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STUDY OF THE BIOLOGY AND ECOLOGY OF
HETERODERA CAROTAE (JONES, 1950)
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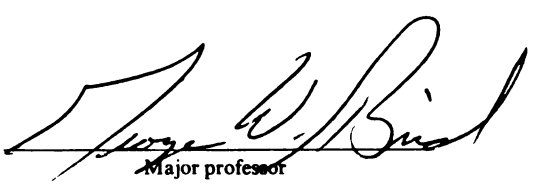

Major professor

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STUDY OF THE BIOLOGY AND ECOLOGY OF HETERODERA CAROTAE (JONES, 1950) IN MICHIGAN

By

Michael F. Berney

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ABSTRACT

STUDY OF THE BIOLOGY AND ECOLOGY OF HETERODERA CAROTAE (JONES, 1950) IN MICHIGAN

By

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Heterodera carotae, carrot cyst nematode, is found throughout carrot production regions of Europe, but, in North America, has only been reported in MI. This host monospecific plant parasitic nematode is of interest for economic reasons, as a model for plant-nematode interactions and agroecosystem studies.

Because of the highly aggregated nature of nematode distributions, and the scale of our interest, detection and description of nematode distributions is difficult. Heterodera carotae was found in all carrot producing regions of MI by survey. The survey sampling method was compared to single aggregate sample and transect sample series. It was a cost effective compromise between simple detection, and the distribution information available from a transect series. Vertical sampling in one field revealed that H. carotae was present in all soil profiles where carrot roots were present, but that most of the population was present in the top 30 cm.

Host range tests in Europe found hosts other than Daucus carota for H. carotae. Host range tests in MI revealed no additional hosts. Any factor affecting the host carrot can

affect the root filtrates which stimulate hatch of H. carotae. Infestation of the root system by Meloidogyne hapla or H. carotae was found to affect hatch. Plants must be at least three weeks old, and a greater percent of hatch as well as two peaks of hatch were reported from plants older than six weeks.

The sequence of plants grown in soil infested with H. carotae was investigated for effect on the carrot cyst nematode population and subsequent carrot plantings. Carrot yields showed less nematode impact after one year of a monocot growth, and less again after two years of nearly any plant type. The continuous planting of carrots for more than two years seems to stabilize populations at levels lower than the highs achieved when plant types change each year. Nematodes are subject to the effects of environmental variation, especially temperature. Heterodera carotae hatches most readily at 10C, most completely at 15C and not at all at 25C. Hatching at 5 and 20C is intermediate.

DEDICATION

This dissertation is dedicated to my wife, Cathy Elizabeth Anne Berney, without whose understanding, patience and support none of it would have been possible

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I wish to express my sincere thanks to Dr. George Bird for his support and encouragement throughout this process. I also wish to thank the members of my guidance committee: Dr. Guy Bush, Dr. Don Hall, Dr. Stuart Gage and Dr. Edward Grafius. Special thanks go to Mr. John Davenport for all of the work he performed in field and lab. Mr. Alan Peterson, former manager of Wm. Bolthouse Farms, Sheridan facility, is gratefully acknowledged for his forbearance, interest and assistance. The Michigan Carrot Research Council is gratefully acknowledged for their financial support. And finally to the faculty and staff of the Department of Entomology, my home for the last few years, I wish to acknowledge my gratitude.

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OVERVIEW

In the literature review there is an overall review of the Heterodera carotae literature, followed by two literature reviews on community ecology. The first of these covers general community ecology, and the second covers nematode community ecology. The final portion of the literature review concerns the population dynamics of Heterodera carotae.

The section on the detection and description of nematode distributions includes three chapters. The first of these is a description of the distribution of Heterodera carotae in Michigan carrot production. The second chapter is an evaluation of the sampling methods for the detection of Heterodera carotae. The final chapter in this section is a study of the vertical distribution of plant parasitic nematodes in Michigan histosols.

The section on ecological studies includes five chapters. The first of these is a report on studies of the host range of Heterodera carotae in Michigan. The second chapter covers the effect of host plant response to nematode infestation on the emergence of Heterodera carotae from cysts. The third chapter concerns the effect of host plant age on the emergence of Heterodera carotae from cysts. The fourth chapter is about the influence of plant growth sequences on Heterodera carotae

populations in Michigan histosols. The final chapter in this section concerns the population dynamics of Heterodera carotae populations in Michigan histosols under continuous carrot production.

The fifth section of this dissertation concerns environmental responses and consists of a single chapter on the effect of temperature on the emergence of Heterodera carotae from cysts.

The final section of this document contains notes and observations made in the course of laboratory and field experiments, which may be important, but are not included elsewhere in the text.

The appendix contains a single chapter on the use of selected primers, PCR and gel electrophoresis on Mitochondrial DNA to differentiate populations of Heterodera carotae.

INTRODUCTION

The Carrot Cyst Nematode Heterodera carotae was discovered and described by Jones (1944 and 1950 respectively). This small lemon shaped cyst, has been reported from 11 European countries, Great Britain, India, Cyprus and the United States. Heterodera carotae was first discovered in the United States in Michigan in 1979 during a survey for Meloidogyne hapla associated with carrot-onion rotations in organic soils. The report of this discovery was first formally made in 1985 (Graney, 1985). To date, Michigan remains the only known location of H. carotae in the Western Hemisphere. Reports of severe crop loss associated with carrot production in H. carotae infested fields testify to the economic impact of this nematode on this important crop (Mathews, 1975).

The thesis of this PhD. dissertation is that an understanding of the biology and ecology of H. carotae is essential to understanding the host-parasite relationship between H. carotae and Daucus carota L., domestic carrot. The experiments outlined in this dissertation are designed to investigate specific selected areas of the population ecology of H. carotae in commercial carrot production in Michigan.

SECTION ONE

Literature Review

INTRODUCTION

Prior to the studies presented here, all of the literature about Heterodera carotae came from Europe. This small lemon shaped cyst is in the same part of the cyst family as Heterodera schactii the sugarbeet cyst nematode. The research presented here reveals differences between the responses of Michigan H. carotae and European members of the same species. Heterodera carotae is a member of the Michigan carrot histosol agroecosystem. Meloidogyne hapla and Pratylenchus penetrans are the two most notable other phyto-nematode members of this community. A large number of other nematodes as well as other microscopic animals inhabit the soil. Diversity is limited in part by limited plant species. Even over an extended series of years, Daucus carota represents the major plant species in this system. This fact heavily favors the preponderant role of H. carotae among the phyto-nematodes.

LITERATURE REVIEW

The literature regarding Heterodera carotae is relatively recent, with only one citation of this species preceding World War II. This reference from Triffit (1935) refers to a cyst forming species of Heterodera associated with carrot roots, but no attempt was made to describe or name this organism. Subsequent work by others leaves little doubt that this nematode was H. carotae. Jones, in 1950, reported the occurrence of a Heterodera species on carrot, and described it as Heterodera carotae (Heteroderinae). H. carotae was first found by Jones on a "small freeholding at Chateris in the Isle of Ely".

Systematic Position

Phylum: Nemata; Class: Secernentea; Order: Tylenchida; Tylenchoidea : Heteroderidae : Heteroderinae : Heterodera Schmidt,1871.

Description

Females. Adults are small with a length 218-625 ($\bar{x}$=408) μ, and a width 165-500 ($\bar{x}$=309) μ. They form lemon shaped cysts with an egg sac that is often as large as the female body. The head is small, consisting of a single annule and a

labial plate. The median oesophageal bulb is rounded, with a distinct valvular apparatus. The excretory pore is located behind the level of the median bulb, 81-119 μ from the anterior end. The ovaries are paired and fill nearly the entire body cavity. The vulval slit is located in a cleft at the top of the vulval cone. The female body changes from a pale white to russet-brown with no intermediate yellow stage. The neck remains distinct, and often appears twisted. The cyst wall pattern consists of irregular zigzag lines forming a close network. A subcrystalline layer is present,but fragile. The vulval cone is prominent, with contours that blend smoothly into the body contour. Bullae are absent. The underbridge is approx. 90 μ long, bifurcate, slender and unsclerotized. The fenestration is indistinct and ambifenestrate.

Males. The males are vermiform with short bluntly rounded tails and a length of 1090-1220 ($\bar{x}$=1154) μ and width of 19-21 ($\bar{x}$=20) μ. The head is slightly offset, and is 7 μ long and 11 μ wide at the widest point, with 6 to 8 indistinct postlabial annules. The cephalic framework is robust. The stomatostyle is strong, 31-38 ($\bar{x}$=35) μ long with rounded basal bulbs. The dorsal oesophageal gland orifice is located 5-7 μ behind the spear knobs. The anterior and posterior cephalids are located at the level of the second and sixth body annules respectively. The median oesophageal bulb is oval with poorly developed valve plates located 85-105 ($\bar{x}$=90) μ from the anterior end. The excretory pore is located 148-161 ($\bar{x}$=153)

μ from the head. The hemizonid is conspicuous, 2-3 annules long and located 6-9 annules anterior to the excretory pore. The hemizonion is inconspicuous. The single testis is uniformly packed with sperm and averages 59% of the body length. The spicules are arcuate with a bulbous anterior part, a tubular part tapering to a twisted posterior part. The spicule tip is bidentate (Clark, 1973). The gubernaculum is slightly curved. The phasmids are located ad-anal. The lateral field is composed of four lines forming three bands, the outer lines being crenated and the outer bands areolate.

Second Stage Juveniles. The overall length is 375-452 ($\bar{x}$=422) μ and the width is 19-21 ($\bar{x}$=20) μ. The head is slightly offset, 4-6 ($\bar{x}$=5) μ long by 9-11 ($\bar{x}$=10) μ wide at the widest point, with four indistinct postlabial annules. The cephalic framework is less heavily sclerotized than the well developed spear. The spear is 22-25 ($\bar{x}$=24) μ long with knobs 2-3 μ long by 4-5 μ wide and having concave anterior surfaces. The cephalids are indistinct, the anterior ones being at the level of the third body segment, and the posterior ones being at the level of the eighth or ninth body annules. Dorsal oesophageal gland orifice, median bulb, hemizonion and lateral field are all nearly identical to those of the male. The phasmids are obscure and located 3-4 annules behind the anus. The excretory pore is located an average of 99 μ from the head. The hemizonid is distinct, 1-2 annules long and located 6-9 annules anterior to the excretory pore.

The genital primordium apparently consist of two cells which are located 60% of the body length from the head. The vast majority of juveniles show typical heteroderoid tail shape, with less than two percent showing variations.

Eggs. The eggs are cylindrical with rounded ends and measure 96-113 ($\bar{x}$=104) μ long by 46-53 ($\bar{x}$=50) μ wide. The ratio of length to width is 1.96-2.28 ($\bar{x}$=2.09). The egg shells are hyaline. The enclosed juveniles are folded four times into five approximately equal parts.

Host Range

This species has been tested on numerous species by Jones (1950),Winslow (1954) and Miller (1976). Only Daucus carota sativa, D. carota carota and D. pulcherrimus were found to be hosts. Vallotton (1980) discovered that H. carotae was able to complete it's development on Torilis arvensis, a wild Umbelliferae occurring in the Valais region of Switzerland, and stated that Mugniery also observed reproduction on T. leptophylla.

Geographic Distribution

Heterodera carotae is generally distributed in Europe where carrots are grown (Greco, 1985). In Great Britain, in addition to the occurrence in England (Jones, 1950) it has been recorded from Northern Ireland (Anon, 1971) and Scotland (Osborne, 1971). Other European countries where H. carotae has been found on cultivated carrots include Holland

(Oostenbrink, 1955), France (Oudinet, 1968), Italy (Ambrogioni, 1969), Switzerland (Vallotton, 1980), the Federal Republic of Germany (Sturhan, 1960), the Democratic Republic of Germany (Decker, 1968, cited in Stelter, 1973), Sweden (Andersson and Nyberg, 1964), Poland (Brzeski, 1970), Czechoslovakia (Sabova' and Valotska, 1976), Hungary (Javor, 1968 cited in Stelter, 1973) and Russia (Kir'yanova and Krall, 1980). The only reports of H. carotae outside of Europe have been from Cyprus (Philis, 1976) and India (Swarup et al., 1964).

Biology and Life History

The currently accepted life history of H. carotae is similar to other cyst nematodes. Eggs are present within a cyst, or in an egg sac or mass. This provides for two separate degrees of exposure to the environment, within a cyst or in an egg sac or mass. The difference is significant in that those eggs in an egg sac are reported to hatch as soon as soil moisture and temperature conditions are suitable, even in the absence of a host plant (Greco, 1985); whereas, those eggs within a cyst are usually delayed in hatching.

Both Winslow (1955) and Greco (1981) state that root exudates from carrot are a necessity to stimulate substantial hatch from eggs within cysts. Aubert (1985) reported that "normal" rates of hatch were obtained by using the root exudates of D. carota (both wild and cultivated), T. arvensis and T. japonica. A root diffusate from Chaerophyllum

temulum," a non-host, non-trap crop" , however, produced the highest recorded hatch of juveniles. The temperature range for egg hatch is reported to extend from 5 to 25 C. Hatch occurs most readily from 15 to 20 C with little occurring at either 5 or 25 C (Greco et al., 1982). The hatching response of eggs within cysts is influenced by the age of the carrot from which the root leachates are collected. The greatest juvenile emergence is stimulated by root leachates collected from carrots 5 to 7 weeks old (Winslow, 1955 and Greco et al., 1982). Practically no emergence occurs from cysts less than two months old (Greco, 1981).

As with all Heterodera spp., the first moult takes place in the egg. Second-stage juveniles hatch and emerge from both eggs and from the cyst. Second-stage juveniles migrate to carrot roots and invade root tissue at temperatures from 5 to 30 C. No H. carotae development occurs within the root below 10 C (Greco, 1985). According to Mathews (1975), root invasion takes place in 36 hours at 18 to 20 C, with development of the egg sac starting about 4 weeks later. Greco (1985) states that "at 20 C a large number of second-stage juveniles invaded roots of the carrot two days after inoculation, and that third-stage juveniles, fourth-stage females and cysts developed after 12, 21, 26 and 36 days from the inoculation, respectively; when 120, 210, 260 and 360 days degrees had accumulated above the developmental basal temperature". In the same publication, Greco states that fourth-stage and adult males are found at 16 and 21 days post-

inoculation, respectively. Females are reported to begin extruding egg sacs with eggs soon after reaching the adult stage, about 31 days post inoculation. The egg sacs continue to enlarge and fill with eggs for 2 weeks, until on day 45 post-inoculation they are full. Newly produced eggs undergo embryonic development almost immediately. Second-stage juveniles were observed in egg masses and cysts at 51 and 57 days post-inoculation respectively (Greco, 1985). Similar results have been reported at 18-20 C (Ambrogioni, 1971). Other authors (Jones, 1950; Oostenbrink, 1955; Sturhan, 1960; Ambrogioni, 1969, 1971; Matthews, 1975) have published developmental information on H. carotae.

