveniles (slender body shape); (2) Stage 2, Intermediate Second-Stage Juveniles (broadened body shape with pointed terminus); (3) Stage 3, Late Second-Stage Juveniles (broadened body with rounded terminus and mucro); (4) Stage 4, Third/Fourth-Stage Juveniles (multiple unshed cuticles and absence of stylet); (5) Stage 5, Adult Female (mature gonads, rounded body shape, and absence of eggs or gelatinous matrix); and (6) Stage 6, Ovipositing Females (as previous stage except egg mass present).

Nematode development was computed by a technique described by Manley (1976). Frequency counts of each stage were plotted against time. It was assumed that nematodes assigned to each stage had completed 50% of the development necessary to complete that stage (i.e., each stage designation represented the midpoint between the previous and subsequent stage). To obtain the estimated median stage value for each sample date, the frequency of each

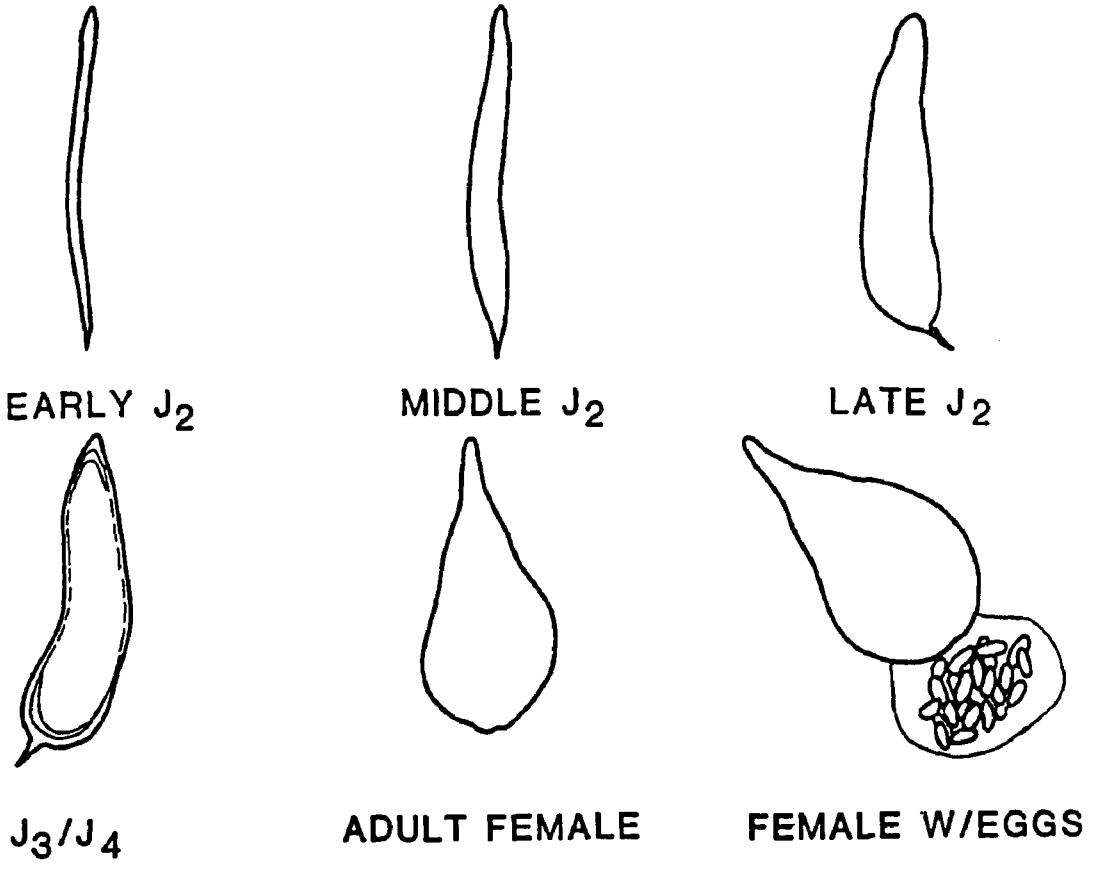


Figure 40. Stage-classes (1,2,...6) used to classify Meloidogyne hapla inhabiting Allium cepa roots.

stage was multiplied by the stage designation number (1, 2, . . . 6), and the resulting product was divided by the total number of nematodes present on that date. The median stage estimates were then plotted against a physiological time scale (degree hours), and a cubic function was fitted to the points to determine the median rate of nematode development.

Egg production by adult female nematodes was monitored from the first appearance of eggs, 24 days following inoculation, until 39 days after the experiment was initiated. On each sample date, egg masses were removed from the roots as the nematodes were counted, agitated in a 1:9 solution of 0.5% sodium hypochlorite in water, and counted under a stereomicroscope. The total number of eggs and empty egg shells was divided by the mean number of ovipositing females to obtain an estimate of the mean number of eggs produced per female. This was done for each treatment and sample date.

RESULTS

All stages of M. hapla occcurred simultaneously (Figures 41, 42, 43). Early second-stage juveniles (EJ_2) were inoculated onto plants. The first appearance of intermediate second-stage juveniles (IJ_2), signifying the commencement of endoparasitic feeding by juvenile M. hapla, was observed after 1075 degree hours (DH) for all treatments (Table 33). Late second-stage juveniles (LJ_2) were first observed on the second sample date (2145 DH) for the P-treated plants and on the third sample date (3314 DH) for the control and VAM-treated plants. The combined third- and fourth-stage (J_3-J_4) and the adult stage were observed after 4360 and 5240 DH, respectively, for all three treatments. Egg production commenced after 8545 DH for the P- and VAM-treated plants, and after 9662 DH for the control plants.

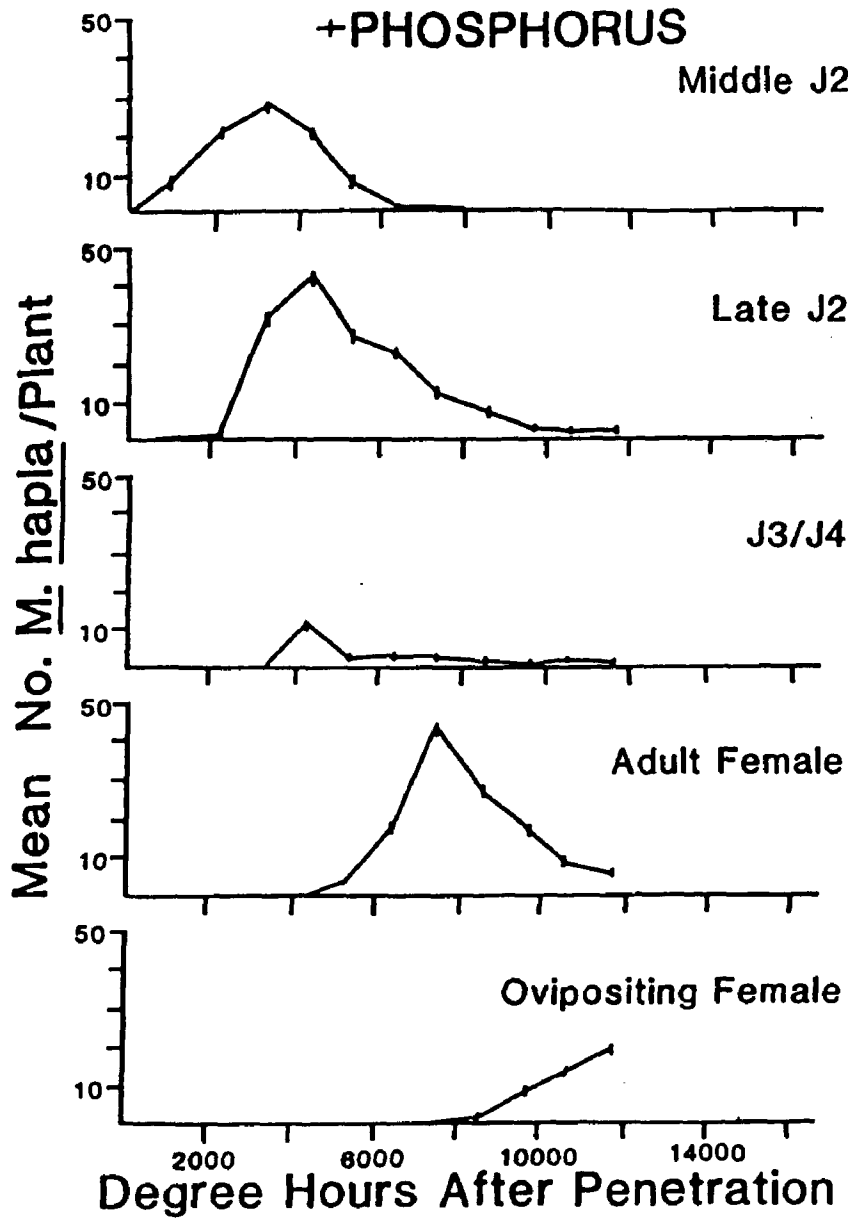


Figure 41. Incidence of five stage-classes of Meloidogyne hapla inhabiting Allium cepa supplemented with phosphorus.

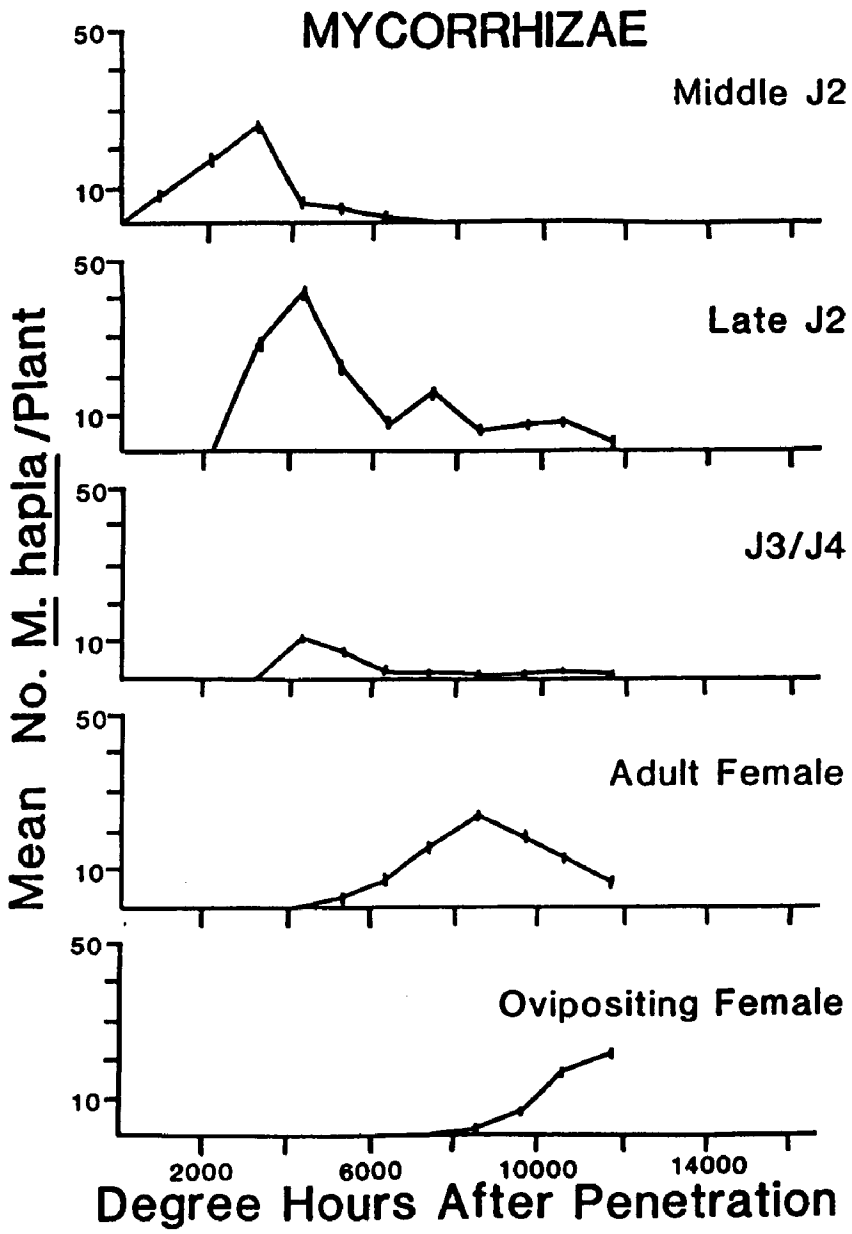


Figure 42. Incidence of five stage-classes of Meloidogyne hapla inhabiting Allium cepa inoculated with Glomus fasciculatus.

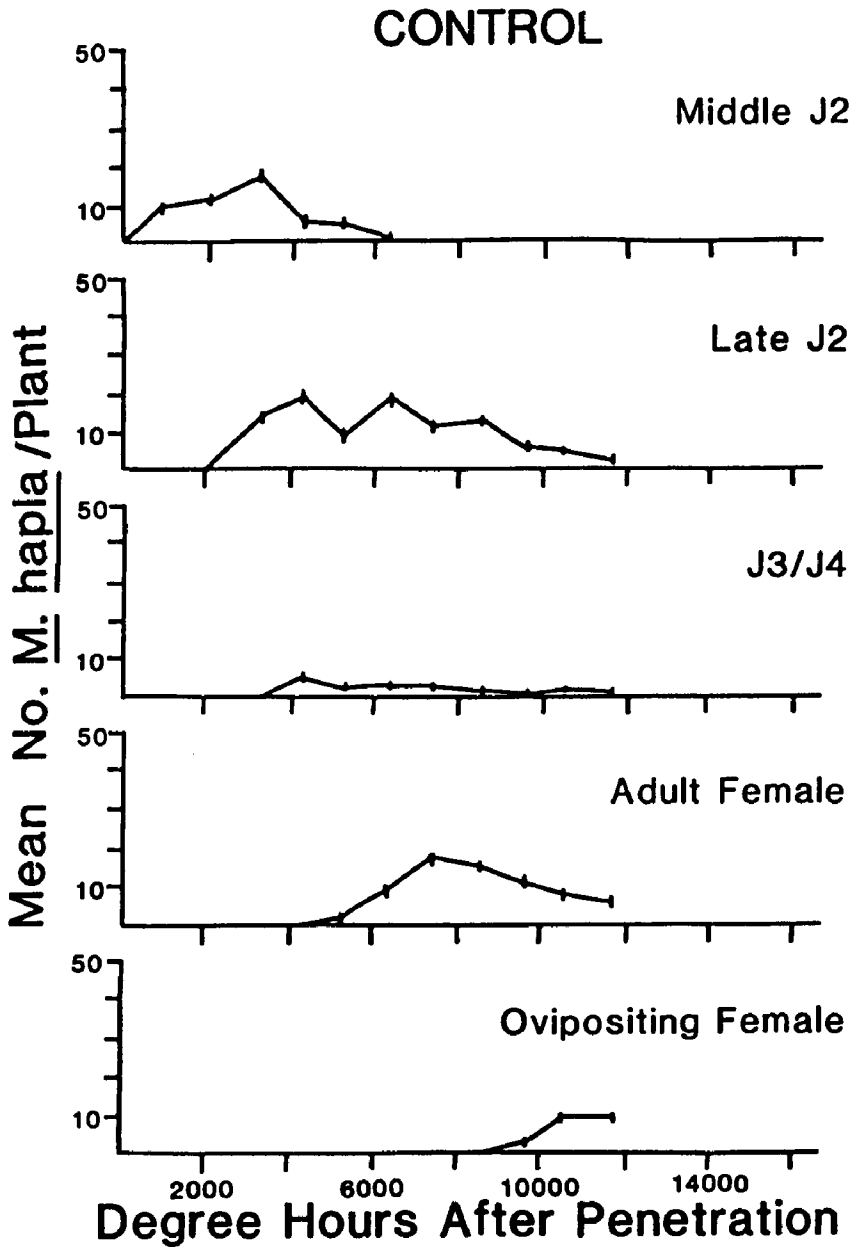


Figure 43. Incidence of five stage-classes of Meloidogyne hapla inhabiting Allium cepa unamended with phosphorus and noninoculated with Glomus fasciculatus.

Table 33. Degree hours (base 9 C) accumulated at the first appearance of five life stages of Meloidogyne hapla associated with Allium cepa.

Treatment	M. hapla stages[a]				
	IJ_2	LJ_2	J_3-J_4	Pre-ovipositing female	Ovipositing female
Control	1075	3314	4360	5420	9662
+ Phosphorus	1075	2145	4360	5420	8545
+ Mycorrhizae	1075	3314	4360	5420	8545

[a] M. hapla life stages:
IJ_2 = intermediate second-stage juveniles
LJ_2 = late second-stage juveniles
J_3-J_4 = third and fourth-stage juveniles combined
Pre-ovipositing female = females without eggs
Ovipositing female = females with eggs

The median developmental stage values were similar for M. hapla reared on control, P-fertilized, and VAM-inoculated plants (Figure 44, 45, 46). The median stage values for the control, P-fertilized, and VAM-treated plants on the final sample date were 5.258, 5.376, and 5.465, respectively. Correspondingly, the degree hours necessary for 50% of the population to attain the IJ_2 through the J_3/J_4 stage were similar (Table 34). The experiment was terminated before 50% of the population in any treatment reached the sixth designated stage, ovipositing female. Development of the median individual (or 50% of the population) to the adult stage occurred slightly earlier in plants receiving P fertilization than in the control or mycorrhizal plants.

Egg production per M. hapla female was not significantly (P=0.05) different between treatments on any sampling date. Adjustment of the egg counts according to the weight of the root system each nematode inhabited using covariance analysis also did not reveal any difference in nematode reproduction between treatments (Figure 47). On the final sample date (13,715 DH), females each produced an average of 134.8, 1150.4, and 209.7 eggs, respectively, in control, P- and VAM-treated plants.

The growth of non-amended control A. cepa over the course of the experiment was less than the growth of P- or VAM-treated plants (Figure 48). Plants fertilized with P were larger than plants inoculated with G. fasciculatus initially but were smaller than the mycorrhizal plants by the end of the experiment. Equations for the regressions appearing in Figure 48 were as follows:

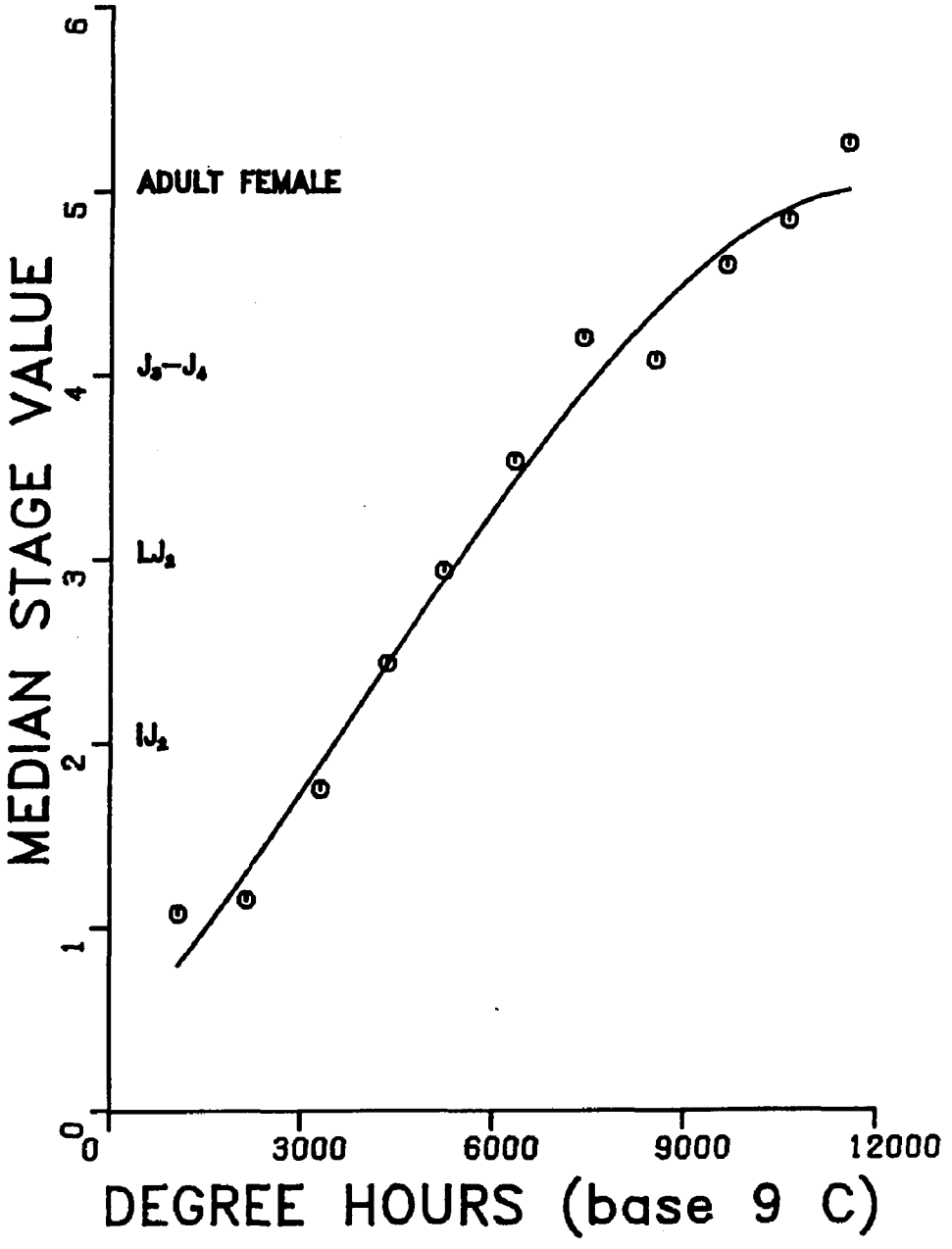


Figure 44. Stage-specific incidence of the median individual (or 50% of the population) of Meloidogyne hapla inhabiting Allium cepa unamended with phosphorus or Glomus fasciculatus (control).