Subsequent generations within the same growing season are presumed to be the result of egg hatch from within egg masses, with 2nd stage juveniles emerging soon after embryonic development and molting are completed. The subsequent pattern of the life cycle is then dependent upon the availability of the carrot host crop, suitable sites within the root system and the soil environment. Thus the number of generations and the synchronization of the development of the nematode with the crop is highly variable within the known range of H. carotae.

The number of generations per year varies from 1-2 in England (Jones, 1950) for a single crop of carrots, to 1-4 in Switzerland (Vallotton, 1980, 1983) if two crops of carrots are sown on the same land in the same year. Temperature of the soil is a major factor. In southern Italy, when carrots

are sown in the warm summer months, H. carotae fails to invade the root systems, even when the crop is irrigated (Greco, 1985).

Host Parasite Relationship

No specific above ground symptoms are associated with carrot plants infested with H. carotae, and shoot system symptoms are typical of those resulting from any root infection or damage (Mathews, 1975). Infection of the root system, however, is uniquely attributable to the carrot cyst nematode. Plants often appear to be growing poorly in patchy distribution throughout the field. Poor stands can also occur. Leaves may be yellowish-red with older leaves becoming necrotic. Below ground the root system may show necrotic symptoms of roots, a substantial increase in rootlets leading to a bearded appearance, an early lignification of the tap root as well as deformities and distortions of the tap root. The growing point of the tap root may be "blinded" resulting in a digitate appearance of the root (Mathews, 1975). Lamberti (1971) also reported a shortened tap root in which the growing point was not blinded, but in which storage organ formation was not allowed to continue down the length of the tap root to anywhere near the normal extent. The result was an extremely short and unmarketable carrot.

As second-stage juveniles penetrate root tissue they move parallel with the longitudinal axis of the root and become sedentary within a few millimeters of the infection court.

The cephalic region of the nematode becomes embedded in pericycle cells. As the juvenile moves into this position, it pierces several cells, moving through the cell walls. These cells become necrotic. From this point on the reactions of the nematode and the plant are typical of most Heterodera spp. host parasite relationships.

A syncytium forms about the cephalic region of H. carotae as it feeds. Up to ten cells may become involved in the syncytium. The enlargement of the syncytium exerts pressure on the vascular elements within a rootlet. When exhausted, a syncytium may become necrotic, and several adjacent necrotic areas may join to kill a root. Several juveniles invading a single rootlet may result in the death of the root. Plant response to the death of rootlets is the formation of additional new rootlets. These may also be invaded in turn, with a resulting "bearded" appearance to the root resulting from a proliferation of rootlets.

As the female nematode body swells in preparation for egg production, the posterior region of the body pushes through the parenchyma and epidermal layers of the root and emerges into the rhizosphere. The cephalic region of the developing female remains embedded in the root, feeding on the syncytium. Once the female body is fully enlarged, the formation of the external egg mass may begin.

Community Ecology

The objective of community ecology is to explain the variety and abundance of organisms at any place and time (Roughgarden, 1986). Though locations are never identical to each other in all particulars, comparisons of their similarities and differences can lead to an understanding of the structures and the forces that form them.

A community can be taken to be all of the organisms in a given area, but this definition is so inclusive that it exceeds the bounds of most scientific inquiries. Various definitions have been proposed (Bowers and Brown 1982, Abele et al., 1984 etc.). No universally accepted unambiguous definition has been broadly accepted. The distinction between community and guild (a small set of species) is frequently unclear. Thus the individual often tailors the definition of community to fit a particular situation. It can in general be seen that these individual species must be living close enough together for the potential interaction to occur. A large number of potential interactions among species can occur, including: mimicry, symbiosis, resource partitioning, plant secondary compounds, pollination and fruit dispersal (Roughgarden and Diamond, 1986). Species abundances are codetermined by competition, predation, herbivory, disease, parasitism, mutualism and weather (Diamond and Case, 1986). Other forces may structure communities and adaptive indirect effects (Wilson, 1986) are one group of these.

Many properties of communities have been presented as evidence of structure in communities. These include: species abundance relations, correlations between body size and abundance, food web patterns, distributions of species in ecomorphological space, body size differences, relations between **a**, **b** and **y** diversity, and geographical trends such as latitunal gradients in life history traits and species diversity (Roughgarden and Diamond, 1986).

Many forces can impact community structure. Perturbations of the environment must rank high on the list of potential factors for change of a community. Introductions, extinctions, exterminations and invasions can also effect the structure of a community. Such events have been common with the current destruction of American elms and the near elimination of the American chestnut 50 years ago (Diamond and Case,1986).

The early development of community ecology was stimulated in large part by the suggestion that morphological and geographical patterns among closely related species of terrestrial vertebrates were related to ecological mechanisms of coexistence or competitive exclusion (i.e. Lack, 1947; MacArthur, 1958; among others). The majority of these studies supported the thesis that interspecific competition plays a major, but not necessarily exclusive, role in determining the organization of guilds of closely related, ecologically similar species (i.e. MacArthur, 1972). Since then, many authors have described a variety of phenomena which they have

been attributed to competition (Gilpin and Diamond, 1984). They also recognized that past evolutionary adjustments to competition may minimize present ecological manifestations (this has been referred to by detractors as the "ghost of competition past".) Tests for competition have frequently been tests of whether or not specific patterns consistent with competition theory are present. These patterns may have alternative explanations. Many authors have called for more direct tests of competition (Connell, 1975; Colwell and Fuentes, 1975). Authors criticizing the application of competition theory have largely gathered about the "null hypothesis" as an alternative. Proponents of this view see most of the evidence of competition as incorrect analysis of the data or as data collection which was biased to support the competition theory. They hold that if appropriate null hypotheses of no competition were formed and appropriately tested that the data would support these null hypotheses.

This argument has consumed the efforts of proponents of each view. The controversy hopefully peaked in March of 1981 when a symposium on community ecology was held at Wakulla Springs Fl. Leading proponents on each side lined up to prove how reasonable they were and how unreasonable the opposing side was. The procedures of this symposium are published as "Ecological Communities" by Strong, Simberloff, Abele and Thistle (1984). The argument has been of value in that it stimulated the development of precise hypothesis testing in an

area where certain assumptions regarding competition as a force shaping communities may have become too common.

Nematode Community Ecology

Particular challenges are evident in the study of the community ecology of nematodes. Chief among these is the small size and resultant large number of individuals per unit volume of soil. Second and maybe even more important is the uncertain taxonomy (Triffit, 1935; Ferris & Ferris, 1989). Though solutions to these problems may be available (Ferris & Ferris, 1989) they are still important considerations in the study of nematode communities and the understanding of the literature.

Soil ecosystems are uniquely effected by plants. The level of microbial activity is many times greater within a few millimeters of living roots than in bulk soil. Many soil organisms profit most by living under a healthy fast-growing plant (Coleman, et al., 1983). Included in this group are many diverse taxa of nematodes. The impact of any pathogen (including plant parasitic nematodes) on the plant and thus on the nutrient composition of the rhizosphere soil, effects the community composition of this species rich part of the soil ecosystem.

Nematodes as a group have characteristics which enable them to successfully establish and maintain populations in a wide variety of soil ecosystems, and under a diversity of environments which might restrict the presence of other types

of invertebrates. Among these characteristics are: anhydrobiotic and cryptobiotic adaptations, high intrinsic rates of reproduction and parthenogenetic reproduction.

Community ecology in nematology has fallen into two basic forms. The first of these can be widely described as agricultural crop community ecology. The second can be described as soil community microecology.

Agricultural crop community ecology has involved the study of plant parasitic nematodes. These have been studied to establish the nature of the plant/parasite relationship. Most studies have involved the only the relationship between a single nematode species and a single host plant. These studies are not community studies. Studies involving two or more species of nematodes and their effects upon a crop plant are probably the simplest type of community studies. These studies usually concentrate on the crop yield component of the system. Measurements of the populations of the nematode species are usually secondary. Similarly many studies involving both a nematode species and another plant pathogen have been conducted. These along with nematode as plant pathogen vector studies can be taken as community studies. Few of these studies examine the entire community of nematodes in the soil ecosystem. This is not unusual in community ecology studies. Reviews of these studies are not conducted from a community ecology perspective. These studies have contributed methodology, taxonomy, biology nematode/nematode

and nematode/plant interactions (Freckman, 1982). Exceptions to this pattern are notable (Ferris & Ferris, 1974).

Community microecology related to soil nematodes has been a much more community based science and much less aimed at measurement of plant productivity. The objective of measurement in many of these studies is energy flow and nutrient cycling. Typically these studies lump groups of apparent species and even genera together in guilds which are the components of the communities (Ferris & Ferris, 1989). Recent overviews of this area have been conducted (Procter, 1990, 1984). As reported there these studies are largely aimed at outlining the trends in species composition, densities and ecological roles in different soil ecosystems. The emphasis here can include adaptability, competition, diversity, ecological and competitive release, ecosystem role and the role of nonbiotic stress. The roles of the limiting factors mentioned at the beginning of this section are evident.

The two approaches to nematode community ecology have remained apart from each other in large part. The scientists involved in each type of research have by and large seemed unaware of each other's work. A symposium was held in Tucson Arizona in 1980 (in conjunction with the annual meeting of the American Institute of Biological Sciences) in order to change this situation. The papers presented at this symposium were collected and published (Freckman, 1982). This volume provides an overview of the state of nematode community

ecology at that time, but most of the emphasis is given to the role of nematodes as decomposer organisms.

Changes in the literature during the intervening decade have not been dramatic. The two types of studies remain largely discrete. The major change has been an increase in the breadth of the types of communities included in the plant parasitic nematode communities. The role of alternate plant types (either weeds or crops) over time in the shaping of a nematode community are increasing over time (i.e. Georgi, 1988).

Population Dynamics

The population of H. carotae in a field and the dynamics of the population are affected by temperature, crop and initial population density (P_i) of the nematode. In Italy (Greco and Brandinisio, 1980), it was found that a P_i of 128 eggs/cc of soil led to 100% of plants exhibiting root-system symptoms within six weeks, but that if the P_i was 8 eggs/cc soil only 30% of the plants showed symptoms at harvest. Below this P_i, no symptoms were detected. Estimates of the tolerance limits (term not defined by authors) of a carrot crop to P_i of H. carotae have been made. In Switzerland (Vallotton, 1980) the estimate is 40 cysts/250 cc soil. In Italy the estimates range from 0.19 eggs/cc soil (Ambrogioni and Marinari-Palmisano, 1976) to 0.8 eggs/cc soil (Greco and Brandonisio, 1980).

The time of planting and the number of crops of carrots planted in a field within a single season can substantially affect the population. Late planted carrot crops may effectively serve as trap crops, reducing the field population if insufficient time and falling temperatures prevent the completion of the life cycle before the crop is harvested (Greco, 1985). Reproduction rates of 10-fold in microplots (Greco and Brandonisio, 1980) and 72-fold in pots (Stelter, 1969) have been obtained. In the field estimated population growth of 10-fold has been recorded (Vallotton, 1980). At low population levels, rates of juvenile increase from 2 to 7-fold are reported with the greater rate occurring in lighter soil (Bossis, 1985). An estimated population equilibrium (term not defined by authors) of 50 eggs/cc soil was determined in Italy (Greco and Brandonisio, 1980). In France, an equilibrium of 50-70 juveniles/gram of soil was found, and explained as a consequence of competition for appropriate sites in the root system (Bossis, 1985).

In a late harvested carrot crop, the egg population was found to be 63% cyst eggs and 37% egg mass eggs (Greco, 1985). The loss of egg mass eggs was found to be faster than for cyst eggs. The loss of each over an 8 month period was 78% and 40% respectively.

The population of H. carotae in soil declines with time and the growing of non-host crops. Decreases of 33% (Bossis, 1985)and 49% (Ambrogioni and Marinari-Palmisano, 1976) have been reported after a single season of a non-host crop.

Hatching studies have revealed a conservative reproduction strategy utilizing the eggs in the egg mass and the eggs within the cyst differentially in order to maximize within year reproduction, while balancing this against potential failures of reproduction in the current year by banking a portion of eggs for later within the year or for subsequent years. Aubert (1985) reported that in tap water second-stage juveniles (j2s) emerged promptly from egg masses reaching a maximum cumulative hatch of 6 j2s/egg mass in 20 days. When exposed to carrot root diffusate, egg masses produced a cumulative hatch of 8.4 j2s in 20 days. No further hatch was reported for 20 days, but then hatch recommenced to reach a maximum cumulative of 40 j2s at 60 days. When exposed to tap water j2s virtually failed to emerge from cysts. When exposed to carrot root diffusate j2s did not emerge from cysts for the first 55 days, followed by a period of rapid hatch over 3 days, followed by a slow rate of hatch to reach a mean cumulative rate of hatch of 15 j2s/cyst at 82 days. In these experiments the mean content of egg masses (390 eggs) was almost twice that of the parental cysts (210 eggs) but the % emergence of j2s from egg masses in water and carrot root diffusate (1.6% and 10.6% respectively) are still higher than the % emergence of j2s from cysts, 0.1% and 7.1% in water and carrot root diffusate respectively. Similar delays in the emergence of j2s from cysts are reported (Greco, 1981), with practically no hatch occurring when cysts less than two months old were used.

Second-stage juveniles emerged from cysts collected at different times of the year, but the fewest j2s emerged from cysts collected during fall or winter (Greco, 1985). This suggests a temperature related overwintering mechanism. Hatching of j2s from egg masses was suppressed by high summer temperatures, but began again when the temperature dropped in the fall (Greco, 1981).

The association of H. carotae with other species of nematodes has only been reported once (Lamberti, 1971). In this instance the co-occurrence of H. carotae was with Meloidogyne incognita (Koifoid and White, 1919) Chitwood, 1949. It was reported as common in carrot production fields of southern Italy.