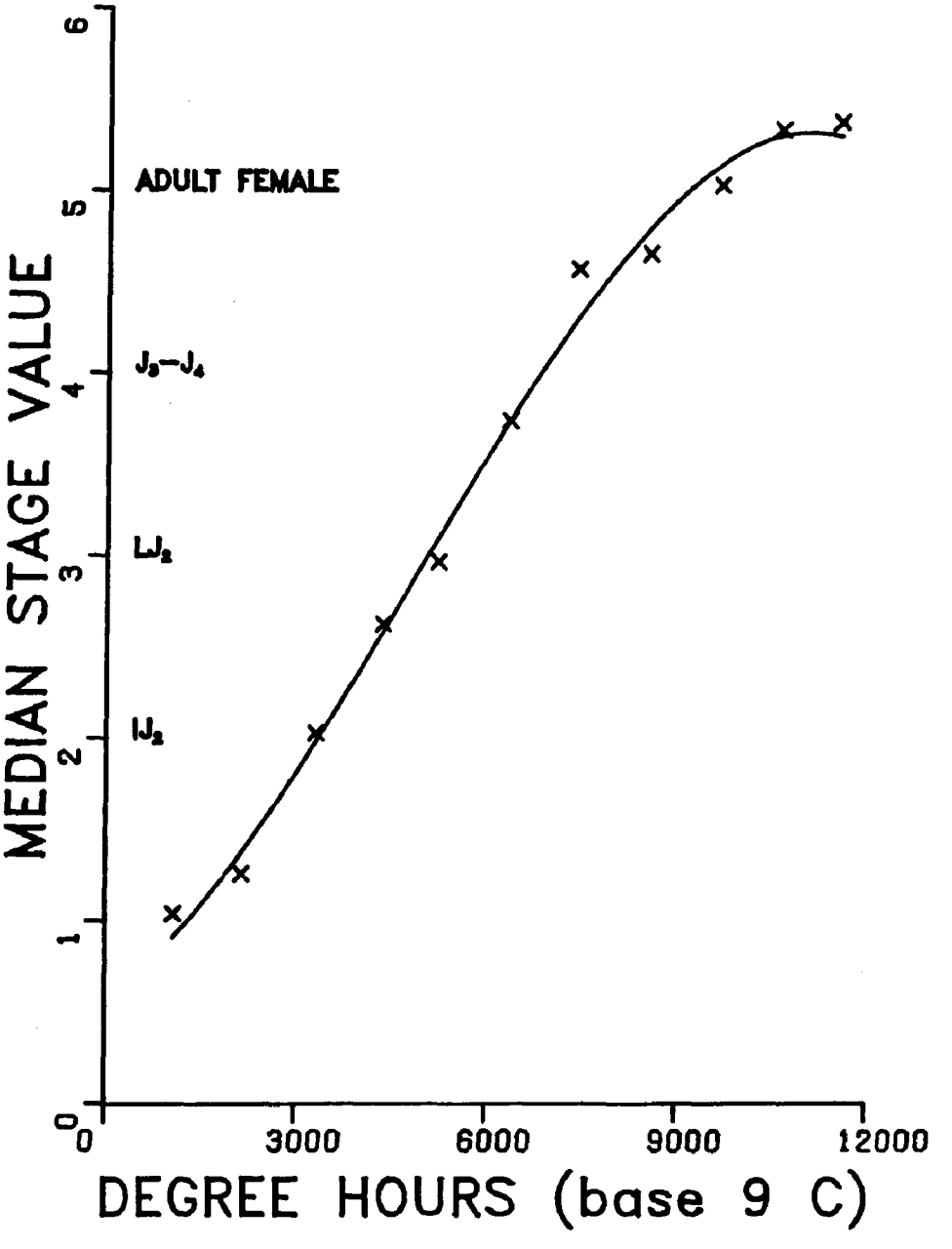


Figure 45. Stage-specific incidence of the median individual (or 50% of the population) of Meloidogyne hapla inhabiting Allium cepa supplemented with phosphorus.

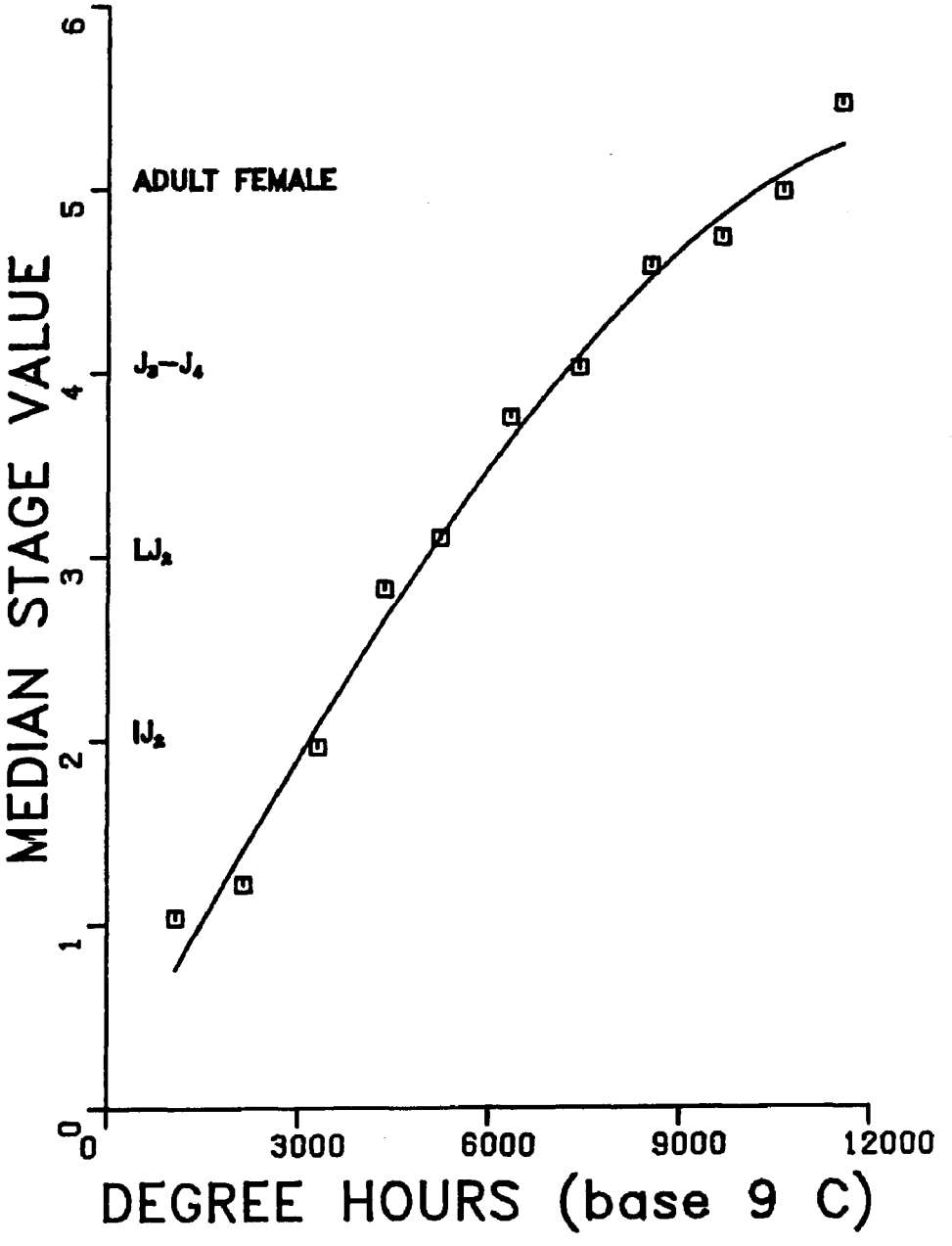


Figure 46. Stage-specific incidence of the median individual (or 50% of the population) of Meloidogyne hapla inhabiting Allium cepa inoculated with Glomus fasciculatus.

Table 34. Degree hour (base 9 C) requirements for the development of the median individual or 50% of the population of Meloidogyne hapla associated with Allium cepa to attain five life stages.

	M. hapla stages[a]				
Treatment	IJ_2	LJ_2	J_3-J_4	Pre-ovipositing female	Ovipositing female
Control	3534	5482	7645	11502	----
+ Phosphorus	3179	5033	7192	10256	----
+ Mycorrhizae	3345	5068	6831	9137	----

[a] M. hapla life stages:

IJ_2 = intermediate second-stage juveniles

LJ_2 = late second-stage juveniles

J_3-J_4 = third and fourth-stage juveniles combined

Pre-ovipositing female = females without eggs

Ovipositing female = females with eggs

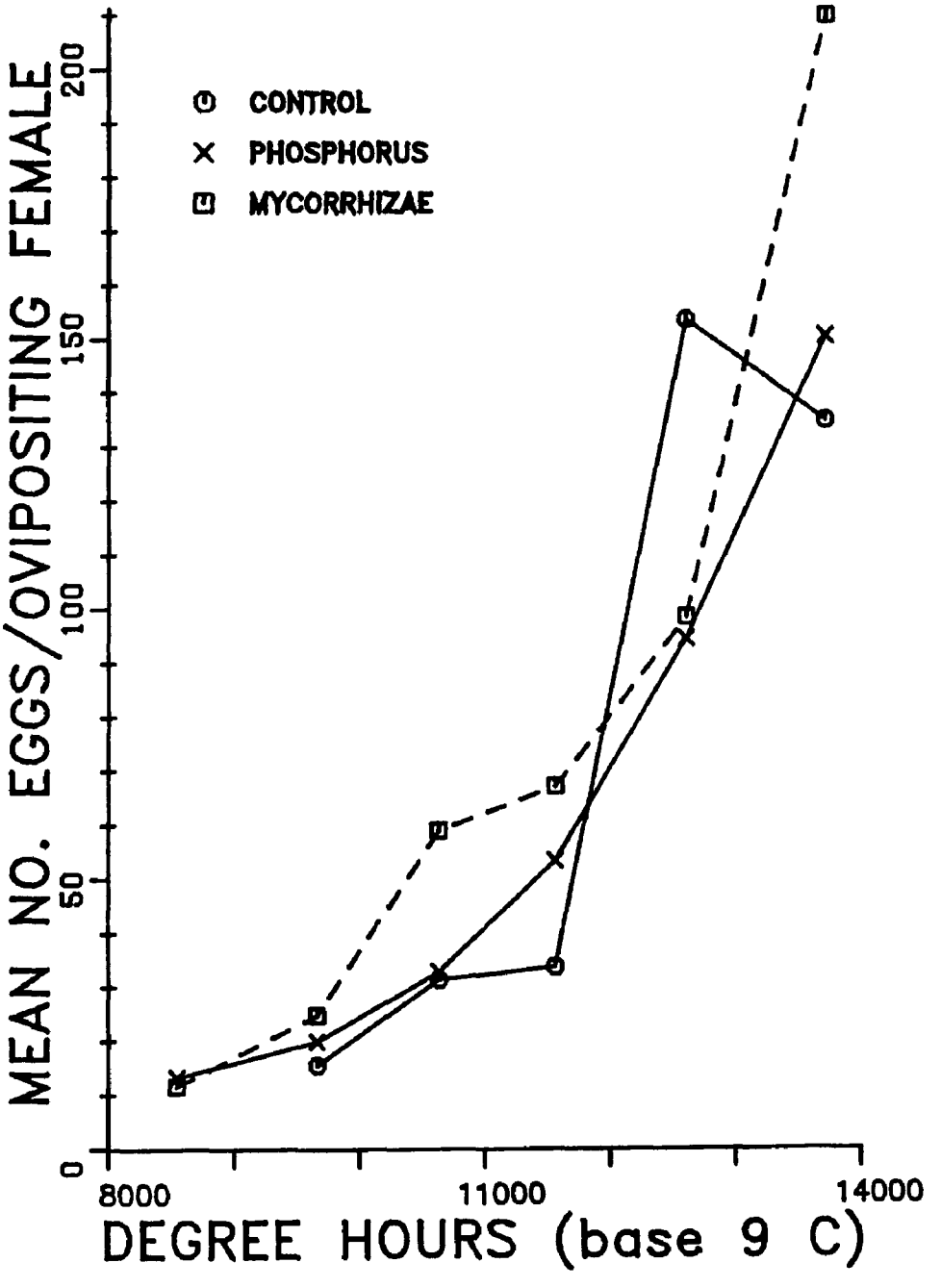


Figure 47. Number of eggs produced per ovipositing Meloidogyne hapla female inhabiting Allium cepa supplemented with phosphorus, inoculated with Glomus fasciculatus (mycorrhizae), and unamended control.

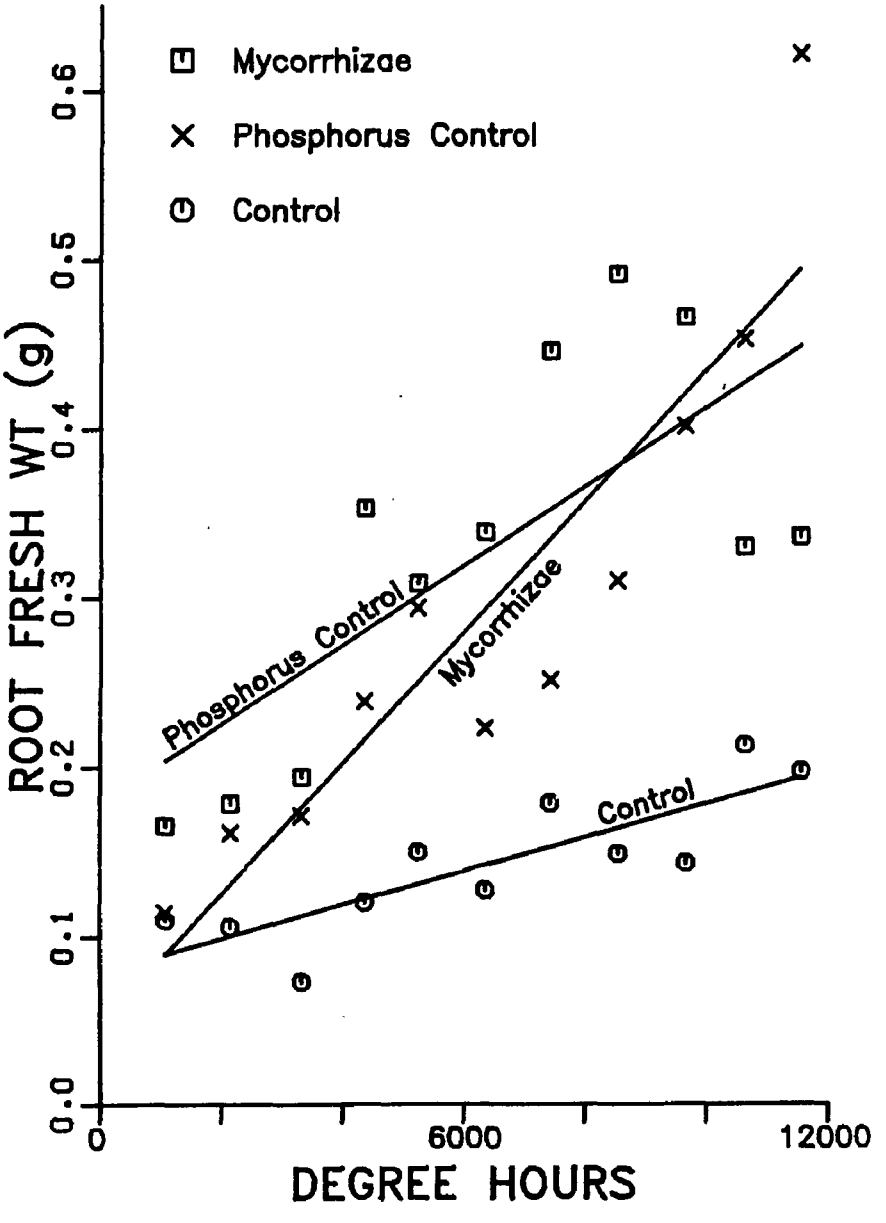


Figure 48. Fresh weight of the roots of Allium cepa supplemented with phosphorus, inoculated with Glomus fasciculatus (mycorrhizae), and unamended control.

G. fasciculatus-inoculated plants:

$Y = 0.00002X + 0.17890 \quad (R^2 = .5225)$

P-fertilized plants:

$Y = 0.00004X + 0.04796 \quad (R^2 = .8311)$

Control plants:

$Y = 0.000001X + 0.07895 \quad (R^2 = .6943)$

where: Y = root fresh wt

X = degree hours (base 9 C)

Initial densities of all combined stages of M. hapla were greatest in the control plants (Figure 49), both before and after nematode densities were adjusted by an analysis of covariance using root weight as a covariate. The total number of nematodes per root decreased considerably within the first six days following root penetration and were not significantly different between treatments on any sample date, according to analyses of both variance and covariance.

DISCUSSION

The population dynamics of phytoparasitic nematodes is dependent on the rates of nematode survival, development, and reproduction. For M. hapla and many other phytoparasitic species, the developmental process occurs primarily within the host plant. The immobility of these nematodes during the majority of the life cycle facilitates the determination of population parameters such as generation time, expected mortality, and fecundity. Few studies, however, have attempted to examine and quantitate the ontogeny of M. hapla or other

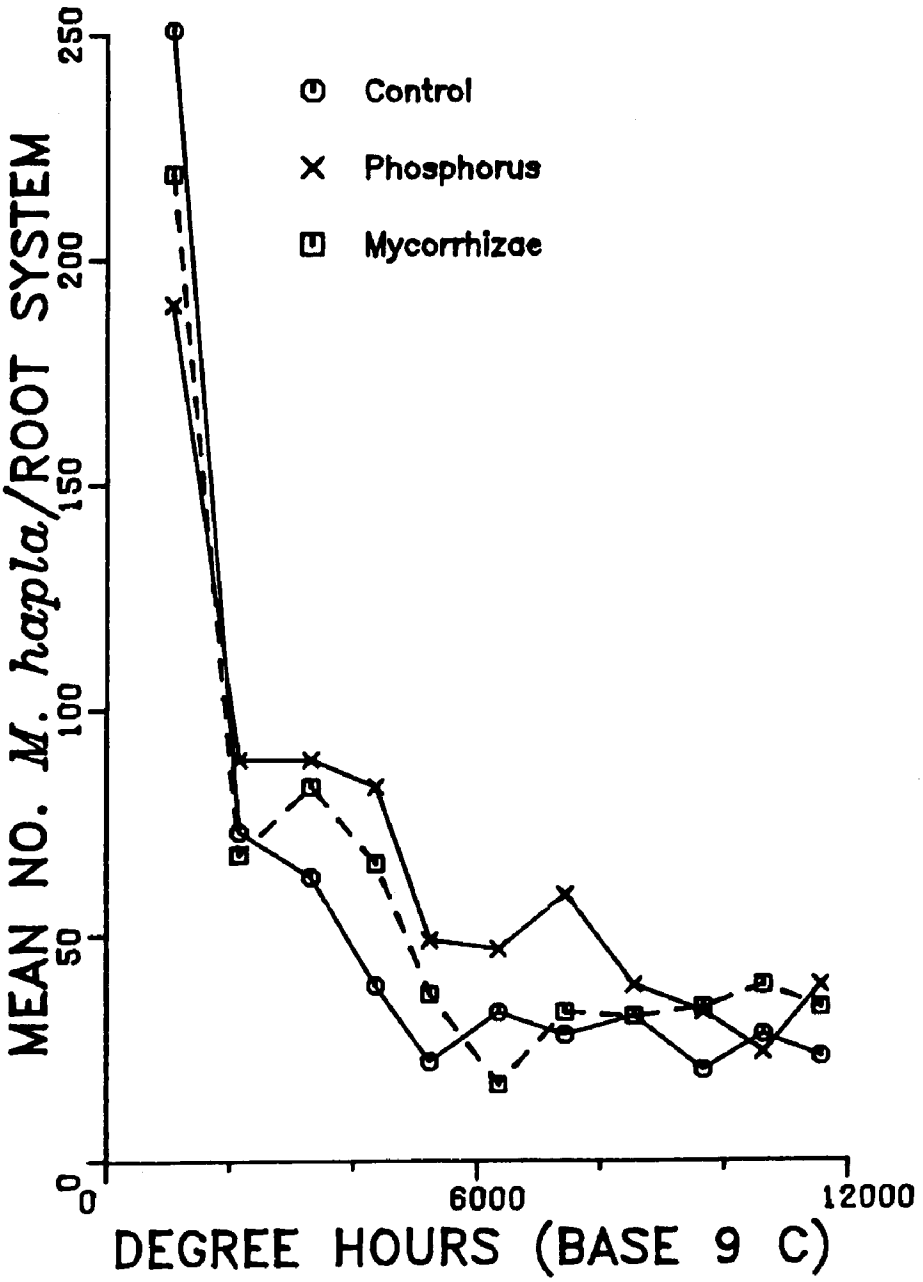


Figure 49. Number of Meloidogyne hapla in the root systems of Allium cepa supplemented with phosphorus, inoculated with Glomus fasciculatus (mycorrhizae), and unamended control.

nematodes. The research reported here is unique not only because an attempt was made to examine the development of each M. hapla life stage, but to relate the stage-specific development of this nematode to the presence of a concomitant root organism, G. fasciculatus.

Fifty percent of the M. hapla population in this study required an additional ca 1000 DH to develop to the adult stage on mycorrhizal A. cepa as compared to plants fertilized with P. The difference in developmental rates between the two treatments seems to be related to some attribute of the host plant other than P levels or nematode density, since P nutrition was augmented in both cases by either fertilization or mycorrhizal colonization and nematode levels within root systems were similar (Figure 49). Plant size also did not seem to influence the development of M. hapla since the developmental rates of nematodes within VAM-treated plants was greater initially, despite the fact that mycorrhizal plants tended to be smaller than P-supplemented plants early in the experiment (Figure 48). Results by Sikora (1978), showing a 10% reduction in the growth of M. incognita infecting mycorrhizal Lycopersicum esculentum, support my findings that mycorrhizal colonization influences the developmental processes of M. hapla. The mechanism responsible for this influence is not known, although Sikora (1979) demonstrated that the formation of giant cells is retarded in the presence of Glomus mosseae, indicating that root tissue is altered physiologically by VAM. The retarded developmental rate of M. hapla inhabiting control plants is not readily explained, but may have been due to the high nematode density relative to plant size.

A comparison between the development of the fastest and median individuals illustrates the variation in ontogeny inherent in nematode populations (Ferris

and Hunt, 1979). No differences between the P- and VAM-amended treatments were apparent when the first appearance of each stage was the criterion for developmental progress. The estimated median developmental rate is a better indicator of the time required to complete each stage, since differences in developmental rates less than ca 1000 DH could not be estimated by the sampling scheme employed in this experiment.

Survival rates for different stages of M. hapla were not determined in this experiment, although some indication of mortality trends is apparent from examining nematode levels through time (Figure 49). Mortality was greatest in all treatments during the early portion of the second stage, as nematodes began the feeding process. Although soil population levels were not monitored, it seems likely that juveniles died, since root population levels did not increase during the experiment as would be expected if nematodes exited and re-entered root systems. A comparison of the final to initial (P_f/P_i) root densities indicates that ca 8.7, 18.5, and 21.0% of the nematodes which entered roots survived for the duration of the experiment in control, VAM-treated, and P-fertilized plants, respectively. Survival seemed to be reduced slightly on mycorrhizal A. cepa, although not as greatly as for M. incognita infecting L. esculentum (Sikora, 1978; 1979).

Reproduction by M. hapla was not influenced by the presence of G. fasciculatus. An examination of the trends shown by the data presented in Figure 46, however, suggest that nematode reproduction may have been greatest in mycorrhizal plants had the experiment continued. At the time of the final harvest, less than 50% of the M. hapla observed had commenced oviposition. Further studies should be conducted to determine if the fecundity of slow-

developing M. hapla is increased in VAM colonized plants, and if so, whether a relatively high reproductive rate can compensate for the slightly increased nematode generation time and lead to higher population levels than would be attained if VAM were not present.

GENERAL DISCUSSION

The studies reported here demonstrate the complexity of the association between nematodes, VAM, and the host plant. Population growth of M. hapla can be reduced in the presence of VAM. Data from all three studies suggests that if M. hapla levels are reduced in mycorrhizal plants, it is most likely due to the inhibition of root penetration by soil-inhabiting juveniles. The mechanism by which VAM influences nematodes was not identified in these experiments, although the data indicate that VAM do not directly affect M. hapla outside or within A. cepa roots.

Hussey and Roncadori (1978) suggested that nematodes and VAM may compete for space within plant roots. Decreased population growth of M. hapla in my studies, however, did not seem to be related to the amount of root tissue available for nematode entry or occupancy. Considering the discrepancy in size between the control and mycorrhizal plants in some of my experiments, it is not unreasonable to propose that the amount of tissue not colonized by fungi in the VAM-inoculated plants was not much greater than the total root tissue in control plants. Furthermore, if the amount of tissue unoccupied by the fungus was the critical factor in determining nematode population increase, one would expect that plants fertilized with P would be the most conducive to nematode penetration and would support the highest nematode densities, since they were

consistently larger than control plants and equal in size to mycorrhizal plants. This did not occur in the microplot experiment (Figure 35). The developmental rates for the juvenile stages of M. hapla were slightly greater in P-fertilized plants (Table 34), but reproductive rates were not (Figure 47), indicating that increased space and nutrients were not the only factors influencing nematode development and maturation.