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SUMMARY

Heterodera carotae is a small lemon shaped cyst that limits carrot production in much of Europe. Until recently it had not been reported in North America. *Daucus carota*, wild or cultivated carrot, is the only known host plant for this species in Michigan. *Heterodera carotae* is found in Italy in association with *Meloidogyne incognita*, among other nematode species. *Heterodera carotae* can be a severe problem in carrot growing, and tends to be a cool season problem in Italy.

SECTION TWO

Detection and Description

of Nematode Distributions

INTRODUCTION

Nematodes tend to be highly aggregated organisms. The scalar difference between humans and nematodes makes this problem worse. When several thousand individual nematodes can live a full life cycle on 1/2 of a plant's root system in a month, and man's concern is a 200 acre field with 3 million plants, there are problems of detecting and describing distributions.

The efforts reported here include attempts to; describe the distribution of Heterodera carotae in Michigan, after extensive sampling, an explanation of the sampling method used in this survey, and its relationship to the distribution of the nematode, and a small scale in field description of the vertical distribution of H. carotae.

DISTRIBUTION OF *HETERODERA CAROTAE* AND *MELOIDOGYNE HAPLA* IN MICHIGAN CARROT PRODUCTION

Abstract

Selected farms in all major carrot growing counties of Michigan were surveyed to determine the extent of infestation by *Heterodera carotae* and *Meloidogyne hapla*. Both species were found in all counties surveyed, but not on all farms.

Introduction

Heterodera carotae, the carrot cyst nematode (Jones, 1950) was first detected in Michigan in 1979 during a survey of organic soil carrot-onion rotations. This find was first reported in 1985 (Graney, 1985). To date, Michigan remains the only area to report *H. carotae* in North or South America.

Meloidogyne hapla, the northern root-knot nematode (Chitwood, 1949) has long been recognized as an economic pest of carrot production (Slinger, 1976; Townshend, J.L. and T.R. Davidson, 1962; Vrain, 1982). The presence of *M. hapla* in Michigan has been well documented (Brody, 1972). The discovery of a second important nematode pest in Michigan carrot production led to this survey to determine

the extent of the range of these two species within the carrot production regions of Michigan (Mathews, 1975).

Materials and Methods

Surveys were conducted during the growing season. All sampling was done late in the season, within two weeks of harvest. Fields to be sampled were identified through Cooperative Extension Service field staff and direct grower contacts. In all cases, an effort was made to sample only fields identified by the grower as having a nematode problem impacting carrot production. This bias was introduced to limit the number of fields sampled in which no plant-parasitic nematodes would be found. Preference was also given to fields planted to carrots in the year of the survey. A brief field history was taken, including: cropping history, past problems, results of previous nematode samples and current year's nematicide applications. All of these factors were used in field selection.

Once selected for sampling, each field was divided into subsections (sites) of eight hectares or less. A modified timed sampling method, weighted to damaged carrots, was used to determine the intensity of sampling in each field. Fifteen minutes was allocated to each site. As the sampler moved into the site the time began. Randomly moving through the site, each carrot encountered that exhibited root system symptoms of nematode infection was placed in a plastic bag, complete with its rhizosphere soil. A site - sample -

sampler identification number was then written on the bag. A carrot plant was judged to be a candidate for nematode infection sampling purposes if: the shoot system was shorter than the surrounding plants, or if the tops were discolored (red or yellow), or if the stand was thinner than the surrounding areas. Only carrots showing root system deformation were retained as potentially nematode infected carrot samples. Root system deformation included: forked roots, stubby roots or excessive lateral root formation (Lamberti, 1971). The time factor entered into the sampling process when no carrots exhibiting shoot or root system symptoms were encountered. This decision was made every five minutes, for the fifteen minute sampling period. At the end of any five minute period when no damaged carrots were found then a sample was taken randomly. Thus, the minimum number of samples per site is set at three by this default function.

All samples were stored at 10 C until processed. All samples were processed by modified sugar flotation - centrifugation and the extracted nematodes counted under a light microscope at 40x. Additionally each carrot root system was examined for the presence of root-knot and cyst nematodes and rated for root deformation. After processing, the remaining soil was used in a bioassay by planting a single carrot (cv. Chancellor) in a one liter pot containing the remaining soil. These pots were placed in the greenhouse an the carrots allowed to grow for four months.

At the end of this time, the carrot was removed from each pot and visually evaluated for the presence of M. hapla and H. carotae. The results presented are the combined data from the sugar flotation-centrifugation and bio-assay procedures.

Results

During the two years of the survey, 592 samples were taken from 514 hectares in 43 fields belonging to 11 carrot growers in 8 counties in MI (Fig. 1, Table 1). These samples represented 15% of the acreage planted to carrots in Michigan in the period as well as 13% of the growers.

At least one field in each county sampled was found to have both Heterodera carotae and Meloidogyne hapla. At least one field belonging to each grower was found to be infested with M. hapla. There was only one farm where H. carotae was not detected. With the exception of one farm, H. carotae was always detected concomitantly with M. hapla. H. carotae was recovered from 29.7% of the samples and 67.4% of the fields (Table 1). M. hapla was detected in 24.8% of the samples and 69.7% of the fields (Table 1). Meloidogyne hapla and H. carotae were not recovered from 57.7% of the fields and 18.6% of the samples.

Discussion

H. carotae is widely distributed throughout the carrot growing regions of MI. The survey confirmed that all

regions had fields infested with M. hapla. 82.7% of all fields inhabited by H. carotae were also inhabited by M. hapla. All of the fields that were exceptions to this trend were on a single farm where field corn had been the exclusive crop grown for three or more years. This rotation was unique among the fields sampled. 80.0% of all M. hapla infested fields were also found to be infested with H. carotae. These fields were distributed among growers and regions. Of the fields sampled, most were organic (muck) soil. A limited number of fields with mineral (sandy) soil were sampled. None of these fields were found to be infested with H. carotae. These mineral soil sites, however, had a greater level of infestation by the M. hapla.

Conclusion

In conclusion, the degree of co-occurrence of Heterodera carotae and M. hapla within the carrot growing regions of Michigan is very high. The widespread geographic occurrence of H. carotae suggests that either this species has been passively spread at a relatively rapid rate, or has been present in Michigan for an extended period of time. The lack of aggregation of H. carotae by region or grower suggests the latter.

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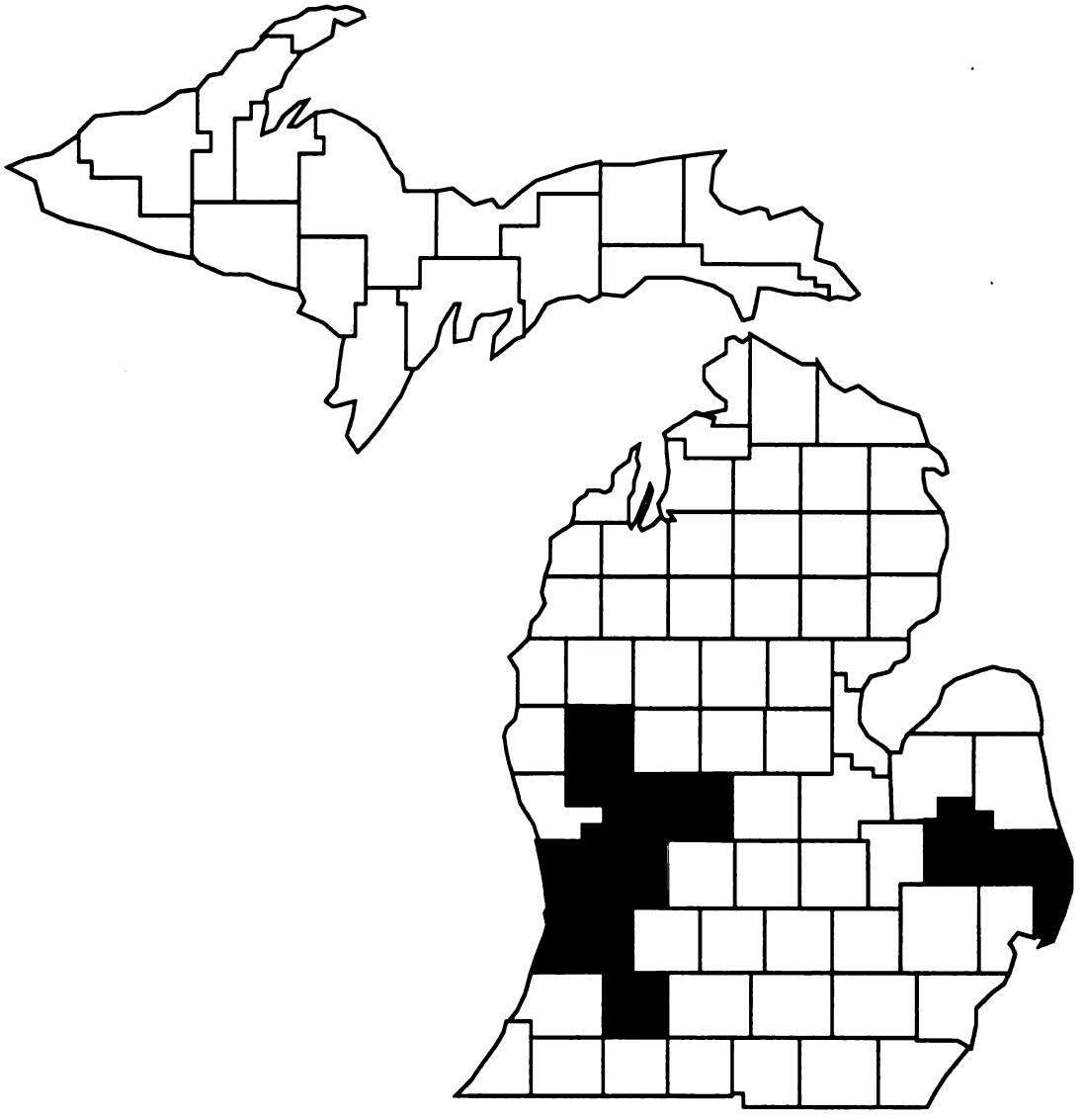

Figure 1. Michigan counties sampled (shaded) for Heterodera carotae and Meloidogyne hapla.

Table 1. Frequency of occurrence of *Heterodera carotae* and *Meloidogyne hapla* in the Michigan carrot nematode survey, 1986-1988.

Year	*Heterodera carotae* alone		*Meloidogyne hapla* alone		*H. carotae* and *M. hapla* both		No detection	
	Samples	Fields	Samples	Fields	Samples	Field	Samples	Fields
1986	42	0	71	5	47	18	168	3
1988	61	5	3	1	26	6	174	5
Total	103	5	74	6	73	24	342	8
%	17.4	11.6	12.5	14.0	12.3	55.8	57.8	18.6

SAMPLING AND VERTICAL DISTRIBUTION OF *HETERODERA CAROTAE* IN MICHIGAN

Abstract

Sampling strategies for soil inhabiting nematodes are difficult to optimize because of the high degree of aggregation and differences in the distribution pattern and population densities. *Heterodera carotae* (Jones, 1950; carrot cyst nematode) was used to investigate the problem of sampling; comparing three strategies in objective dependent and time/cost analyses. Four 10 acre replicated blocks and 1.5 acre sub-plots in a known infested site were used for the study. Sampling methods included: composite, timed and transect sampling. The analysis indicated that all methods were capable of detecting *H. carotae* at the levels present in the study site. Differences in interpretation of the site, sampling objective and time expenditure were the major differences among the methods.

Experiments were conducted for three years at a *H. carotae* research site near Sheridan, Montcalm Co. MI. to determine seasonal, annual and multi-year changes in the vertical distribution of *H. carotae*. Soil samples were taken at fifteen centimeter intervals from soil surface to a depth of ninety centimeters. Sampling was done in the spring and

fall. Soil was analyzed and all plant parasitic nematodes counted. Heterodera carotae was detected in all soil profiles where carrot roots were present. Seasonal variation included decreases in the number of cysts and number of occupied profiles over the winter and increases in the number of occupied profiles after the growing season. After two consecutive years of growing Daucus carota (domestic carrot), the relative population density of cysts stabilized from season to season.

Introduction

Problems associated with field sampling to estimate nematode population distributions are generally understood (Ferris et al., 1990). Numerous papers have been published on the design of sampling strategies and tactics for estimation of nematode population density and distribution (Bird & Warner, 1990; Boag et al., 1987; Ferris, 1984; McSorley & Dickson, 1991; McSorley & Parrado, 1982; Southwood, 1980). Most of these deal with intense sampling of relatively small areas (Boag et al., 1987; Ferris, 1984; McSorley & Dickson, 1991; McSorley & Parrado, 1982; Noe & Campbell, 1985; Wheeler et al., 1987). In practice, both survey and diagnostic service sampling efforts do not always deal with such plots or fields, or comparable intensities of sampling (Berney & Bird, 1992).

Heterodera carotae (Jones, 1950; carrot cyst nematode) is a sedentary endoparasitic nematode with a high degree of aggregation (Boag et al., 1987).

A survey was conducted to compare the relative efficacy of three different sampling methods in the detection and enumeration of H. carotae populations. Nematode samples are taken for a variety of reasons; including: detection (survey-regulatory activities) and population density enumeration (management decisions or research). The first objective of this research was to compare three sampling methods in their efficiency for detection of H. carotae. The second objective was to compare the relative estimates of population density obtained using the three sampling methods.

Heterodera carotae is found throughout The United Kingdom and Europe, where carrots are grown (Mathews, 1975). Michigan is the only known location for H. carotae in North America (Berney & Bird, 1992). Heterodera carotae has one primary host, Daucus carota the common wild or domestic carrot (Aubert, 1985). Eggs within cysts are largely unresponsive to any factors other than appropriate root exudates. Thus only passive movement disseminates second-stage juveniles, eggs or cysts, over any great distance.

Carrot cyst nematodes can cause a wide variety of symptoms in carrot plants including: leaf discoloration, stunting, tap root reduction, fine root proliferation, tap root deformation and plant death. Yield reduction can be as little as a minor loss in tap root quality to complete crop

failure (Lamberti, 1971; Greco, 1980). Since carrot tap roots frequently extend to 40 cm and more below the soil surface, and fine roots may extend to 90 cm, the vertical distribution portion of this study was designed to help identify those soil profiles containing H. carotae and to describe seasonal variation, if any.