VAM reportedly have an antagonistic effect on Thielaviopsis basicola (Baltruschat et al., 1973). In contrast, VAM do not seem to be antagonistic to nematodes. Mycorrhizal plants in my experiments supported some nematode penetration (Figure 38) and development (Figure 42) and did not seem to adversely affect nematode survival (Figure 49). Furthermore, the high M. hapla density attained in mycorrhizal plants grown in microplots demonstrates that the presence of VAM does not always inhibit nematode population development.

It seems, then, that impeded penetration of M. hapla and subsequent reduction in population growth in mycorrhizal plants is not due to the direct competition or antagonism between nematode and fungus. It is more likely that VAM colonization alters the physiology of the host plant which, in turn, produces changes in the quantity and quality of food reserves and living environment for nematodes. A host-mediated influence of VAM would be subject to all edaphic conditions that affect plant growth. The differential effects of VAM on M. hapla individuals and populations in the greenhouse and field environments supports the thesis that it is the physiological and nutritional state of the plant itself, and not VAM per se, that was the major determinant of the dynamics of nematode population growth in my experiments.

The specific physiological alteration(s) of A. cepa by VAM that most affect M. hapla was/were not identified in my studies. Although some plants fertilized

with P grew comparably with mycorrhizal plants, similarities between the P-amended and mycorrhizal plants with respect to nematodes cannot be definitively attributed to increased P levels, since the roots were not assayed for P content. The data from the microplot experiment (Figure 35), however, suggest that P levels in plant roots were a major factor in determining the host status of A. cepa, since mycorrhizal plants supplied with additional P supported much lower levels of M. hapla than did unfertilized mycorrhizal plants.

Several investigators proposed that the inhibiton of VAM colonization by high root P levels is associated with a membrane-mediated decrease in the exudation of root cell metabolites (Ratnayake et al., 1978). Colonization of roots by VAM, in turn, increases root P levels and decreases root permeability and exudation (Graham et al, 1981). Evidence for the attraction of nematodes to root diffusates was recently reviewed by Prot (1980). It is possible that a reduction in root exudates due to VAM is sufficient to inhibit the location and penetration of roots by nematodes. In addition, an increase in lignification of mycorrhizal roots (Dehne, 1982) might further inhibit root penetration or the establishment of a feeding site by endoparasitic nematodes.

The complexity of the relationship between environmental factors, root membrane permeability, and VAM colonization has only recently been examined (Graham et al., 1981). Further studies characterizing and quantifying the separate and joint impact of soil and root P levels, temperature, moisture, and other factors on plant growth and VAM colonization may elucidate the seemingly inconsistent response of nematode populations to host plants with a VAM association.

SUMMARY AND CONCLUSIONS

M. hapla is commonly associated with onion in Michigan muck soils and is particularly prevalent in areas where carrot is grown in rotation with onion. Nematodes occurring concomitantly with M. hapla in Michigan onion acreage are Pratylenchus spp., Paratylenchus spp., and Tylenchorhynchus spp. Mycorrhizal fungi, Glomus spp., are ubiquitous in agricultural soils and colonize roots of A. cepa coincidentally with M. hapla.

M. hapla does not seem to be very pathogenic to A. cepa under commercial onion production conditions, due in part, to the tolerance of some A. cepa cultivars to M. hapla parasitism and to the relative inability of M. hapla populations to increase using A. cepa as a food source. The latter factor seems to be very important in describing the relationship between M. hapla and A. cepa and was supported by data from my experiments. Levels of M. hapla associated with A. cepa were never comparable to those associated with D. carota. The reasons for this are not clear.

The generation-time for M. hapla seems to be greater on A. cepa than on D. carota and other crops. The differences between the rate of nematode development observed in my studies and reports in the literature, however, may be due to the fact that most developmental studies use the first appearance of a stage as the criterion for developmental progress. Reproduction by M. hapla infecting A. cepa is comparable to M. hapla reproduction on tomato (Tyler, 1938) and other crops. Correspondingly, mortality rates of M. hapla do not seem to be greater on A. cepa than on other plant hosts, although not much information on this point is available. What then limits the growth of M. hapla populations associated with A. cepa?

The presence of mycorrhizal fungi was one factor identified by my studies to adversely affect the population dynamics of M. hapla. There is evidence that VAM can impact all M. hapla life stages to some degree, probably by altering the physiology of the host plant. The major effect of VAM, however, seems to be in limiting the penetration of roots by M. hapla juveniles. The presence of VAM colonization in roots of A. cepa may be an important factor impeding the occurrence of a second nematode generation. Reproduction by first generation M. hapla females commences generally in late July. Second-generation nematodes not successful in invading A. cepa roots do not produce progeny, and probably do not live long themselves, since there is little evidence of diapause in M. hapla or other nematodes. Even if the inhibition of root penetration by VAM is short-lived, the delayed entrance of nematodes into roots may be sufficient to reduce the number of eggs and progeny produced before the natural senescence of A. cepa roots.

The influence of VAM on the population dynamics and pathogenicity of M. hapla was variable between experimental environments. Under greenhouse conditions, the deleterious effect of M. hapla parasitism to A. cepa was not alleviated by the presence of VAM unless the mycorrhizal association was well established before the introduction of nematodes. The data showed that plants colonized by VAM prior to inoculation with M. hapla offered a relatively unsuitable environment for the penetration of roots by nematodes. In contrast, plants which were colonized by VAM prior to nematode inoculation and grown in a field environment were more subject to nematode invasion that non-mycorrhizal plants. The different results of these experiments may have been due to the methodology of the inoculation or transplanting procedures, although

it is also likely that other factors, such as soil moisture stress and soil and root P levels, tempered the relationship between nematode and fungus.

If the major influence of VAM is to inhibit the attraction and penetration of nematodes to roots, as I propose, then the direction of future research should be to further investigate the stimuli that elicit host finding behavior by nematodes and to elucidate the effect of VAM colonization on those stimuli. Such research should clarify many of the discrepant results obtained in my and others' research.

Although the research described here concerned only the relationship between M. hapla, A. cepa, and VAM it is important not to lose sight of the other factors present in the onion agroecosystem that are potentially important as determinants of nematode population growth. Attributes of the host plant, such as the pattern and distribution of root growth and biochemical properities, may influence the increase of M. hapla. The presence of soil organisms, predaceous nematodes and other phytoparasites may be important in limiting the distribution of this nematode. Soil moisture, soil and plant nutrient levels, and other abiotic factors undoubtedly influence the population dynamics of M. hapla, both directly and indirectly through their impact on VAM and other organisms.

The methodology of systems science can be used to study the joint and combined influence of agroecosystem components on the relation between M. hapla and A. cepa. The conceptual model I developed did much to increase my understanding of the interaction between nematodes and plants. The modeling process was insightful in identifying research needs concerning the ecology of M. hapla.

The complexity and number of biological components present in the onion agroecosystem support the need for further study on the ecology of M. hapla and

other phytoparasitic nematodes. Hopefully, the experiments I performed will be useful in understanding the relationship between many nematodes and their associated hosts. The influence of G. fasciculatus and other VAM may not be limited to M. hapla infecting A. cepa. A systematic evaluation of the interrelationships between nematodes and their environment should be of high priority to nematologists, since it will expedite our understanding of pests such as M. hapla and our ability to anticipate the response of plants to their presence.

APPENDICES

APPENDIX I

```
      PROGRAM HAPLA (INPUT,OUTPUT,TAPE1,TAPE2,TAPE3,TAPE4,TAPE5)
C     ************************************************************************
C     ********************   DRIVER PROGRAM   ********************************
C     ************************************************************************
C
C    ****   VARIABLES:   ***************************************************
C *
C *
C *      AVETIME=  AVERAGE TIME (HRS) BETWEEN ITERATIONS
C *      BASE=  LOWER DEVEL. THRESHOLD FOR NEMA - USER MAY VARY
C *      CUMDH=  CUMULATIVE DEGREE HOURS CALC. FROM VARIABLE "BASE"
C *      DH=  DEGREE HOURS ACCUMULATED IN EACH ITERATION
C *      DT=  ITERATION TIME STEP EXPRESSED AS FRACTION OF 1 DAY
C *      INTTEMP=  AVG. TEMP BETWEEN READINGS TAKEN AT EACH ITERATION
C *      INTTIME=  BASED ON DAILY CLOCK  (EX.: IF TIMEINT=4 THEN
C * +      INTTIME= 4,8,12,16,20,24
C *      JDAY=  JULIAN DAY
C *      LX=  VARIABLE USED TO DISTINGUISH 1ST ITERATION (VARIABLES
C * +      INITIALIZED) FROM SUBSEQUENT ITERATIONS
C *      NDAYS=  NO. OF DAYS IN SIMULATION (USER INPUT)
C *      NDTS=  NO. OF ITERATIONS PER DAY
C *      NUMDAY=  NO. OF DAYS SINCE PLANTING
C *      OWEGG=  OVERWINTERING EGGS (USER INPUT)
C *      PDAY=  DAY OF PLANTING (JULIAN DAYS)
C *      SOILARV=  STATE VARIABLE: 2ND-STAGE JUVENILE IN SOIL
C *      STEMP=  SOIL TEMP READ FROM TAPE1 (RECORDED BIHOURLY) - 3 INCH
C *      SUMTEMP=  USED TO CALC. TEMP WHEN ITERATION = 1 DAY
C *      TEMP=  SOIL TEMP CALC. FOR EA. ITERATION (INTERPOL. FROM STEMP)
C *      TIMEINT=  NO. OF HOURS IN ONE ITERATION
C *      TOTNEM=  TOTAL NO. OF NEMAS IN ROOTS
C *
C *
C *
C ************************************************************************
C
C
      COMMON/DRIVE/DT,LX,NUMDAY,TEMP,OWEGG,BASE,DH
      COMMON/ONION/NEMSITE,TOTSITE,NEWROOT,ROOTWT
      COMMON/NEMA/EGG,SOILARV,EL2,LL2,L3L4,FEM,OVIFEM,TOTNEM
      COMMON/NEMFEED/NEMAFED,FEEDNEM
C
      DIMENSION STEMP(13)
      REAL INTTIME,INTTEMP
      DATA BASE/9.0/,LX/0/
```

```
45
                REWIND 1
                REWIND 2
50              REWIND 3
                REWIND 4
                REWIND 5
          C
          C USER INPUTS
55        C
                PRINT*,"PLANTING DATE (JULIAN DAYS) = "
                READ*,PDAY
                PRINT*,"NO. OF DAYS IN SEASON = "
                READ*,NDAYS
60              PRINT*,"TIME INCREMENT FOR SIMULATION (HRS./24) = "
                READ*,DT
                PRINT*,"INITIAL EGG POPULATION = "
                READ*,OWEGG
          C
65        C INITIALIZE VARIABLES
          C
                TIMEINT=DT*24.
                NDTS=1/DT + 0.5
                CUMDH=0.0
70              NUMDAY = 1
          C
          C *****   MAIN-DAY LOOP   *****
          C
                JDAY=0
75        C
                DO 1000 I=1,NDAYS
          C
                INTTIME=0.0
          50    CALL SOILTMP(JDAY,STEMP,LX)
80        C
          C RUN THRU TAPE1 UNTIL TEMP DATA FOR PDAY IS LOCATED
          C
                IF(JDAY.LT.PDAY) GO TO 50
          C
85        C
          C *****   WITHIN-DAY LOOP   *****
          C
                DO 500 J=1,NDTS
          C
90        C TEMP FOR ITERATION = 1 IS CALC. BY AVERAGING 13 TEMP READINGS
          C TEMP FOR ITERATION LT 1 IS CALC BY FUNCTION FNL
          C
```

```
      IF(NDTS.EQ.1) GO TO 75
C
95    INTTIME=INTTIME + TIMEINT
      INTTEMP = FNL(STEMP,0.,2.,12,INTTIME)
      AVETIME=INTTIME - 0.5*TIMEINT
      TEMP = FNL(STEMP,0.,2.,12,AVETIME)
C
100   GO TO 200
C
75    SUMTEMP=0.0
      DO 100 K=1,13
      SUMTEMP=SUMTEMP + STEMP(K)
105   100 CONTINUE
      TEMP=SUMTEMP/13.
C
200   CONTINUE
C
110   C CALCULATE DEGREE HOURS
C
      TEMP=20.
      IF(TEMP-BASE) 300,300,400
300   DH=0.00001
115   GO TO 450
400   DH=(TEMP-BASE) * TIMEINT
450   CONTINUE
      CUMDH=CUMDH + DH
C
120   C
      CALL ONION
      CALL NEMA
      CALL NEMFEED
C
125   C CHANGE LX TO 1 AFTER 1 ITERATION - VARIABLES NOW INITIALIZED
C
      LX=1
500   CONTINUE
C
130   C ONION DATA TRANSFERRED TO TAPE2
C DATA FOR JUVENILE STAGES TRANSFERRED TO TAPE3
C DATA FOR ADULT STAGES TRANSFERRED TO TAPE4
C DATA FOR TOTAL SOIL AND ROOT NEMATODES ON TAPE5
C
```

```
135               NUMDAY=NUMDAY + 1.
                  WRITE(2,700) NUMDAY,CUMDH,ROOTWT,TOTSITE,FEEDNEM
            700   FORMAT(1X,I5,4(2X,F14.4))
                  WRITE(3,800) NUMDAY,CUMDH,EL2,LL2,L3L4
            800   FORMAT(1X,I5,4(2X,F14.4))
140               WRITE(4,900) NUMDAY,CUMDH,FEM,OVIFEM,EGG
            900   FORMAT(1X,I5,4(2X,F14.4))
                  WRITE(5,950) NUMDAY,CUMDH,SOILARV,TOTNEM
            950   FORMAT(1X,I5,3(2X,F14.4))
            C
145         C
            600   CONTINUE
            1000  CONTINUE
            C
            C
150               PRINT*,"FINAL DENSITY OF SOIL NEMATODES = ", SOILARV
                  PRINT*,"FINAL DENSITY OF ROOT NEMATODES = ", TOTNEM
                  END
```

```
C
C
C
C   ************************************************************************
C   ********************   SUBROUTINE ONION   ******************************
C   ************************************************************************
C
C *****   VARIABLES:   ***************************************************
C *
C *
C *      FOR VARIABLES NOT LISTED HERE SEE SUB. DRIVER OR NEMA
C *
C NEMAFED = PROP. ROOT REDUCTION DUE TO NEMA FEEDING
C NEMSITE = NO. FEEDING SITES AVAILABLE FOR NEMA PENETRATION
C *      NEWROOT=  ROOT GROWTH (G) OCCURING IN 1 ITERATION
C OLDGROW = ROOT WT AT (T - 1)
C OPTROOT = OPTIMAL ROOT GROWTH FOR (T)
C *      RATE=  RGROWTH (1) OR (2)
C ROOT = REGRESS. EQ. OF OPTIMAL ROOT GROWTH
C GROWNOW(1) = ROOT GROWTH RATE FOR 1 - 54 DAYS AFTER PLANTING
C *        OBTAINED FROM GREENHOUSE EXP. AND COMPARED TO LIT.
C GROWNOW(2) = ROOT GROWTH RATE FOR 54 - 140 DAYS AFTER PLANTING
C *        OBTAINED FROM LIT -- BOTH RATES ARE FOR TEMP = 15 (OPT)
C ROOTWT = TOTAL ROOT WT (NEWROOT SUMMED)
C *      TEMP=  TEMP FOR ITERATION CALC (INTERPOLATED) FROM STEMP
C *      TEMPFAC=  TEMP FACTOR TO REDUCE RGROWTH WHEN TEMP LT OR GT
C *+       THAN OPTIMAL
C TOTSITE = TOTAL NO. OF NEMA FEEDING SITES
C XNUMDAY = NUMDAY = NO. OF DAYS SINCE PLANTING
C *
C *
C *
C ************************************************************************
C
      SUBROUTINE ONION
C
      COMMON/DRIVE/DT,LX,NUMDAY,TEMP,OWEGG,BASE,DH
      COMMON/ONION/NEMSITE,TOTSITE,NEWROOT,ROOTWT
      COMMON/NEMA/EGG,SOILARV,EL2,LL2,L3L4,FEM,OVIFEM,TOTNEM
      COMMON/NEMFEED/NEMAFED,FEEDNEM
C
      DIMENSION GROWNOW(2)
      REAL NEWROOT,NEMSITE,NEMAFED
C
```

```
C INITIALIZE VARIABLES IF LX = 0
C
      IF(LX.GT.0) GO TO 80
      OLDGROW = 0.0
      TOTSITE=0.0
      ROOTWT=0.0
      NEMAFED=0.
80    CONTINUE
C
C OPTIMAL ROOT GROWTH FOR THIS ITERATION
C EQ. 1 IF FOR GROWTH ON DAYS 1 - 54
C EQ. 2 IS FOR GROWTH ON DAYS 54 - 140
C
      XNUMDAY = NUMDAY
      GROWNOW(1) = (0.0095537 * EXP(0.0924842 * XNUMDAY)) * DT
      GROWNOW(2) = (0.000002694 * (XNUMDAY ** 3.340064)) * DT
C
C CALC. TEMPFAC
C
C IF THEN
      IF((TEMP.LT.10.).OR.(TEMP.GT.30.)) GO TO 100
      TEMPFAC=-9.4925 + (1.8461*TEMP) - (0.1115*TEMP**2) +
     + (0.00265*TEMP**3) - (0.00002092*TEMP**4)
      GO TO 200
C ELSE
100   TEMPFAC=0.08
C END IF
200   CONTINUE
C
C SET ROOT = OPTIMAL ROOT GROWTH FOR NDAY
C
      IF(NUMDAY-54) 300,300,400
300   ROOT = GROWNOW(1)
      GO TO 500
400   ROOT = GROWNOW(2)
500   CONTINUE
C
C CALC NEW ROOT GROWTH ( ROOT GROWTH (T) - ROOT GROWTH (T-1)
C
      OPTROOT = ROOT - OLDGROW
C
C MODIFY OPTIMAL ROOT GROWTH BY TEMP FACTOR
C
      NEWROOT = OPTROOT * TEMPFAC
```

```
90          C RECALC. NEW ROOT GROWTH BY SUBTRACTING PERCENT REDUC. DUE TO NEMAS
            C
                 NEWROOT=NEWROOT - (NEWROOT*NEMAFED)
            C
            C SAVE THE VALUE FOR ROOT GROWTH FOR THIS ITERATION
95          C
                 OLDGROW = ROOT
            C
            C NEMSITE = CARRYING CAPACITY FOR NEMATODE POPULATION
            C
100              NEMSITE = NEWROOT * 1000
            C
            C TOTAL ROOT GROWTH
            C
                 ROOTWT = ROOTWT + NEWROOT
105              RETURN
                 END
```

```
SUBROUTINE NEMA

      C
      C
      C
      C   ************************************************************************
      C   *********************   SUBROUTINE NEMA   ******************************
      C   ************************************************************************
      C
      C *****   VARIABLES:   ***************************************************
      C *
      C *
      C * FOR VARIABLES NOT LISTED HERE SEE SUBS. DRIVER OR ONION
      C *
      C * DEV(STAGE) = DEV. RATE FOR PARTICULAR STAGE
      C * EGG = EGGS PRODUCED DURING SIMULATION
      C * EL2 = EARLY SECOND-STAGE LARVAE (RECENTLY PENETRATED)
      C * LL2 = LATE SECOND-STAGE LARVAE (SEXUALLY DIFFERENTIATED)
      C * L3L4 = THIRD & FOURTH STAGES COMBINED
      C * FEM = PREOVIPOSITING FEMALES
      C * FEMDENS = NEMA DENSITY FACTOR INFLUENCING EGG PRODUCTION
      C * NEMSITE = NO. FEEDING SITES AVAILABLE FOR NEMA PENETRATION
      C * OVIFEM = OVIPOSITING FEMALES
      C * OVITEMP = TEMP FACTOR INFLUENCING EGG PRODUCTION
      C * OWEGG = OVER-WINTERING EGGS (USER INPUT)
      C * PENLARV = LARVAE WHICH HAVE PENETRATED ROOT (PRECEDES EL2)
      C * SOILARV = SECOND-STAGE JUVENILES IN THE SOIL
      C * SURV(STAGE) = SURVIVAL RATE FOR PARTICULAR STAGE