Materials and Methods

Sampling Study The site chosen or this study was a 160 acre field of organic soil located near Sheridan, Michigan (Montcalm Co.). The east half of the field was planted to corn and the west half to carrots. The carrots were planted in north-south rows, with three beds of three rows per bed, in each planter pass. Four ten acre strips (330 ft. wide by 1320 ft. long) were surveyed in the west half of the field (Fig. 1). The site was known to be infested with H. carotae. Previous sampling had indicated two gradients of H. carotae population density. The first was a gradient of increasing density from west to east, and the second was a gradient of increasing density from south to north. The four strips (replicates), therefore, were parallel to one gradient and perpendicular to the other. The study was conducted one day before carrot harvest; when population densities are highest, and carrot plants are available to orient the sampling.

The four strips were sampled using each of three methods; referred to as composite, timed and transect, respectively.

In the composite sampling method, 15 sub-samples were taken at random along a zig-zag pattern following the length of the 10 acre strip (Fig. 1). Each sub-sample consisted of 300-500 cm3 of rhizosphere soil from the root system of a single carrot. A hand trowel with a 20 cm long blade was used to remove a slice of soil parallel to the carrot tap root to a depth of 20 cm. All 15 sub-samples were placed into a 5 gallon pail and thoroughly mixed. A composite sample of 500 cm3 was removed, and placed in a labeled plastic bag. Each composite sample represented one ten acre strip.

The timed method consisted of a modified timed sampling procedure developed by the authors for survey purposes (Berney & Bird, 1992). Fifteen minutes were allocated to each 10 acre strip. Each carrot encountered within that time exhibiting symptoms of potential nematode infection, was sampled and recorded separately. A carrot was considered to be damaged for sampling purposes if the top was discolored (red or yellow), if the stand was thin or if the plant was shorter than the surrounding plants. All plants which met one or more of these criteria were removed from the soil and the roots examined. If the roots showed deformities, including stubbiness, bifurcation, excessive lateral root formation, or the presence of root galls or cyst females: a rhizosphere soil sample was collected. If no damaged carrots were encountered in the first five minutes, a random sample was taken at that time. This default function applied to each of the three five minute time intervals. Thus, if no damaged plants were

encountered during the entire fifteen minute time of sampling, three samples were collected. With this minimum of three samples per replication, each sample represented 3.33 acres or less.

The transect sampling method consisted of visually drawing a line lengthwise down the center of the plot and removing one rhizosphere soil sample from the center row of each planter pass. This resulted in one sample being taken every twenty feet along a line thirteen hundred and twenty feet long. Sixty six samples were taken per replication. Each sample represented 0.15 acres.

The eastern most 1.5 acres (200' x 300')of each plot was sampled as a separate plot. These sites were sampled using the composite, timed and transect methods described above. In addition, ten samples were taken at random from each of the 1.5 acre plots. Each sample, therefore, was representative of 0.15 acres.

All samples were taken from the rhizosphere of growing carrot plants, and were individually labeled. All samples were processed using modified sugar flotation/centrifugation ("heavy" sugar) (Jenkins, 1964) and the resulting nematodes counted at 40x under a light microscope. Only the data for cysts of H. carotae are presented.

Vertical Distribution A field research site was maintained for three years at the site described above. The soil profiles to ninety centimeters were sampled before and after second third and fourth consecutive crops of D. carota,

domestic carrot, were grown at this location. Relative population densities were compared before and after carrots were grown at six depths. The carrots were planted in north-south rows, with three scatter chute rows 12 inches apart in each three row bed. Each sub-sampling site was one bed twenty feet long. Weekly/bi-weekly samples to twenty centimeters were taken for the duration of the study, and are reported elsewhere. Vertical distribution samples reported here were taken at fifteen centimeter intervals from the exposed sides of a ninety centimeter deep trench dug in a east-west direction across each sub-sampling site. There were six sub-sampling sites. Subsequent sampling trenches were dug in other portions of the sub-sampling site. Approximately 200 ml of soil were taken from the center of each fifteen centimeter sampling zone. These samples were marked and refrigerated until processing. Modified sugar flotation / centrifugation was used to extract the nematodes from the soil. All plant parasitic nematodes present were identified and counted.

Results

Sampling Methods Heterodera carotae cysts were detected in all of the 10 acre blocks, using the composite sampling method. Densities ranged from 25 to 109 cysts per 100 cm3 in replicates 4 and 2 respectively. A mean of 72.2 cysts per 100 cm3 of soil with a standard deviation of 35.8 was obtained using the composite method (Table 1). With the

timed sampling procedure, H. carotae cysts were not detected in all samples. No cysts were recovered from 2 of the 24 timed samples, and the population density estimates for the remaining samples ranged from 1 to 203 cysts per 100 cm3 of soil. The mean was 47.2 cysts per 100 cm3 of soil with a standard deviation of 48.8 (Table 1). No cysts were recovered from 12.6% of the transect samples. In the remaining 87.4% of the samples the densities ranged from 1 to 421 H. carotae cysts per 100 cm3 of soil (Table 1), with a mean of 56 cysts per 100 cm3 of soil and a a standard deviation of 48.8.

Sub-plot Analysis The results from the 1.5 acre samples included H. carotae cysts in all of the random samples taken, the densities ranged from 22 to 425 cysts per 100 cm3 of soil, with a mean of 127.2 cysts per 100 cm3 of soil and a standard deviation of 83.8 (Table 2). The timed sample taken in replicate 4 of the 1.5 acre samples included no cysts, the densities of the other timed samples ranged from 20 to 43 with an overall mean of 25.25 cysts per 100 cm3 of soil. Cysts were recovered from all of the transect samples taken in the 1.5 acre plots. The population density estimates ranged was from 7 to 421 cysts per 100 cm3 of soil with a mean of 91.7.

Pairwise comparison of the random and transect sample values from the 1.5 acre plots revealed that the random values in replicates two and three were significantly (P=.06) greater than the transect values. Pairwise comparison for all replicates revealed that the random values were significantly (P=.07) higher than the transect values.

The time required to achieve these estimates varies with method. The following sampling time estimates are for the 40 acres sampled in this study, and do not include the fixed time costs of transportation and site identification. They do, however, include the variable time costs of sample acquisition, processing and nematode counting: Composite sampling method (4 samples 289 cysts): 130 minutes, Timed sampling method (24 samples 1,133 cysts) 290 minutes, Transect sampling method: (264 samples 14,784 cysts) 2350 minutes (Table 3).

Vertical Distribution Figure 1 illustrates the pattern of change in the relative density of H. carotae cysts across all soil profiles over time, in sites where carrots were grown for the term of the experiment. This figure reveals that the number of cysts per volume of soil is relatively constant for the term of the experiment, beyond the initial high at the start of the experiment. This coincides with the end of the second consecutive year of carrot growth at the site. Figure 2 shows the relative proportion of the cyst population present at each depth during the term of this study. This figure shows that the percentage in the top two profiles shifts slightly toward the second level in the later part of the experiment. Figure 3 shows the relative population estimate for H. carotae in all profiles at each sampling time. These population estimates were derived from the soil samples, with an estimate of the number of eggs and second stage juveniles per cyst. These estimates were derived from a series of

samples taken in spring and fall. Replicated sampling from these soils revealed that fall collected cysts had an average of 32 total eggs and j2s. Spring collected cysts had on an average 65 combined eggs and j2s. (This was a major seasonal variation in in the H. carotae population.)

Conclusions and Discussion

Sampling Methods All three methods of sampling detected H. carotae cysts in these plots at the population densities present at that time. The differences between methods were minimal in terms of detection.

Greater time efficiency may be derived from the use of composite sampling methods, if the objectives of the sampling effort can be achieved using these methods. Certainly the objective of detection can be achieved in this way, but no information about the distribution or population density of the nematode species can be drawn from a single composite sample. (i.e., a single sub-sample of 420 cysts mixed into 14 other sub-samples each containing no cysts would result in an estimation of 28 cysts per 100 cm3 of soil for the entire sample.) A series of 4 composite samples as presented here provide only a very small amount of information on the distribution and population density of the target nematode species, that is, that cysts are present in all 4 of the replicates. Comparisons of repeated measure may be made between composite samples from the same area, over time, as the composite samples from different areas may be compared,

but the assumptions made in these comparisons must include: that the nematode densities presented are method relative, that the underlying distributions need not be in any way similar, and that these undetected differences may result in very different field observations than the sample information seems to indicate.

This level of information might be sufficient upon which to base control decisions, or the layout of research plots, if the uniformity of nematode distribution across the area was not a concern, or was available from other sources. This is possible only when other information is entered into the decision making process, i.e.; results from previous sampling, observations of plant growth in the field, etc.

The timed sampling method requires double the time input of the composite sampling method and actually samples less than half the number of individual root systems (6/replicate instead of 15). It provides, however, some information about the distribution of the target nematode species. The relative values for mean and standard deviation indicate a distribution approaching the negative binomial (where variance exceeds the mean), whereas, no interpretation is possible from the results of the composite method. This result is achieved by maintaining individual plant soil samples.

Taking all four replicates together, the decreasing north-south population gradient was confirmed. A trend for the decreasing west to east population gradient was also shown. The third and fourth replicates (south) each have one

sample which contain no cysts. This method is capable of detecting cysts but the greater cost (time) provided no advantage in answering the question of presence/absence. The distribution information provided may be useful, depending on the sampling objective.

The transect sampling method required approximately 2,400 minutes to collect, process and count the nematodes in the samples. This is five times that required for the timed sampling method and ten times that required for the composite sampling method. Considerable information on the distribution of H. carotae in this field can be derived from this sampling method. Both gradients were confirmed using the transect sampling method. With 12.6% of the transect samples containing no cysts, a dispersion constant k can be determined for all the transect samples (Southwood, 1980):

$$\log (N / no) = k \log (1 + x / k)$$

where N= total number of samples, no = the number of samples where no cysts are found, x = the mean value for all samples and k is the value solved for on an iterative basis. The k value for the transect samples was 0.42. Most commonly in ecological studies the variance will be found to be larger than the mean, indicating that the distribution is contagious. Many contagious populations are best described by a negative binomial distribution. Generally values of k are in the region of 2; as they become larger, the distribution approaches and is eventually (at infinity), identical with the Poisson. Fractional values of k indicate a distribution

tending toward the logarithmic series, which occurs when k is zero. The value k is not a population constant, but often increases with the mean (Southwood, 1980).

Common k values for H. carotae were not identified. The k value of the transect samples from replicate four was 0.249, much less than the 0.42 cited for the sum of all four replicates. Previous estimations of k have ranged from 0.04 for survey samples taken in 1986 through a value approaching infinity for 84 samples taken from a single farm in 1988. This lack of a common k makes the calculation of an optimal sampling density for this species impossible. Attempts to use a nearest neighbor method to establish the area within which each cluster of individuals is randomly distributed were unsuccessful even in those cases where k estimates most closely approached 2. Estimates of k do serve to describe the distribution of a species in a particular field, and measurement of changes in k over time may be as significant as the changes in the size of each cluster of individuals. Thus in studies of the population dynamics of nematode species in response to biotic and abiotic factors, sampling methods like the transect method described here, which provide sufficient data to allow estimates of k, may be necessary to meet the objectives of a particular nematode sampling effort.

The objective of each sampling effort must be clearly described before a sampling method is selected. Composite sampling methods are the most time efficient, if the objective of the study is to determine presence or absence of a specific

species. But no further information may be derived from a single sample obtained using composite sampling. If the sampling objective is to obtain population distribution information, a series of samples is needed. The usefulness of this information is enhanced by knowing the spatial relationship of each sample to the others. In this case even a series of composite samples may provide less data than a series of discrete samples. A field scale estimate of absolute population density probably cannot be made with any degree of reliability, given the limitations of sampling at field scale. In sampling containers or very small plots, these differences probably approach the insignificant. Similarly as distributions approach uniform, in presence even if not in density, the same is probably true.

Vertical Distribution The H. carotae population as measured appears to be relatively stable on a seasonal basis beyond the second consecutive year of carrot growth. The apparent seasonal variation in the egg/j2 content of cysts may be a function of the increased destruction of the smaller size class cysts over the winter. There appears to be an increase in the percent of samples from the lower profiles that contain carrot cyst nematodes over time. The proportion of the total population resident in the second (30 cm) profile increased over time. It was observed that all samples which contained carrot roots contained individuals of H. carotae. Thus the spread of carrot cyst nematode vertically through the soil profiles may be directly linked to active movement of second

stage juveniles toward and along growing carrot roots. This assumption is supported by the observation that the samples which contain no individuals of H. carotae appear not to contain carrot roots either. When considering studies of this type it is important to keep in mind that only relative population estimates are being used here and that the minimum detection level with the sampling methods used may be relatively high. In the case of this study we have determined that an absolute population density of seven cysts per one hundred cubic centimeters of soil is necessary to achieve a ninety five percent probability of detecting at least one cyst.

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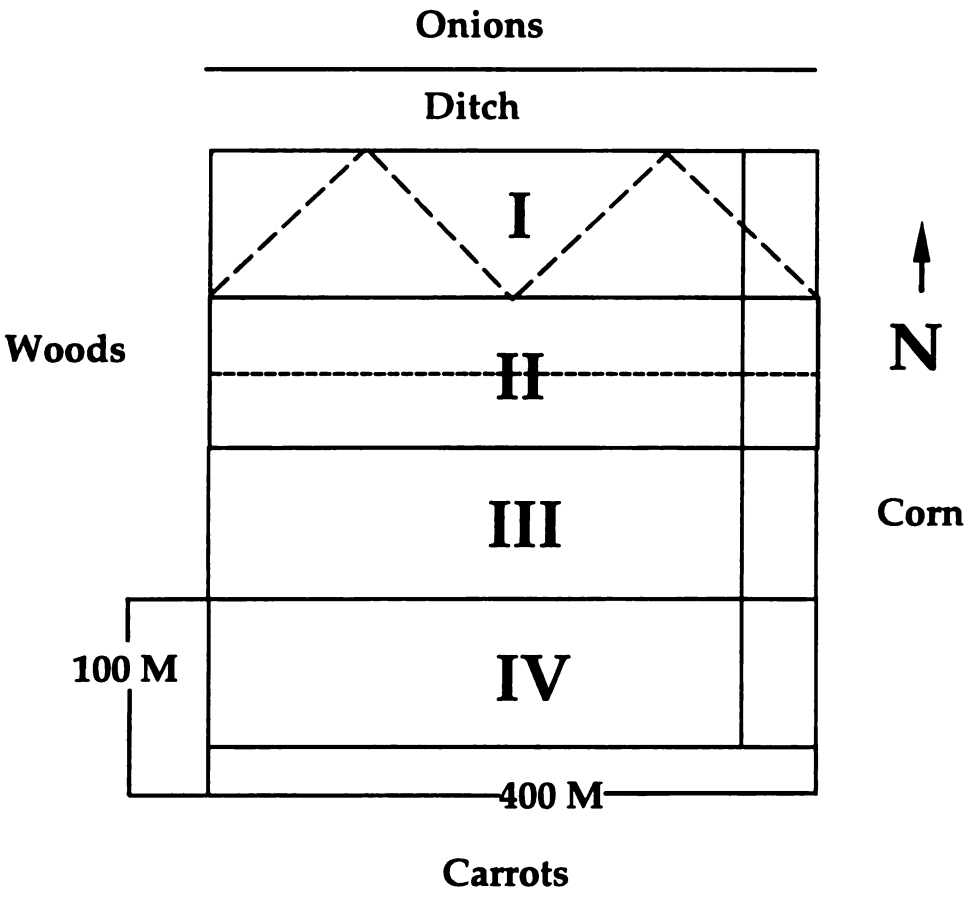

Figure 1. View of sampling methods, superimposed over map of study site.