      C * TEMPEN = TEMP FACTOR INFLUENCING ROOT PENETRATION
      C * VIABLE = ARRAY NAME USED IN TEMPEN
      C * VIALARV = VIABLE LARVAE = NO. AVAILABLE TO PENETRATE
      C *
      C *
      C ************************************************************************
      C
            SUBROUTINE NEMA
      C
            COMMON/DRIVE/DT,LX,NUMDAY,TEMP,OWEGG,BASE,DH
            COMMON/ONION/NEMSITE,TOTSITE,NEWROOT,ROOTWT
            COMMON/NEMA/EGG,SOILARV,EL2,LL2,L3L4,FEM,OVIFEM,TOTNEM
            COMMON/NEMFEED/NEMAFED,FEEDNEM
      C
            REAL L1,EL2,LL2,L3L4,NEMSITE
      C
```

```
      DIMENSION X1(10),X2(10),X3(10),X4(10),X5(10),X6(10),X7(10)
      DIMENSION VIABLE(11)
      DIMENSION STORE(10)
      DIMENSION AUX(10)
      DATA VIABLE/.01,.05,.10,.25,.50,.75,1.00,.75,.50,.25,.01/
C
C INITIALIZE VARIABLES
C
      IF(LX.GT.0) GO TO 100
C
      DO 5 I=1,10
      X1(I)=0.0
      X2(I)=0.0
      X3(I)=0.0
      X4(I)=0.0
      X5(I)=0.0
      X6(I)=0.0
      X7(I)=0.0
5     CONTINUE
C
      EGG=0.0
      SOILARV=0.0
      TOTGONE=0.0
      EL2=0.0
      LL2=0.0
      L3L4=0.0
      FEM=0.0
      OVIFEM=0.0
      FEMDENS=1.0
      OVITEMP=1.0
      SURVSL = 0.0
      SLL2 = 0.0
      SL3L4 = 0.0
      SFEM = 0.0
      SOFEM = 0.0
      SURVX1= 1.00
      PENLARV = 0.0
      AXTOT1=0.0
      AXTOT2=0.
100   CONTINUE

C CALC. EGG FROM OVERWINTERING EGGS (1ST ITERATION ONLY) AND
C+ OVIPOSITING FEMALES (14 EGGS/FEMALE/DAY AT OPTIMAL TEMP AND DENSITY)
C
C
```

```
90
            EGG= (DT* (OWEGG + OVIFEM*14.)) * FEMDENS * OVITEMP
            OWEGG=0.0
          C
          C CALC. DEV.RATE FOR EGG STAGE BASED ON TEMP
95        C
          C IF THEN
            IF(TEMP.LT.10) GO TO 200
            DEVEGG=(-0.0564 + 0.00564*TEMP) * DT
            GO TO 300
100       C ELSE
          200   DEVEGG=0.0001
          300   CONTINUE
          C END IF
          C
105         CALL MODEL1(X1,STORE,DEVEGG,AXTOT1,L1)
            PRINT*,"EGG =  ",L1
          C
          C ADD EGG INPUT TO EGG DEVELOPMENT PROGRAM
          C
110         X1(1)=X1(1) + EGG * 0.30
          C
          C KILL OFF 70 PERCENT OF ALL EGGS - GUESSTIMATE
          C
          C TEMP FACTOR FOR SOILARV MOBILITY
115       C
            TEMPEN = FNL(VIABLE,10.,2.,10,TEMP)
          C
          C CALC. NO. OF LARVAE CAPABLE OF PENETRATING ROOTS BASED ON TEMP
          C
120         SOIL=SOILARV-TOTGONE
            IF(SOIL.LT.0.) SOIL=0.0
            IF(TEMP-20.)400,400,500
          400   DIESOIL=(SOIL*0.142857/2)*DT
            GO TO 700
125       500   DIESOIL=(SOIL*0.142857)*DT
            GO TO 700
          700   CONTINUE
            AVAIL=(SOIL-DIESOIL)*TEMPEN
            IF(AVAIL-NEMSITE)800,900,900
130       800   PENLARV=AVAIL
            GO TO 1000
          900   PENLARV=NEMSITE
          1000  CONTINUE
            GONE=DIESOIL+PENLARV
135         TOTGONE=TOTGONE + GONE
```

```
C
C
C CALC. RATE OF SOILARV DEV
C THE DEVELOPMENT PROGRAM IS USED HERE TO PROGRESS NEMAS
C+TO DEATH.  ONLY NEMAS STILL IN DEVELOPMENT ARE USED TO CALC. THE
C+THE NO. OF LARVAE CAPABLE OF PENETRATING ROOTS
C SOILARV DIE IN 14 DAYS IF TEMP LT 20 AND 7 DAYS IF TEMP GT 20
C SOILARV DIE AT VERY SLOW RATE (NEGLIGIBLE) WHEN TEMP IS LT 9
C
      IF(TEMP-20.)1020,1020,1030
1020   DECSOIL = (0.142857/2.) * DT
      GO TO 700
1030   DECSOIL = 0.142857 * DT
      GO TO 1050
1050  CONTINUE
C
C SURVIVAL RATE OF SOILARV
C
C
      CALL MODEL3(SOILARV,STORE,DEVEGG,DECSOIL,SURVSL)
      PRINT*,"SOILARV =  ",SOILARV
C
C CALC. DEV RATE FOR PENETRATED LARVAE - EARLY 2ND-STAGE
C
      IF(TEMP.LT.BASE) GO TO 1300
      IF(TEMP-25.) 1100,1200,1200
1100  DEVEL2 = DT * (0.10/(18192.04377 * TEMP ** (-3.04976)))
      GO TO 1400
1200  DEVEL2= 0.10 * DT
      GO TO 1400
1300  DEVEL2=0.0001
1400  CONTINUE
C
      CALL MODEL1(X3,AUX,DEVEL2,AXTOT2,EL2)
      PRINT*,"EL2 =  ",EL2
C
```

```
      X3(1)=PENLARV
C
C CALC DEV RATE FOR LATE 2ND STAGE JUVENILES
C
      IF(TEMP.LT.BASE) GO TO 1700
      IF(TEMP-25.) 1500,1600,1600
1500  DEVLL2 = DT * (0.167/(TEMP/(-16.538 + 1.72811 * TEMP)))
      IF((TEMP.GE.9.).AND.(TEMP.LT.11.)) DEVLL2=0.05 * DT
      GO TO 1800
1600  DEVLL2=0.167 * DT
      GO TO 1800
1700  DEVLL2=0.0001
1800  CONTINUE
C
      SURVLL2=1.00
C
      CALL MODEL2(DEVEL2,DEVLL2,AUX,X4,SURVLL2,SLL2,LL2)
      PRINT*,"LL2 =  ",LL2
C
C CALC. DEV RATE FOR 3RD AND 4TH STAGE JUVENILES (COMBINED)
C
      IF(TEMP.LT.BASE) GO TO 2100
      IF(TEMP-25.) 1900,2000,2000
1900  DEVL3L4 = DT * (0.50/(8.756129 * EXP(-0.08549 * TEMP)))
      GO TO 2200
2000  DEVL3L4=0.50 * DT
      GO TO 2200
2100  DEVL3L4=0.0001
2200  CONTINUE
      SURVL34=1.00
C
      CALL MODEL2(DEVLL2,DEVL3L4,AUX,X5,SURVL34,SL3L4,L3L4)
C
C CALC. DEV RATE OF PRE-OVIPOSITING FEMALES
C
```

```
      IF(TEMP.LT.BASE) GO TO 2500
      IF(TEMP-25.) 2300,2400,2400
2300  DEVFEM = DT * (0.125/(4.01124 - 0.1177928 * TEMP))
      GO TO 2600
2400  DEVFEM=0.125 * DT
      GO TO 2600
2500  DEVFEM=0.0001
2600  CONTINUE
      SURVOVI=1.00
C
      CALL MODEL2(DEVL3L4,DEVFEM,AUX,X6,SURVOVI,SFEM,FEM)
C
C CALC. DEV RATE OF OVIPOSITING FEMALES - DEVELOPMENT LEADS TO DEATH
C
      IF(TEMP-25.) 2700,2800,2800
2700  DEVOFEM = (0.02/2.) * DT
      GO TO 3000
2800  DEVOFEM = 0.02 * DT
3000  CONTINUE
C
      DEADFEM=1.00
      DECFEM=.05
C
      CALL MODEL3(OVIFEM,AUX,DEVFEM,DECFEM,DEADFEM)
C
C CALC. EFFECT OF NEMA DENSITY ON EGG PRODUCTION
C
      FEMDENS=1.00
C
C CALC. EFFECT OF TEMP ON EGG PRODUCTION
C
      OVITEMP=1.00
C
C CALC. TOTAL NO. OF NEMAS OCCUPYING ROOT
C
      TOTNEM = EL2 + LL2 + L3L4 + FEM + OVIFEM
C
      RETURN
      END
```

```
SUBROUTINE NEMFEED
C
C
C   ************************************************************************
C   ********************   SUBROUTINE NEMFEED   ****************************
C   ************************************************************************
C
C *****   VARIABLES:   ***************************************************
C *
C DH = DEGREE HRS FOR THIS ITERATION
C *     FEEDFAC= INFLUENCE OF NEMATODE DENSITY ON THE FEEDING FUNCTION
C *+      OF ONE NEMATODE (EXPRESSED AS PERCENT GROWTH REDUCTION)
C *     FEEDNEM=  TOTAL NO. OF NEMATODES INHABITING THE ROOT SYSTEM
C *+      EXCEPT L3L4 STAGE JUVENILES
C *     FEM=  STATE VARIABLE: PRE-OVIPOSITING FEMALE STAGE
C *     LL2=  STATE VARIABLE: LATE 2ND STAGE
C *     L3L4= STATE VARIABLE: 3RD,4TH STAGE (COMBINED)
C *     NEMAFED=  PERCENT GROWTH REDUCTION RESULTING FROM NEMA FEEDING
C *     OVIFEM=  STATE VARIABLE: OVIPOSITING FEMALE STAGE
C PERNEMA = FEEDING EFFECT OF EACH NEMA DEPENDENT ON NEMA DENSITY
C *     SOILARV= STATE VARIABLE: 2ND STAGE JUVENILES IN SOIL
C *     X3=  STATE VARIABLE: EARLY 2ND STAGE JUVENILES IN ROOT
C *
C *
C *
C ************************************************************************
C
      SUBROUTINE NEMFEED
C
      COMMON/DRIVE/DT,LX,NUMDAY,TEMP,OWEGG,BASE,DH
      COMMON/ONION/NEMSITE,TOTSITE,NEWROOT,ROOTWT
      COMMON/NEMA/EGG,SOILARV,EL2,LL2,L3L4,FEM,OVIFEM,TOTNEM
      COMMON/NEMFEED/NEMAFED,FEEDNEM
C
      REAL NEMAFED,LL2
C
C NO. OF NEMATODES FEEDING WITHIN ROOT SYSTEM
C
      FEEDNEM = EL2 + LL2 + FEM + OVIFEM
      IF(FEEDNEM.LT.1.) GO TO 100
```

```
40          C
            C CALC. FEEDING FACTOR BASED ON NEMATODE DENSITY
            C
                  IF(DH-8000.) 40,40,50
            40    PERNEMA = (0.0005019 + 0.65623/FEEDNEM) * DT
45                GO TO 60
            50    PERNEMA = ((0.0005019 + 0.65623/FEEDNEM)/2.5) * DT
            60    CONTINUE
            C
                  IF(FEEDNEM.GT.125.) PERNEMA = 0.00022122 * DT
50                NEMAFED = (PERNEMA * FEEDNEM) * DT
            C
            C LIMIT EFFECT OF NEMAS TO 70 ROOT REDUCTION
            C
                  IF(NEMAFED.GT.0.70) NEMAFED = 0.70
55                GO TO 200
            100   NEMAFED = 0.0001
            200   CONTINUE
                  RETURN
                  END
```

```
SUBROUTINE SOILTMP

      C
      C
      C
      C
      C   ************************************************************************
      C   ********************   SUBROUTINE SOILTMP   ********************************
      C   ************************************************************************
      C
      C *****   VARIABLES:   ***************************************************
      C *
      C *      JDAY=  JULIAN DAY
      C *      LX=  PARAMETER USED TO DISTINGUISH 1ST ITERATION OF SIMULATION
      C *        (VARIABLES INITIALIZED) FROM SUBSEQUENT ITERATIONS
      C *      STEMP=  SOIL TEMP READ FROM TAPE1 (RECORDED BIHOURLY)
      C *
      C *
      C *
      C ************************************************************************
            SUBROUTINE SOILTMP(JDAY,STEMP,LX)
      C
            DIMENSION STEMP(13)
      C
      C JULIAN DAY COUNTER
      C
            JDAY=JDAY + 1
      C
      C INITIALIZE VARIABLES
      C
            IF(LX.GT.0) GO TO 100

            DO 10 I=1,13
            READ (1,50) STEMP(I)

            STEMP(I)= (STEMP(I)-32.) *5./9.

      10    CONTINUE
      C
            GO TO 30
```

```
40          C
            100   CONTINUE
            C
                  STEMP(1)=STEMP(13)
                  DO 20 I=2,13
45                READ (1,50) STEMP(I)
                  STEMP(I)= (STEMP(I)-32.) *5./9.


            20    CONTINUE
50          30    CONTINUE
            50    FORMAT(F2.0)
                  RETURN
                  END
```

SUBROUTINE MODEL1

```
      C
      C
      C
      C
      C   ************************************************************************
      C ********************   SUBROUTINE MODEL2  ****************************
      C   ************************************************************************
      C
      C   VARIABLES:
        SUBROUTINE MODEL1(X,AUX,RATE,AXTOT,TOT)
        DIMENSION X(10),AUX(10)
        AXTOT=0.
        DO 10 I=1,10
        AUX(I)=X(I)
        AXTOT=AXTOT+AUX(I)
        X(I)=0.
   10   CONTINUE
        DO 15 I=1,10
        DO 9 J=1,I
        YMIN=.1*(J-1)
        YMAX=.1*J
        IF(RATE-YMIN)9,9,12
   12   IF(RATE-YMAX)13,13,9
   13   IJ=I-J+1
        IJ1=IJ-1
        IF(IJ1)9,30,31
   30   X(I)=10.*(YMAX-RATE)*AUX(IJ)
        GO TO 9
   31   X(I)=10.*((YMAX-RATE)*AUX(IJ)+(RATE-YMIN)*AUX(IJ1))
    9   CONTINUE
   15   CONTINUE
        TOT=0.
        DO 40 I=1,10
   40   TOT=TOT+X(I)
        RETURN
        END
```

SUBROUTINE MODEL2

```
      C
      C
      C
      C
      C   ************************************************************************
      C ********************   SUBROUTINE MODEL   ****************************
      C ************************************************************************
      C
      C ******   VARIABLES   ***************************************************
      C * A = DEV. RATE OF STAGE (I-1)
      C * B = DEV. RATE OF STAGE (I)
      C * C2 = AUXILLARY VARIABLE USED TO STORE C3 AT (T-1)
      C * C3 = ARRAY OF NO. OF INDIVIDUALS IN STAGE I
      C * MORT = SURVIVAL RATE
      C * TOT = TOTAL NO. PASSING FROM I (T-1) TO I (T)
      C * SUM = TOTAL NO. IN STAGE (I) AT TIME (T)
      C *
      C *
      C ************************************************************************
            SUBROUTINE MODEL2(A,B,C2,C3,SURV,TRTOT,TOT)
            DIMENSION C2(10),C3(10),TAUX(10),BU(10)
            DO 70 I=1,10
      70    BU(I)=0.
            CALL MODEL1(C3,TAUX,B,DUMB,DUM)
            DO 300 I=1,10
            YMIN=(1.-(10.-(I-1))/(A*10))*10.*B
            YMAX=(1.-(10.-I)/(A*10.))*10.*B
            IF(YMAX.LE.0) GO TO 300
            DO 290 K=1,10
            IF(YMAX-K)50,100,100
      50    IF(YMAX-(K-1))290,290,80
      80    IF(YMIN-(K-1))110,120,120
      110   C3(K)=C3(K)+A/B*(YMAX-(K-1))*C2(I)*SURV
            BU(K)=BU(K)+A/B*(YMAX-(K-1))*C2(I)*SURV
            GO TO 290
      120   C3(K)=C3(K)+A/B*(YMAX-YMIN)*C2(I)*SURV
            BU(K)=BU(K)+A/B*(YMAX-YMIN)*C2(I)*SURV
            GO TO 290
      100   IF(YMIN-K)150,290,290
```

```
      150   IF(YMIN-(K-1))130,140,140
      130   C3(K)=C3(K)+A/B*C2(I)*SURV
            BU(K)=BU(K)+A/B*C2(I)*SURV
            GO TO 290
      140   C3(K)=C3(K)+A/B*(K-YMIN)*C2(I)*SURV
            BU(K)=BU(K)+A/B*(K-YMIN)*C2(I)*SURV
      290   CONTINUE
      300   CONTINUE
            TOT=0.
            DO 60 I=1,10
      60    C2(I)=TAUX(I)
            DO 310 I=1,10
            TOT=TOT+C3(I)
      310   TRTOT=TRTOT+BU(I)
            RETURN
            END
```

SUBROUTINE MODEL3

```
      C
      C
      C*************************************************************************
      C**********************SUBROUTINE MODEL3**********************************
      C*************************************************************************
      C
            SUBROUTINE MODEL3(X8,AUX,RATE1,DEC,SURV)
            DIMENSION AUX(10),XI8(10)
            ADIN=0.
      10    DO 10 I=1,10
      10    XI8(I)=0.
            DO 160 IM=1,10
            IF(10-IM-10.*RATE1)53,160,160
      53    RVAL=RATE1-.1*(10-IM)
            IF(RVAL-.1)54,55,55
      54    XI8(IM)=RVAL*10.*AUX(IM)
            GO TO 57
      55    XI8(IM)=AUX(IM)
      57    ADIN=ADIN+XI8(IM)*SURV
      160   CONTINUE
            X8=EXP(-DEC)*X8+ADIN
            RETURN
            END
```

FUNCTION FNL

```
1          C
           C   ************************************************************************
           C   *********************   FUNCTION FNL   ******************************
           C   ************************************************************************
5          C
           C DVAL = NAME OF DATA ARRAY
           C XS = FIRST X VALUE
           C DX = DELTA FOR INDEPENDENT VARIABLE
           C N = NO. OF DATA PTS - 1
10         C X = NAME OF INDEPENDENT VARIABLE
           C
                 FUNCTION FNL(DVAL,XS,DX,N,X)
                 DIMENSION DVAL(1)
                 IF(X-XS)10,10,5
15         5     IF(X-XS-N*DX)20,15,15
           10    FNL=DVAL(1)
                 GO TO 25

           15    FNL=DVAL(N+1)
20               GO TO 25
           20    XD=X-XS
                 I=1.0+XD/DX
                 FNL=(XD-FLOAT(I-1)*DX)*(DVAL(I+1)-DVAL(I))/DX+DVAL(I)
           25    RETURN
25               END
```

APPENDIX II

Efficiency of a Method for Extracting Meloidogyne hapla from Soil

INTRODUCTION

The purpose of this experiment was to assess the efficiency of the sugar flotation-centrifugation method used in the described experiments to extract Meloidogyne hapla juveniles from muck soil. Earlier, Kotcon (1979) concluded that the equipment used and the timing of the various stages of the procedure were important variables in determining the number of M. hapla recovered from muck soil. In agreement with Seinhorst (1962), he found that the length of the nematode in relation to the size of the sieve used in the procedure was also important. Kotcon's extraction efficiency for M. hapla was 28.1%, as compared to 36.7% for Pratylenchus penetrans and 37.4% for Tylenchorhynchus spp., nematodes considerably larger than M. hapla. Moriarty (1960) found that in addition to the sources of experimental error mentioned above, there was considerable variation between different observers counting nematode samples. For this reason I felt it important to establish a measure of the extraction efficiency for myself and my employees, even though the technique employed was identical to that of Kotcon. A secondary, but perhaps more important, objective of the experiment was to assess the influence of nematode density per soil sample on the efficiency of our extraction procedure.

METHODS

Freshly-hatched M. hapla second-stage juveniles were counted and placed in test tubes containing ca 10 ml of water. The following nematode densities were isolated (number of replications in parentheses): 1(10), 5(10), 25(5), 50(4),

75(4), 100(4), and 200(4). One hundred cm^3 of muck soil, steamed for eight hours, was placed into each of 51 plastic bags. One tube of nematode inoculum was added to each bag; the tube was rinsed in ca 5 ml of water and also added to each bag; and the soil within the bag was agitated thoroughly. The sample was then processed by a modified sugar flotation-centrifugation technique (Jenkins, 1964) by an employee who had also processed the majority of the soil samples for the experiments described previously. The resulting samples were counted by myself using a stereomicroscope. The bags containing soil and the tubes containing the processed sample were coded so that neither the employee nor myself was aware of the nematode density for a specific sample.

RESULTS AND DISCUSSION

There was a highly significant (P=0.01) linear relation between the initial nematode density and the number of nematodes recovered per sample (Figure 50A). According to regression analysis, an average of 34.95 of the nematodes within a sample were recovered. Data for the proportion of nematodes recovered were transformed by an arcsine transformation and regressed on initial nematode density (Figure 50B). There was no linear relation between the percentage of nematodes obtained after extraction and initial nematode density, indicating that a constant proportion (0.33) of the nematodes in each sample were recovered.

Inspection of the untransformed data showed that the variances of the observations for different nematode densities were not equal. For this reason, a weighted least squares analysis of the data was also performed to fit the model:

$$E(y) = bX$$

where:

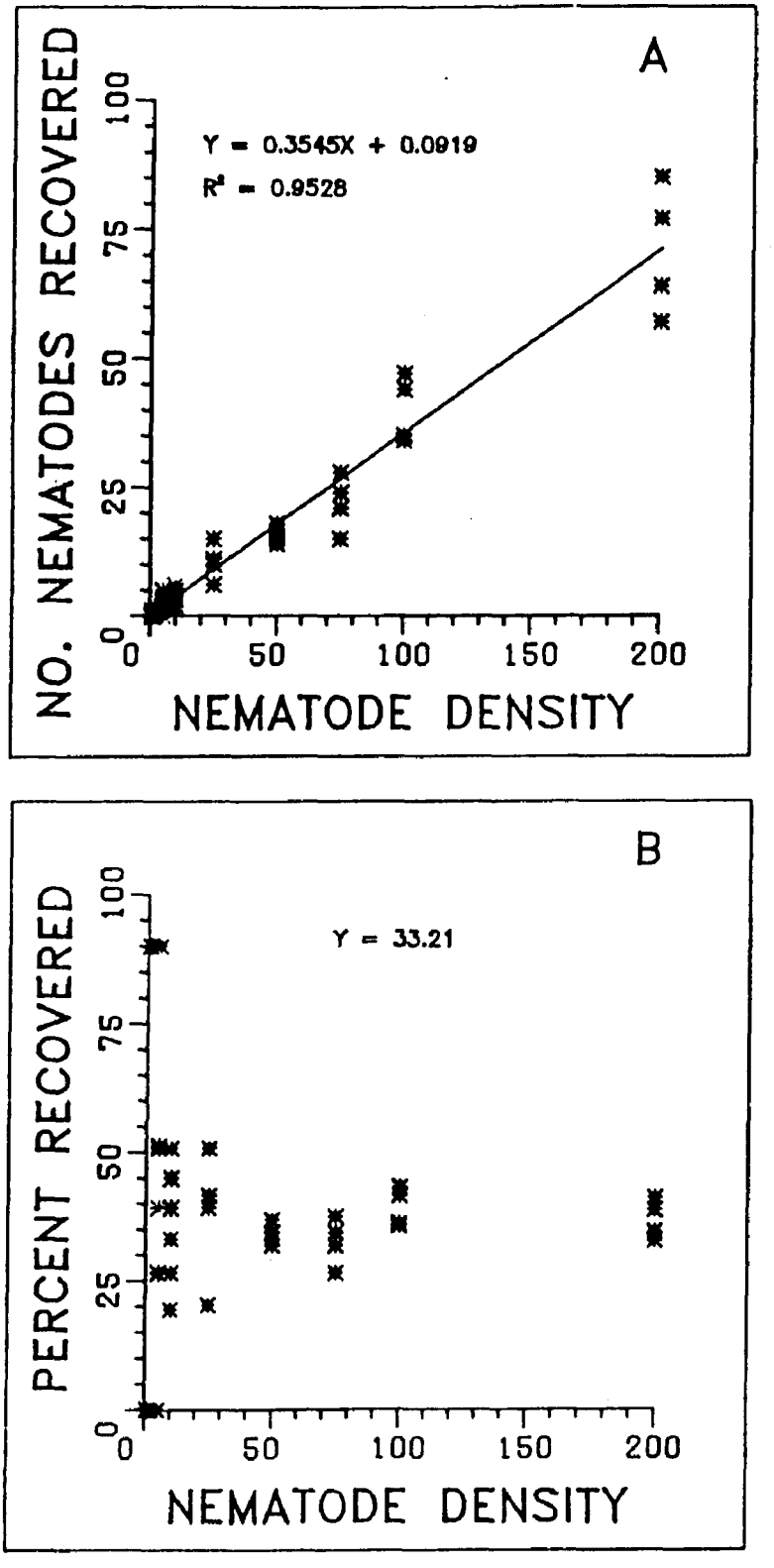


Figure 50. Number (A) and percentage (B) of Meloidogyne hapla recovered per 100 cm^3 soil given initial densities of 1 - 200 nematodes.

$b = \Sigma w_i x_i y_i / \Sigma w_i x_i^2$

w_i = weighting value δ^2 / δ_i^2

x_i = actual nematode density

y_i = nematodes recovered

Y, the number of nematodes recovered from a sample, can be described by a binomial distribution, according to the following equation:

$Y_i = Px_i (1-Px_i) x_i$ + error (E(error)=0) (V(error)=kx_i)

where:

Px_i = probability of finding a nematode (constant)

E = mean

V = variance

$k = Px_i(1-Px_i)$

Substituting kx_i in the denominator of the weighting value, w_i, b can be estimated by:

$$\Sigma y_i / \Sigma x_i = \bar{y} / \bar{x}$$

According to this analysis, 35.68% (V(b)=0.9107%) of the initial number of M. hapla present in a sample were recovered by the technique employed.

The three techniques used to analyze extraction efficiency: (1) regression analysis of counts, (2) regression analysis of transformed (arcsine) counts, and (3) weighted least squares analysis, produced similar results. It seems then, that nematode density (for densities less than 200 nematodes per 100 cm^3 soil) was not a factor influencing the results of the experiments presented here. Many other potential sources of sampling error do exist, however, and may not be consistent between different experimental environments. Additional sources of variability in research procedures should be analyzed before an attempt is made to directly relate the data collected in greenhouse experiments to observations made in the field.

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