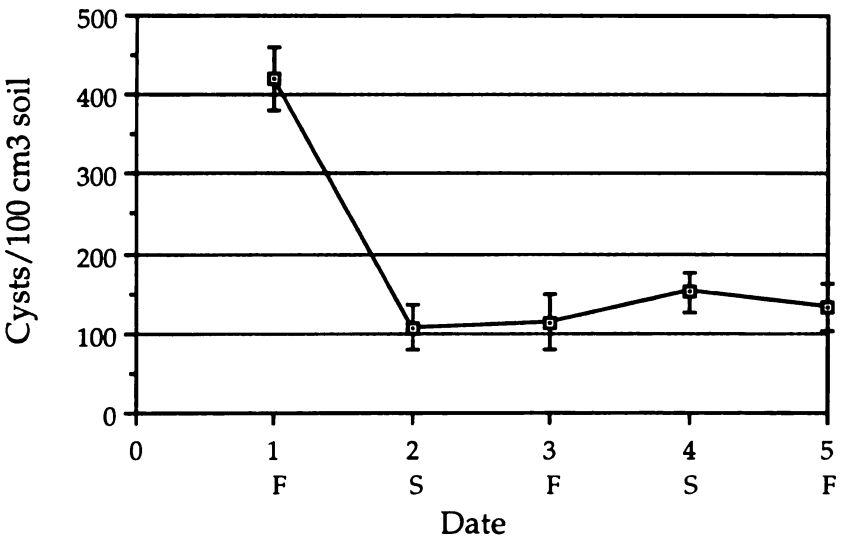

Figure 2. Heterodera carotae population density in cysts on five dates.

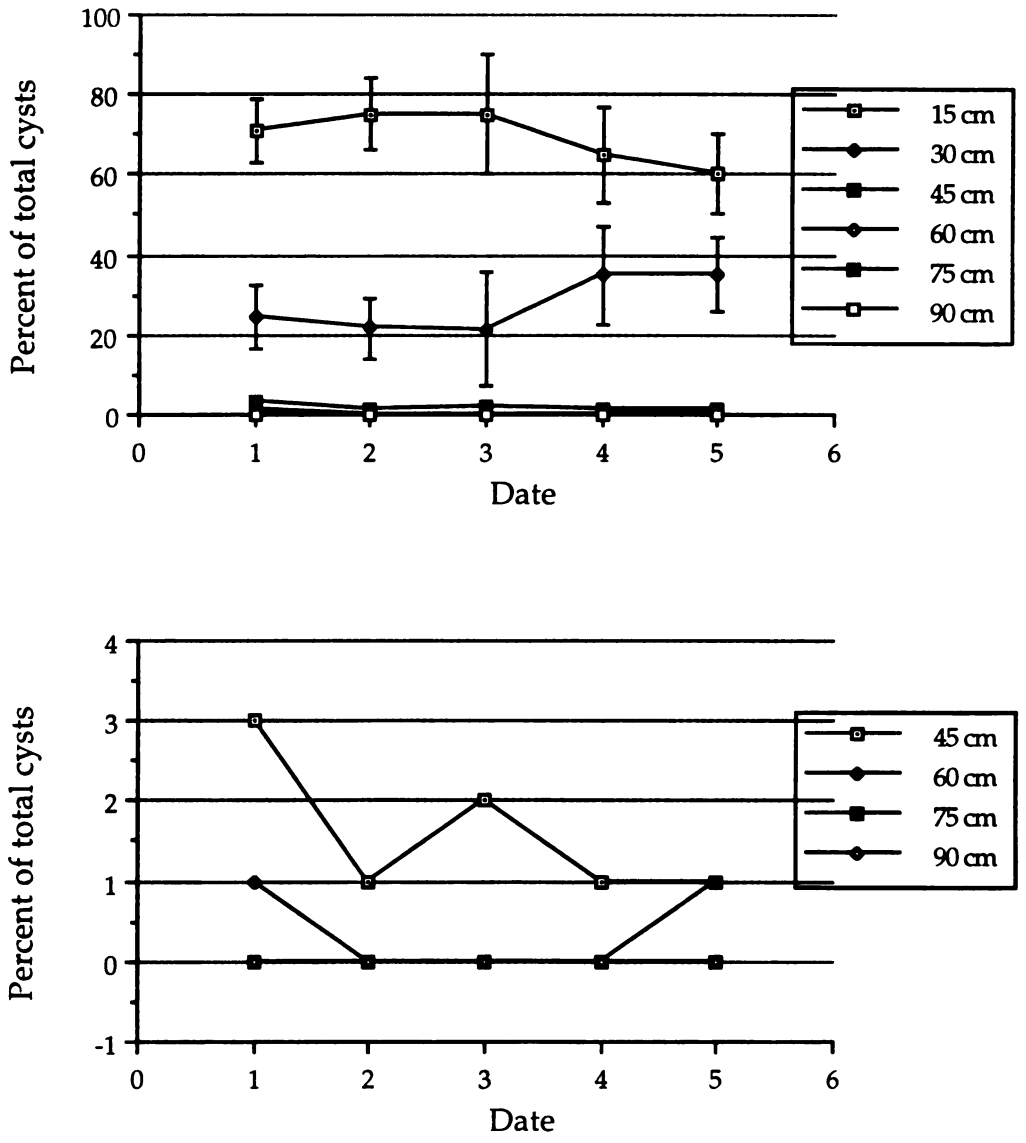

Figure 3. Heterodera carotae cyst distribution by depth as a percentage of total on five dates.

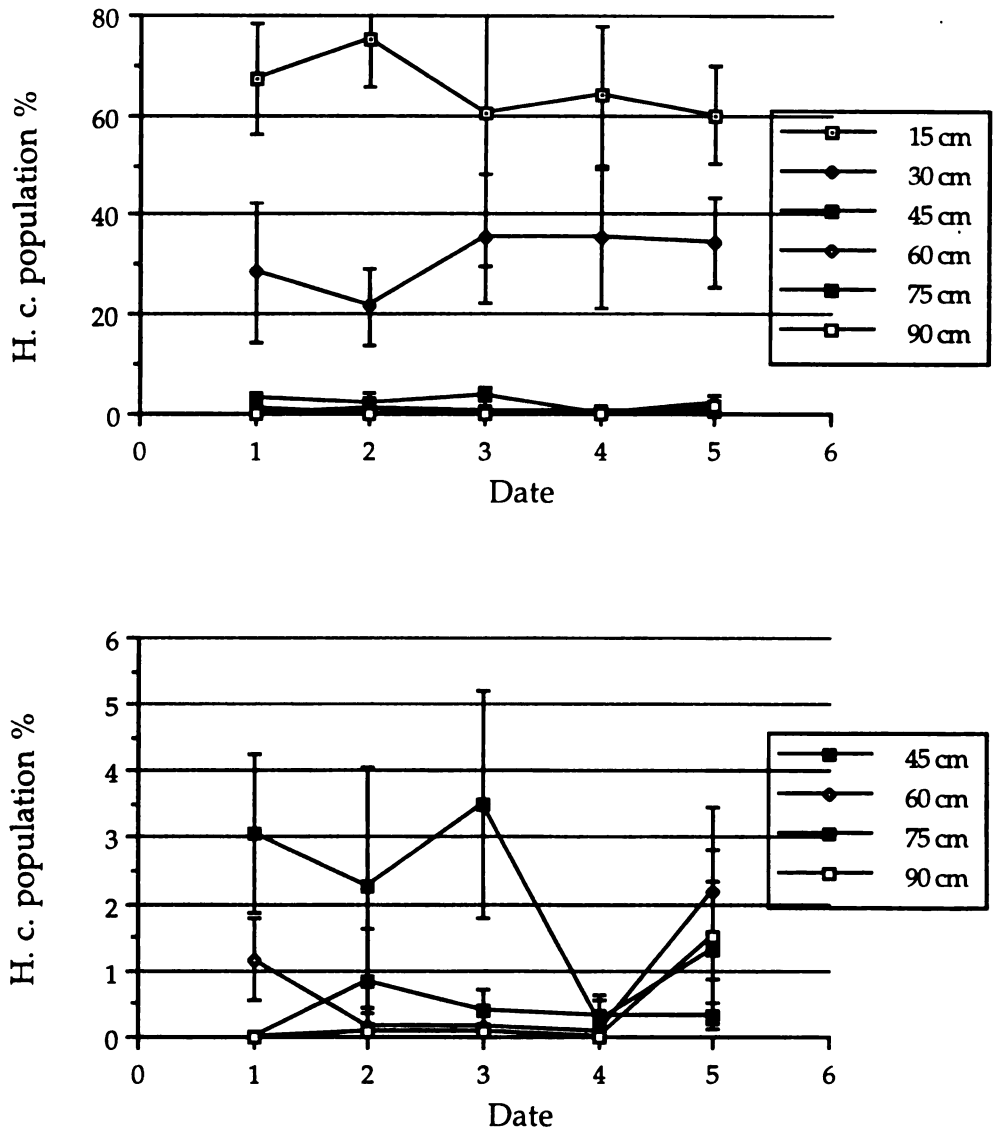

Figure 4. Heterodera carotae population percentage by depth on five dates.

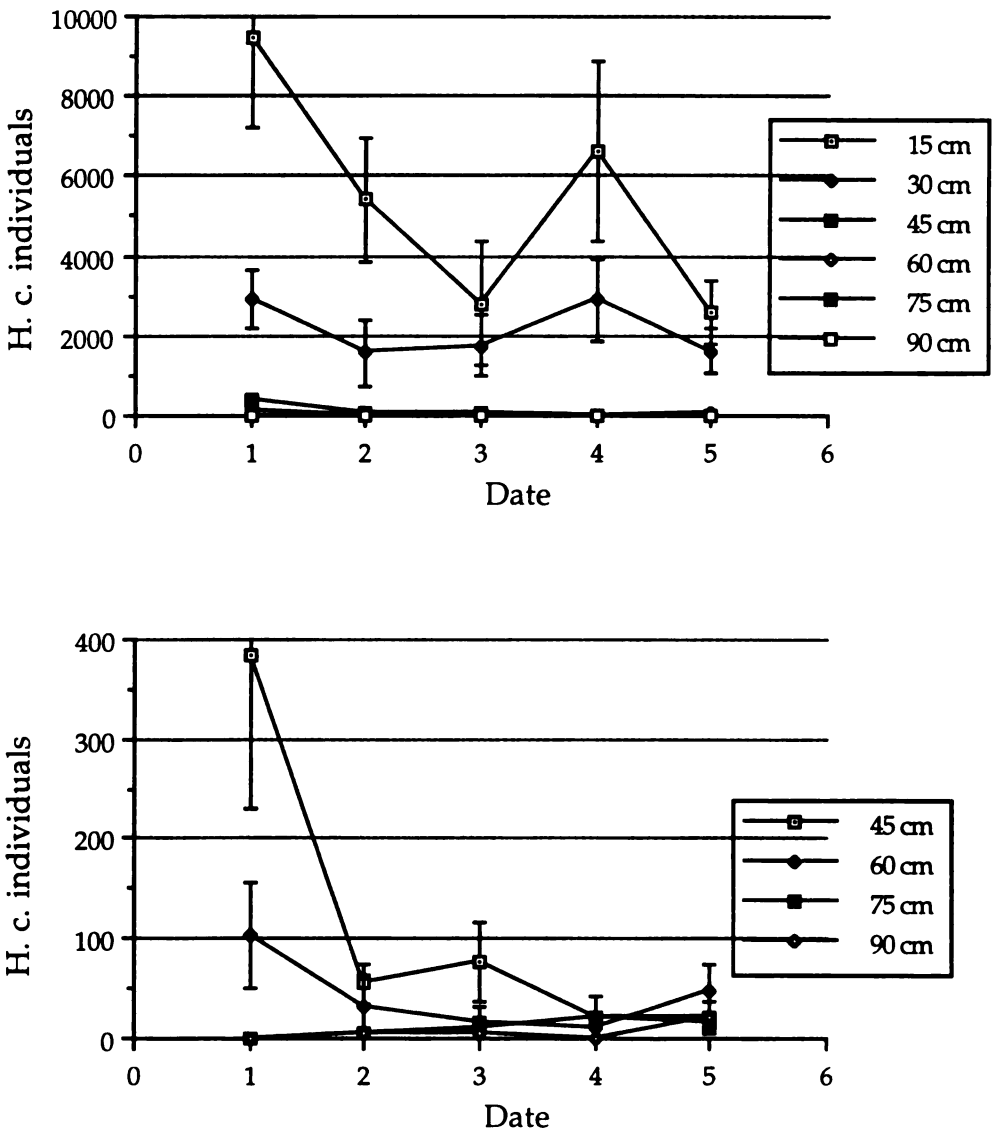

Figure 5. Heterodera carotae population distribution of individuals by depth on five dates.

Table 1. Cysts per 100 cm3 of soil for each sampling method, by replicate for the 10 acre sampling plots. (1)

Rep.	Sampling method		
	Composite (N=1)	Timed means (N=6)	Transect means (N=66)
1	67	36 (32)	92.3 (93.6)
2	109	81.1 (75.5)	63.0 (60.25)
3	88	45.5 (37.45)	48.6 (57.26)
4	25	31.1 (28.8)	30.1 (48.40)
Mean	72.2 (35.8)	47.2 (48.8)	56.0 (68.8)

(1) values in parantheses are standard deviations for preceeding means

Table 2. Cysts per 100 cm3 of soil by sampling method and replicate for the 1.5 acre sampling plots (1).

Rep.	Method			
	Timed (N=1)	Transect (N=1)	Random (N=10)	Composite (N=1) (2)
1	43	145.1 (108.83)	164.1 (97.39)	67
2	38	100.0 (38.92)	145.7 (61.29)	109
3	20	72.4 (38.19)	132.2 (83.16)	88
4	0	49.4 (42.05)	52.0 (20.64)	25
mean	22.25 (19.51)	91.7 (78.4)	127.2 (83.8)	72.2 (35.8)

(1) values in parentheses below means are standard deviations

(2) Extension values are from entire 10 ac. of each rep.

Table 3. Results of time analyses by method.

Method	ttl min/4 reps	min/ sampl	min/ cyst	% postive samples	min/pos. sample
Extension	130	32.5	.449	100	32.5
Timed	290	12.0	.255	91.6	13.18
Transect	2350	8.9	.158	87.4	10.17

SUMMARY

Heterodera carotae was detected in every carrot growing region in Michigan. It was almost always accompanied in the field by Meloidogyne hapla. Nematode soil sampling is an objective dependent process. If detection is the objective, then single samples composed of numerous pooled sub-samples is the most cost effective. If a description of density or distribution is needed, then the most costly alternative of a series of discrete samples is required, and previous experience can help define an optimal sampling strategy. Heterodera carotae is found throughout the soil profile wherever carrot roots are present. The largest part of the population is found where most of the carrot roots are found, in the top 30 cm of soil.

SECTION THREE

Ecological Studies

INTRODUCTION

Previous host range studies of Heterodera carotae have indicated that it reproduces only on Daucus carotae, wild and cultivated carrot, and two species of Torilis. None of these plants are native to Michigan. The carrot is widely grown in MI, and the wild carrot, Queen Anne's Lace, is a very common plant in old field and recently disturbed sites. An ecological approach to finding other plant species on which H. carotae might reproduce was taken. Field, greenhouse and growth chamber studies of those plants closest in time and space to the commercial carrot production system were selected and evaluated as H. carotae hosts. Host plant root filtrates (or exudates) stimulate the hatching of plant parasitic nematodes. This response is particularly important in the hatch of cyst nematode eggs present within the cyst body. Studies were conducted to see if nematode infestation of the host plant roots led to a change in the root filtrates, as reflected in an alteration of hatching patterns of eggs within cysts. The quality of a host plant changes over time, from the perspective of the plant parasite. A series of studies were conducted to see if the age of a carrot plant influences the timing, intensity or degree of total hatch.

Different plant sequences or successions tend to influence plant parasitic nematode community structure. In

the short term, plant sequences seem to effect the population density and readiness to hatch of Heterodera carotae eggs within cysts. Studies were conducted to study this relationship in typical sequences found in the carrot agro-ecosystem, and the effect on yield in subsequent carrot crops. A second parallel study looked at changes in the Heterodera carotae population over time as plant sequences change.

THE POPULATION DYNAMICS AND HOST RANGE OF *HETERODERA CAROTAE* IN MICHIGAN HISTOSOLS

ABSTRACT

Heterodera carotae, carrot cyst nematode, populations were sampled year around for three consecutive years at the *H. carotae* field research site. Sampling was on a weekly basis from April through November and bi-weekly December through March.

Parallel sampling of plots where other plant sequences were grown was also conducted. In the first study the relative population estimate, measured as monthly mean cyst number per one hundred milliliters of soil, rose almost steadily to February of the year in which the third consecutive crop of carrots was to be planted. After this point the relative population density measurement fell through spring and rose only modestly during the growing season.

In the second study the relative population measure showed a similar pattern of change. The population reached its peak in February of the year before the second consecutive crop of carrots. The population estimate fell less and rebounded more in the second year, than for the previous study.

The pattern of relative population density variation observed in this population of H. carotae indicates that at least three years of continuous carrot production would be required to achieve relative stability of the population. This should not be unexpected from a obligate plant parasite with a single host plant, where that host plant is a biennial with a greatly reduced potential as a host in its second year.

A parallel series of host range studies were conducted at the field research site, in the greenhouse and in growth chambers. This was done to determine if any plants present in the Michigan carrot histosol ecosystem were hosts of H. carotae. Plants to be tested were selected on the basis of their presence in or near cultivated carrot histosol fields. Twenty four plants were tested. Heterodera carotae reproduced on Daucus carota, wild and domestic carrot, only.

INTRODUCTION

Heterodera carotae (Jones, 1950), the carrot cyst nematode, is widely distributed throughout the commercial carrot production areas in europe and Michigan (Mathews, 1975; Berney and Bird, 1992). In combination with Meloidogyne hapla, the northern root-knot nematode, H. carotae represents the most serious challenge to commercial carrot production in Michigan in the current status of the production system. Michigan is the sole reported location for H. carotae in North America. Daucus carota is the sole known host of H. carotae in Michigan, and grows here in two forms; the cultivated

carrot and as Queen Anne's Lace the familiar weed of old fields and roadsides. Both are biennial plants with extensive vegetative growth in the first year and limited vegetative growth associated with bloom and seed formation in the second year. In MI carrot production the carrot is rarely allowed to grow for two years.

Different plant sequences have been observed to have differential impacts upon the populations of carrot cyst nematode.

Plants other than carrot which were grown at the site were selected from alternative plant sequences practiced in different regions of Michigan, or because of their presence in on near histosol carrot production fields. This was done to determine the effect upon populations of H. carotae of different plant sequences, or the potential of the plant as a host for H. carotae.

MATERIALS AND METHODS

Field Research

All field research reported here was conducted at the H. carotae research site near Sheridan, Montcalm Co. MI. The study site is a 160 acre histosol, "muck", field which has been in commercial carrot production for over a decade. Each sampling area was 1 M wide and 6.3 M long. The long axis of each was north to south. Each sampling area was repeated six times, and all were surveyed and mapped to fixed points to allow for return to specific areas from year to year. Each

sampling area was rototilled to a depth of 0.4 M each spring before planting. Plants grown at the site, in addition to carrots, included: corn, onion, sudax, oats and a variety of weeds. Some carrots were also grown for two years at the site, to determine relative reproduction on carrots in their first year of growth, as opposed to during their second year of growth.

Sampling was conducted on a weekly basis from April to November and on a biweekly basis from December through March of each year. A sample was a composite of 5 sub-samples,each a slice of soil from the surface to 20cm deep and approximately 0.1 L in volume. All subsamples were taken from the root zone of the current or most recent plants. The sub-samples from each sampling area were mixed together and stored in plastic bags. 0.1 L of soil from each sample was subject to sugar flotation/centrifugation (Jenkins, 1964) and the resulting nematodes counted, under a dissecting microscope.

2 Year Series The year previous to the start of the study reported here this area was planted to carrots. In the spring of year 1 of this study 36 sampling areas were laid out as described above. These areas were planted to planted to: carrot, onion, sudax (cut), corn, sudax (not cut) and fallow. The carrot treatment was composed of three rows of carrots (cv. chancellor), the onion treatment was composed of 2 rows of onions (cv. yellow globe), the sudax (not cut) was planted with 0.5 kg of hybrid sorghum/sudangrass cv. "haygrazer" broadcast and incorporated, allowed to grow and head out, the

corn plantings were composed of two rows of pioneer field corn spaced 70 cm. apart, the sudax (cut) plantings were identical with the sudax (not cut) above except that they were cut once, just before pollination began, and the cut material was laid back on the sampling areas, the fallow sampling areas were kept plant free by manual removal of all plants emerging there, cultivation and herbicide application. All sampling areas were hand weeded and received appropriate herbicide applications where this could be done without undue risk of injury to adjacent areas. All plants and the fallow areas were maintained through their normal growing season. All portions of the site not planted as above were planted to onions or carrots. In the spring of the second year of this study all sampling areas, including those which had been in plants other than carrot the previous year were planted to carrots (cv. chancellor) with 3 rows per sampling area.

3 Year Series In the spring of the second year of this study an area adjacent to the 2 year series, but located 10 m to the north was laid out to replicated sampling areas of seven alternative plants. These were carrot, onion, sudax (not cut), corn, sudax (cut) fallow and oats. Each of these plant choices was repeated in six sampling areas. All of these were included in a randomized complete block design. All of the area planted to the 3 year sampling areas had been planted to onions the previous year and carrots the year before. The sampling areas were planted in the methods described above except that oats at 0.25KG per sampling area,

broadcast and incorporated and tomato, cv "Rutgers" were transplanted at one per M of row were added. All sampling areas were maintained as for the previous year in the 2 year series. In the spring of the following year the 3 year series areas were planted to carrots, maintained and harvested with the methods used in the 2 year series the previous year.

Greenhouse and Laboratory

A literature review revealed that all of the plants reported as alternative hosts of H. carotae (Aubert, 1985) were not recorded in MI, and no members of their genera were reported except as "limited escapes from cultivation" (J. Beaman, Beal Darlington Herbarium, personal communication). A survey of five cultivated histosol fields and their uncultivated margins was conducted.

This information in consultation with farm managers and employees was used to construct a list of wild and cultivated plants most likely to be present in the MI histosol carrot production system. The availability of seed and the limited greenhouse and growth chamber space influenced the number of species grown. The final list of species included in the field and greenhouse / growth chamber studies appears in Table 1. Where possible, the seeds of wild plants were collected at the research site. All the seeds or cultivated plants were purchased. All tests were repeated twice.

The greenhouse tests were conducted in the Michigan State University Nematology Program greenhouses. All plants were

maintained under twelve hours of artificial light to supplement natural light. Studies were conducted spring and fall when temperature control is most predictable. Spring collected field soil containing at least seventy H. carotae cysts per liter was placed in one liter pots, with several seeds. The emerging plants were thinned to a single plant per pot. Each plant species was replicated ten times. All plants were allowed to grow to the onset of flowering, or the onset of senescence. They were then removed from the soil and the roots stained by McBrydes modified (Barker et al., 1985) and examined under the stereo microscope for presence of H. carotae.

Growth chamber experiments were conducted as described or the greenhouse studies except the growth chambers were held at 15C, with twelve hours of mixed fluorescent and incandescent light. 15C was chosen as the optimum temperature for the hatch of H. carotae.

RESULTS

Field 2 Year Series

Mean numbers of H. carotae cysts per 0.1 L of soil for the 2 year series of samples from continuous carrot planting are presented in Fig. 1. The almost continuous increase in relative cyst numbers from May of the first year through February of the second year is followed by a precipitous fall in relative cyst numbers for the next three months. This low point occurs about the same time as carrot planting for the

third consecutive year. From planting to harvest there is a substantial increase, followed by a drop in October and a stable population estimate through the next six months.

The relative population estimates (relative to the continuous carrot sampling area estimates) of the non-carrot sampling areas are presented in Fig. 2. In the first year of the series, only the fallow areas exceed the carrot planted sampling areas in relative cyst density. This is in part because the cyst numbers are seen to decline in all other measurement areas, but not in the fallow sampling areas. This trend ends by October of the first year. From October of the first year through April of the second year all other plant sequences have lower relative cyst population estimates than the continuous carrot planting areas. In May of the second year the relative population estimates from the sampling areas where sudax and corn had been grown the previous year exceed that from the areas of continuous carrot growth. The pattern of all other plant sequences having relative cyst population estimates higher than the estimates from the continuous carrot sampling areas extends through the end of the comparisons of these plots, with very few exceptions.

Field 3 Year Series

The population estimates from the three year series begin in the second year of the studies reported here. The relative mean monthly estimates of cyst density per 0.1 L of soil for the continuous carrot measurement areas are presented in Fig.

3. The general trend is a fluctuating up and down, with more up than down, from may of the second year through February of the third year. The next five months show a general downward trend to July, after which there is a recovery to a level above that of the previous July, but less than the high in February.

The relative population estimates from the sample areas for the other plant sequences are presented in Fig. 3. In these comparisons only those sample areas planted to onion were detected to have a relative cyst density in excess of that of the continuous carrot planting in the first 14 months of this series of samplings. Between July and September of the final year of the trial, all of the other plant sequences exceeded the mean cyst concentration of the continuous carrot sampling areas.

Greenhouse and Growth Chamber

In both greenhouse and growth chamber Daucus carota, both domestic and wild carrot supported reproduction by H. carotae. Reproduction was greater and more consistent on carrots in their first year of growth than in their second year. Reproduction was also greater on domestic carrot than on wild carrot. No other plant species supported reproduction by H. carotae in any of the studies.

CONCLUSIONS

Field Studies

Two or more years of continuous carrot planting seems to stabilize a H. carotae population, or at least reduce the level of relative cyst density fluctuations. This seems to be the case regardless of the plant sequence which preceded the carrot plantings. Carrot cyst densities seem to stabilize at levels much below (50 to 85% below) the highest levels reached during the highest fluctuations. Within and between season variability is reduced to less than 50% of the of its previous range. Two years without carrots had less stabilizing influence on H. carotae populations than two years of carrots did, when measured in the third year when carrots are planted in both. One year without carrots led to wider variation among responses after carrots were planted again than did two years without carrots.

Greenhouse and Growth Chamber

Heterodera carotae did not reproduce on any of the species of plants in this study, except Daucus carota. No members of any genera known to support reproduction of H. carotae are present in Michigan to any extent, except D. carota.

Discussion

Field Studies Previous observations as well as the field and greenhouse/growth chamber studies reported here have all

indicated that carrot plants in their second year of growth are relatively poor hosts. The reasons behind this are presumed to be the greatly reduced volume of fine roots maintained by the plant as well as the mobilization of nutrients from the storage organ of the tap root to the flowers and seeds formed above. This is the reverse of the nutrient flow in the first year of carrot plant growth. This difference in host quality is expressed in part in the response of carrot cyst nematode eggs within cysts in response to carrot root filtrates. Heterodera carotae may also be adapted in part to a biennial plant which is a poor host in its second year. This may explain why it takes two or more years of continuous carrots to stabilize the population. Selection for a biotype may be occurring, or adaptation may be occurring, or stabilization of an unmeasured portion of the population might be occurring, before stabilization of the part of the population we are measuring, the cysts. Eggs exist not only in cysts but also in egg sacs and free in the soil. Eggs which are free in the soil must have come either from cysts or egg sacs. Eggs from egg sacs are supposed to be more readily responsive to carrot root filtrates, not requiring a delay of months or longer from the time that they are born until they will hatch, like eggs in cysts. Whether this is a physiological difference between the two sets of eggs or if it is just a difference in the degree of exposure to the environment, we have been unable to determine. But it is possible that H. carotae eggs free in the soil, are a

factor in the rate of reproduction, and its eventual stabilization, and the further reflection of this stabilization in the relative cyst concentration in the soil. The precipitous decline in cyst numbers detected in soil between February and June of each year could be explained in part by the microbial degradation of the cyst walls and the release of many of the eggs into the soil. It is very difficult to recover cyst nematode eggs from soil, much less identify them to species. It is a reasonable hypothesis that at planting time each year, the majority of the H. carotae population in the soil may be present as essentially undetectable eggs free in the soil. Released from the confines of the cyst body, the embryonated eggs are more likely to hatch in response to carrot root filtrates? The eggs are also more easily subject to passive movement by water and air.

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Table 1. Plants used in host range test of *Heterodera carotae*.

Common Name	Scientific Name	Host Range Test	Results
Domestic Carrot	*Daucus carota* "Chancellor"	1,2,3	+
Domestic Carrot - 2nd yr	*Daucus carota* "Chancellor"	1,3	+
Oats	*Avena sativa*	1,2,3	-
Sudax	*Sorgum halapense* X *S. sudanese* cv. "Haygrazer"	1,2,3	-
Onion	*Alium cepa*	1,2,3	-
Corn (grain)	*Zea mays*	1,2,3	-
Barley	*Hordeum vulgare*	3	-
Tomato	*Lycopersicon esculentum*	3	-
Turnip	*Brassica rapa*	2,3	-
Beet	*Beta vulgaris*	2,3	-
Mangle	*Beta vulgaris*	2,3	-
Parsnip	*Pastinacca sativa*	2,3	-
Cannola	*Brassica napus*	3	-
Lettuce	*Lactuca sativa*	2	-
Wild Carrot-Queen Anne's Lace	*Daucus carota*	1,2,3	+
Lambs Quarter - common	*Chenopodium album*	1,2,3	-
Purslane whole plants cuttings	*Portulaca oleracea*	1,2,3 3	- -
Teasel	*Dipsacus fullonum*	3	-
Prostrate Pigweed	*Amaranthus blitoides*	1,2,3	-
Wild Parsnip	*Pastinaca sativa*	2	-
Night Shade, Black Eastern	*Solanum ptycanthum*	1,2	-
Common Mallow	*Malva neglecta*	2	-
Common Burdock	*Arctium minus*	2	-
Prostrate knotweed	*Polygonum aviculare*	2	-

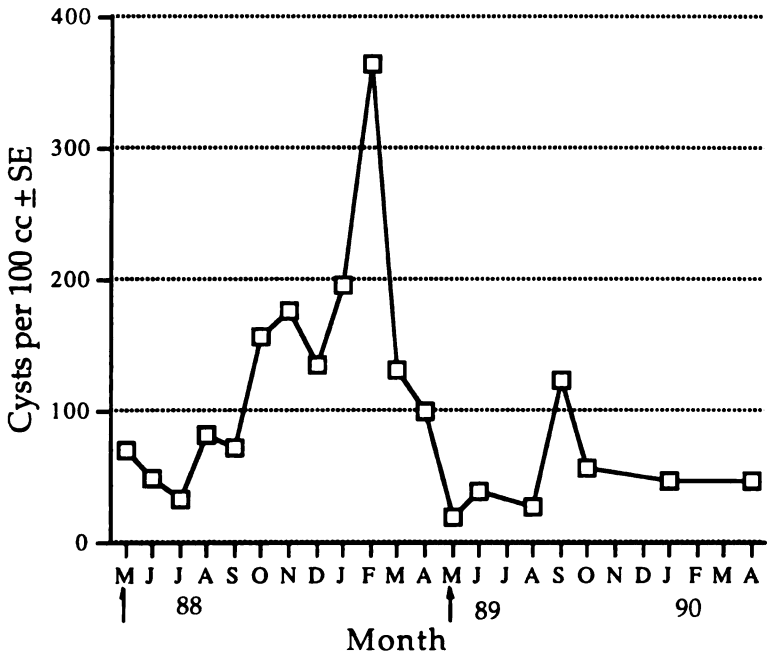

Figure 1. Monthly mean cyst concentration for two year series carrot planted areas. Carrot planting months are indicated by arrows.

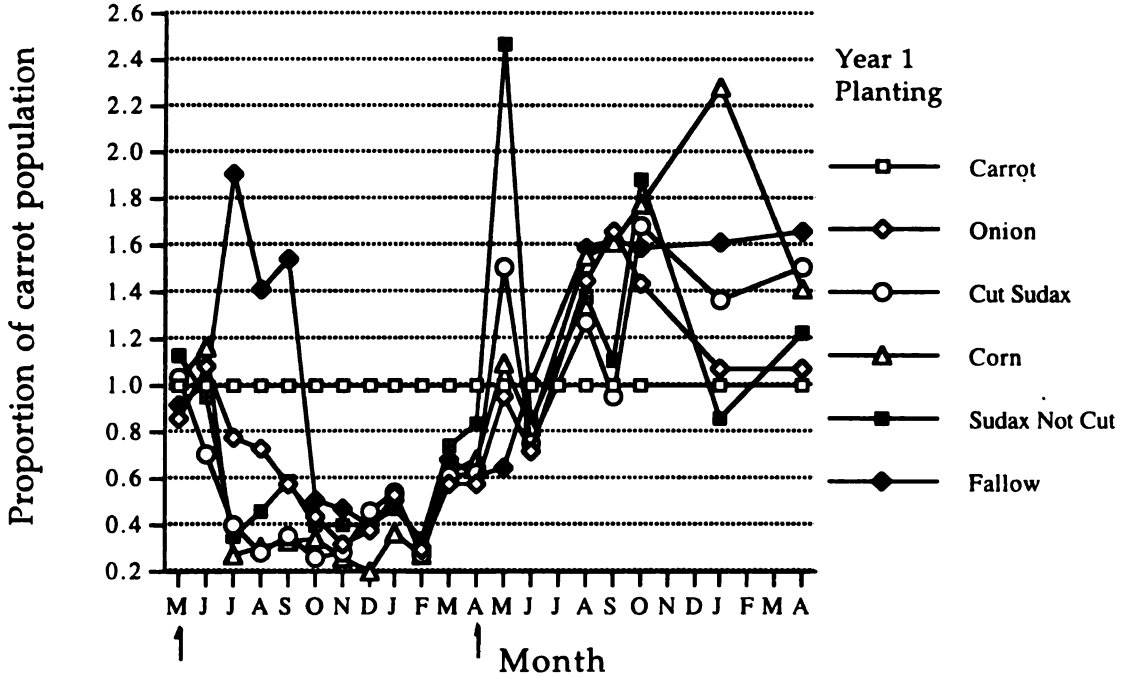

Figure 2. Monthly mean cyst concentrations for five plant sequences, adjusted to a percentage of the carrot sequence. Months of planting indicated by arrows.

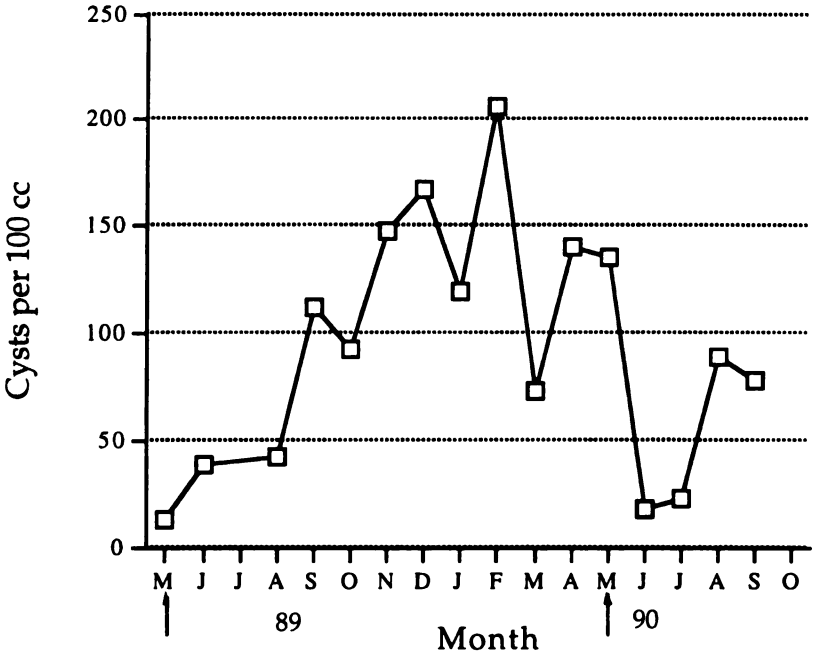

Figure 3. Monthly mean cyst concentration for last two years of three year series carrot planted areas. Months of planting indicated by arrows.

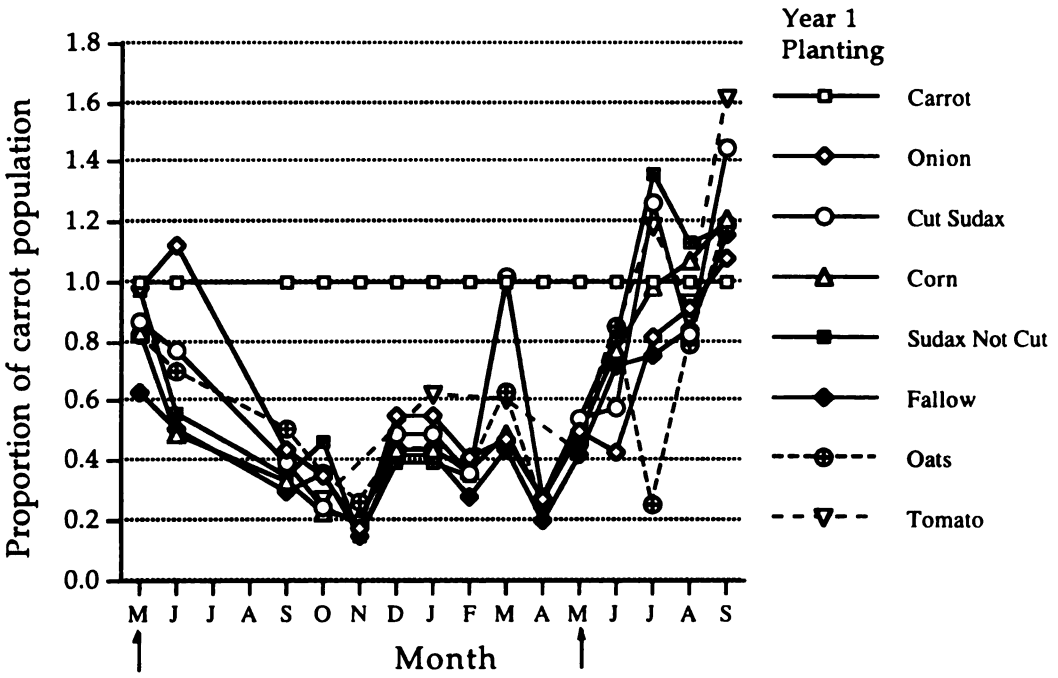

Figure 4. Monthly mean cyst concentrations for seven plant sequences, adjusted to a percentage of the carrot sequence. Planting months indicated by arrows.

EFFECT OF HOST PLANT RESPONSE TO NEMATODE INFESTATION ON THE EMERGENCE OF *HETERODERA CAROTAE* FROM CYSTS

Abstract

Heterodera carotae (carrot cyst nematode) cysts were held at 15C in carrot root filtrates. Filtrates were collected from carrots grown in sterile sand, as well as sand infested with *H. carotae* or *Meloidogyne hapla* (northern root-knot nematode). Filtrates were collected on a weekly basis by overwatering the plants and collecting the excess water as it passed through the sand. Cysts were selected from two research populations, one collected from a commercial carrot field and one that had been maintained under greenhouse conditions for five years. The two populations differed significantly in their response to root filtrates. The greenhouse culture population did not respond differentially to the root filtrates when measured at the number of second stage juveniles on peak hatch day. The field research population showed differences between exudate responses at this point, with the healthy carrot eliciting the most intense response. The two populations were clearly separable at this measure, with all of the field population responses being significantly greater than those of the culture population. The total percent hatch also separates the two populations,

but the field population shows no differential response to the exudate source here, where the culture population does. The exudate from the healthy carrot elicits the highest percent hatch from the culture population, almost comparable to the response in the field population.

Introduction

Heterodera carotae, the carrot cyst nematode, is found in Europe, Cyprus and India as well as Michigan (Mathews, 1975; Berney and Bird, 1992). It has one primary host, Daucus carota the common wild or cultivated carrot (Aubert, 1985). Carrot cyst nematode can cause a variety of symptoms in carrot plants including: leaf discoloration, stunting, tap root reduction, fine root proliferation, tap root deformation and plant death. Yield reduction can be as little as a loss in quality up to complete crop loss (Lamberti, 1971).

Field and laboratory experiments identified significant differences, in the response of eggs within cysts to external stimuli, of two populations of H. carotae. These were: a greenhouse culture population of carrot cyst nematode, maintained for five years in the Michigan State University nematology program greenhouse and a populations of H. carotae recently collected from the field research site at Sheridan, Montcalm Co, MI. The Michigan Greenhouse Population, MGP, had been maintained in a greenhouse where temperatures ranging from 7 to 40C were recorded on a seasonal basis. Carrots were grown in the same soil for several years without other plants

or fallow periods. The Michigan Field Population, MFP, was collected from a commercial carrot production field where surface soil temperatures during the growing season were observed to exceed 40C on a sunny day. At the same time the temperature at 20 cm below the soil surface was never observed to exceed 20C during three growing seasons. Further, at 90 cm below soil surface, the temperature never fell below 0C in two consecutive winters. This field was planted sequentially to carrots, onions and corn in three year cycles. This variation in temperatures and plant sequences did not prevent reproduction in either population. MGP cysts did not respond as expected to preliminary controlled temperature hatching experiments. Eggs within cysts from MGP continued to hatch in response to root exudates from carrot plants already infested with H. carotae, when eggs within field collected cysts from MFP stopped hatching. These findings among others, led to the series of experiments reported here. All experiments were conducted twice. Results reported here are from the second trial set. Identical trials were conducted at 5, 10, 20 an 25C, with results reported elsewhere.

The terms emerge and hatch are used interchangeably in this paper. For the purposes of this paper, both are taken to mean the process whereby a second stage juvenile (j2) of H. carotae leaves the confines of its egg shell, and removes itself beyond the cyst body. In practice these two processes are sequential with no appreciable delay separating them. On

very few occasions were j2s observed uncoiled within cyst bodies.

The objective of these experiments was to determine the influence of host response to nematode infestation on the further hatch of juveniles from cysts of these two populations of H. carotae. The co-occurrence of H. carotae with M. hapla at all sites in MI where H. carotae has been detected (Berney and Bird, 1992), along with mentions of co-occurrence of H. carotae with M. incognita in Italy (Lamberti, 1971), led to the previous infestation aspects of these experiments.

Materials and Methods

Carrots were grown in the greenhouse and the same carrots were used for both populations. Cysts were maintained in the dark at a constant temperature of 15C in controlled environment chambers. Each treatment was composed of 10 cysts of uniform size and color suspended in 2 ml of carrot root filtrate in a Bureau of Plant Industry (BPI) dish. Each treatment was replicated 7 times, with all replicates held in a glass petri dish lined with a moist filter paper. All cysts were selected for uniformity in size and color. All field population cysts used were spring collected. The number of j2s hatching from the cysts were counted daily. The root filtrates were replaced weekly, when the j2s were removed. All experiments were maintained for at least 200 days. Each exudate/population combination was terminated when two consecutive weeks yielded less than one j2 for seven

replicates. At termination all remaining cysts were crushed and the residual j2s and embryonated eggs counted. This allowed for the calculation of percent of total hatch and other measures of response based upon percent of the possible total.

Analysis

The pattern of emergence from MFP cysts for the three carrot root exudates: Healthy carrot, no nematodes infesting the roots or soil (-Nema), Carrot with roots infested with Heterodera carotae (+Hc), Carrot roots infested with Meloidogyne hapla (+Mh) and the water control (H2O) show a confusing series of patterns (Fig. 1). The difficulty of comparing eight emergence curves over a period in excess of 200 days is obvious. To understand the differences between the emergence patterns of the different population/exudate combinations six points of comparison were used: number of days to 10% emergence, number of days to 50% emergence, number of days to 90% emergence, total percent emergence, number of j2s emerged on peak hatch day and number of days to peak emergence day.

Results

The days to 10% emergence measurement showed no differences among root exudates, including the water control, for MGP (Fig. 2). For this same measurement the MFP showed three groupings, the +Mh exudate being the highest with the -

Nema and +HC together in the middle grouping and finally the water control as the lowest group. There were no significant differences detected between the two populations at this measurement. A similar pattern appears in the 50% emergence measurements, with only the water control giving a significantly different value in the MGP comparison (Fig. 3). The tests of MFP for the +Mh and +Hc exudates are significantly higher than the water controls, and that the -Nema is intermediate and not significantly different from any of the others, at this point. The comparison of the two populations shows a significant difference for the +Hc values, with the MFP taking significantly more days to reach the 50% emerged level, an average of 8.7 days.

The 90% emerged means (Fig. 4) show no differences between exudates for MFP and only the water controls differed significantly from the other exudates in MGP. The contrast of the two populations revealed that for the +Mh exudate, the two populations are different, with MFP needing an average of 30.3 fewer days to reach the 90% emerged value.

MGP shows three distinct groupings at the total percent emerged measurement (Fig. 5). The -Nema treatment has the highest total percent emerged at 92, the +Mh and +Hc exudates are together in the middle at 71 and 65.4% respectively with the water control lowest at 4.3%. MFP has a different separation, all exudates except the water control being nearly uniform. Comparisons of the two populations reveals that for

the +Mh and +Hc exudates the total percent emerged is significantly higher for MFP.

The peak emergence day number measurements (Fig. 6) show a response pattern almost the opposite of the total percent emerged measurement. For MGP, all measurements are statistically inseparable except the water controls, which differ from all of the others. MFP had three distinct groupings, with the +Mh exudate taking the most days to reach the peak of emergence at a mean of 130.7. Both the -Nema and the +Hc come next at 73 and 62 days respectively. Finally the water controls come in at a mean of 4 days. Contrasting the two populations at this measurement shows a significant difference in response to the +Hc exudate and a weak difference in response to the -Nema exudate.

The measurement of peak emergence j2 number for the MGP reveals the low overall intensity of emergence from eggs of this population as compared to MFP (Table 7). The differences between the two populations were significant for all exudates except the water control. MFP had three distinct response groups, with the +Mh and +Hc grouped together between the lower water control and the higher -Nema exudate.

Conclusions and Discussion

The water controls generally showed a clean statistical separation from the other treatments, and the two populations were not separable in the response to the water controls.

The measurements of days to 10, 50 and 90% emergence do not reveal a discernable pattern of differences.

The measurement of the peak emergence day number shows MGP having no significant differences in exudate response, where the MFP response is significantly effected by the +Mh exudate. For the MFP, the +Hc and -Nema exudate responses are similar to each other, but different from the response to the same factors by MGP.

The peak emergence j2 number is a measurement of the intensity of the response of the eggs within cysts to the root exudates. This measure clearly separates the two populations at all three exudates. MFP also showed differences in response to exudates, with significantly more intense response to the -Nema exudate than to the +Mh or +Hc exudates. This difference was not observed in the MGP. The intensity of the responses by the MGP was lower for all exudates.

MFP shows no response to root infestation at the total percent emerged measurement. MGP does show such a response, with the total percent emerged significantly lower for the +Mh and +Hc exudates than the -Nema. This difference, along with a weak difference in response to the -Nema exudate separate the two populations.

The differences at these three points of measurement show that MGP was slower to respond to hatching stimuli and responded less intensely to these stimuli than MFP. In addition MFP and MGP were both influenced by the presence of nematodes in the root systems from which the carrot root

exudates were collected. This indicates the presence of a feed back loop of chemical communication from plant to nematode. That is, the impact of the nematodes infesting the plant is to change the nature of the chemicals released by the plant's root system. The nematodes remaining in the soil have their response modified by this change in the chemicals released by the roots of the plant.

It should be noted that the relationships and differences outlined here are not thermally stable but vary within the temperature range within which significant H. carotae egg hatch occurs.

These results also highlight the danger of assuming that greenhouse culture nematodes represent their "wild" cousins in response to plant mediated (or other) stimuli.

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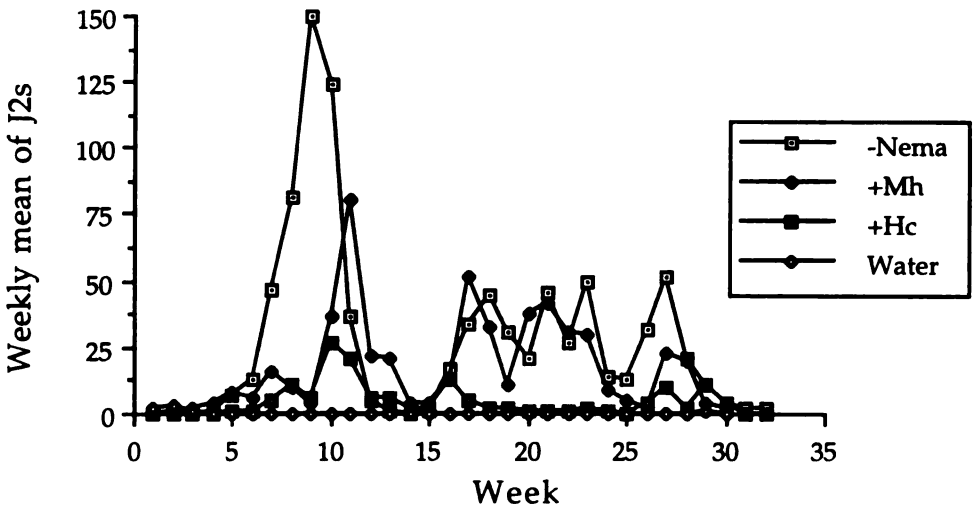

Figure 1. Weekly J2s hatching, by root exudate source, for field collected cysts.

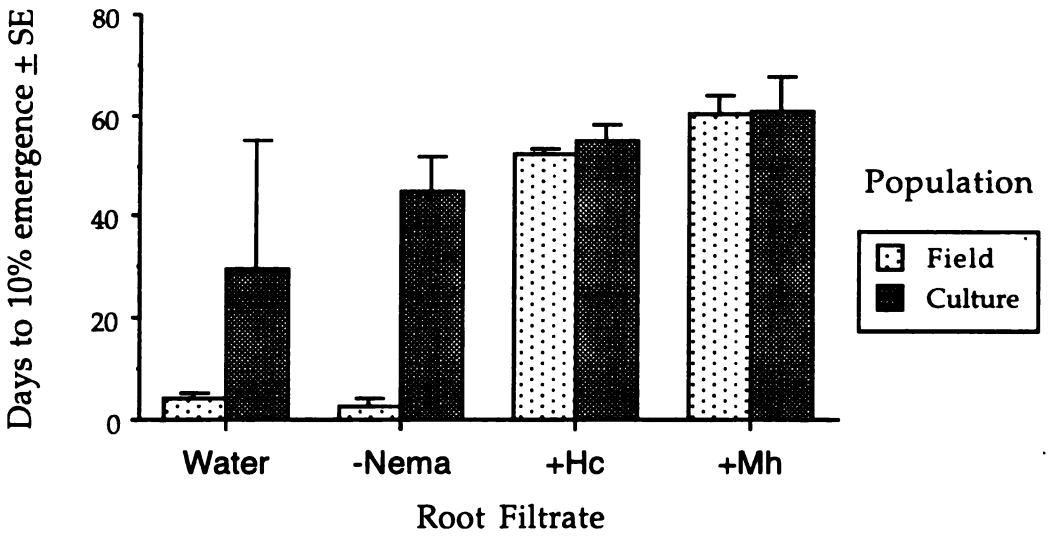

Figure 2. Days to 10% emergence, from cysts at 15C by population and root filtrate source.

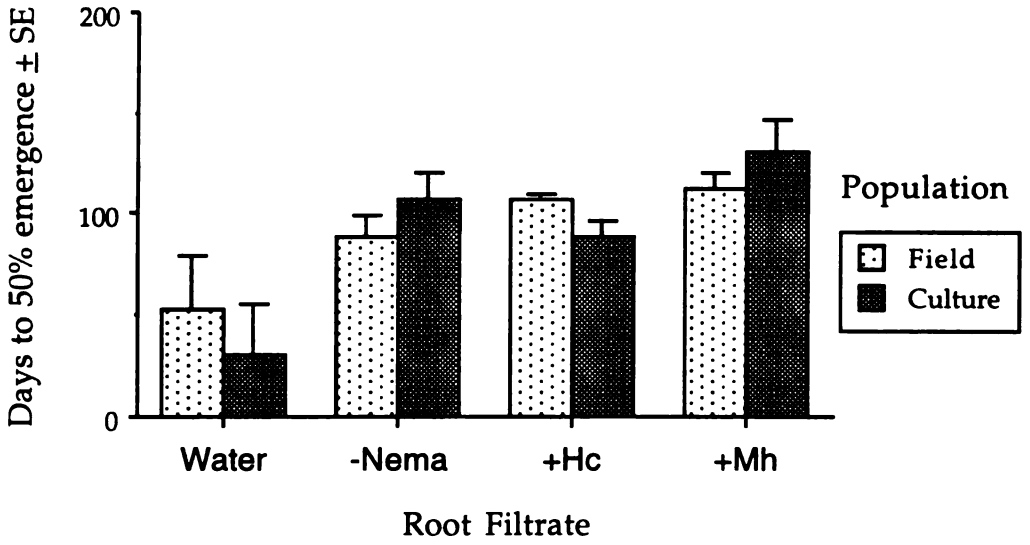

Figure 3. Days to 50% emergence, from cysts at 15C by population and root filtrate source.

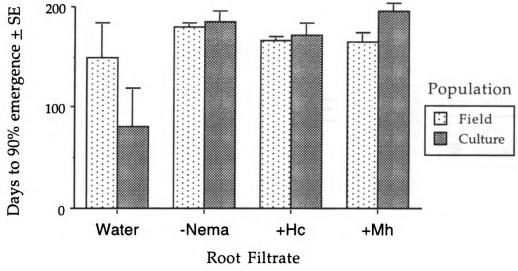

Figure 4. Days to 90% emergence, from cysts at 15C by population and root filtrate source.

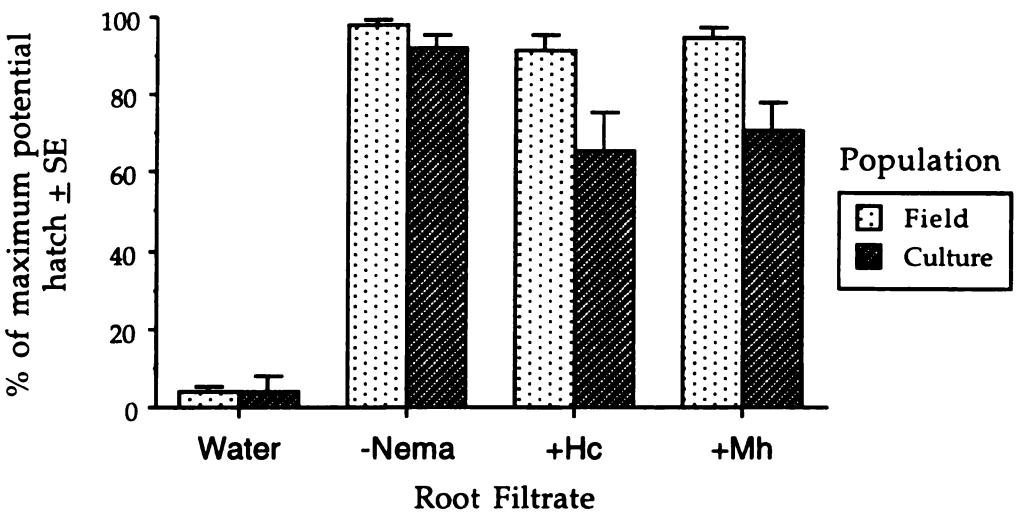

Figure 5. Effect of host plant infestation on emergence of Heterodera carotae from cysts.

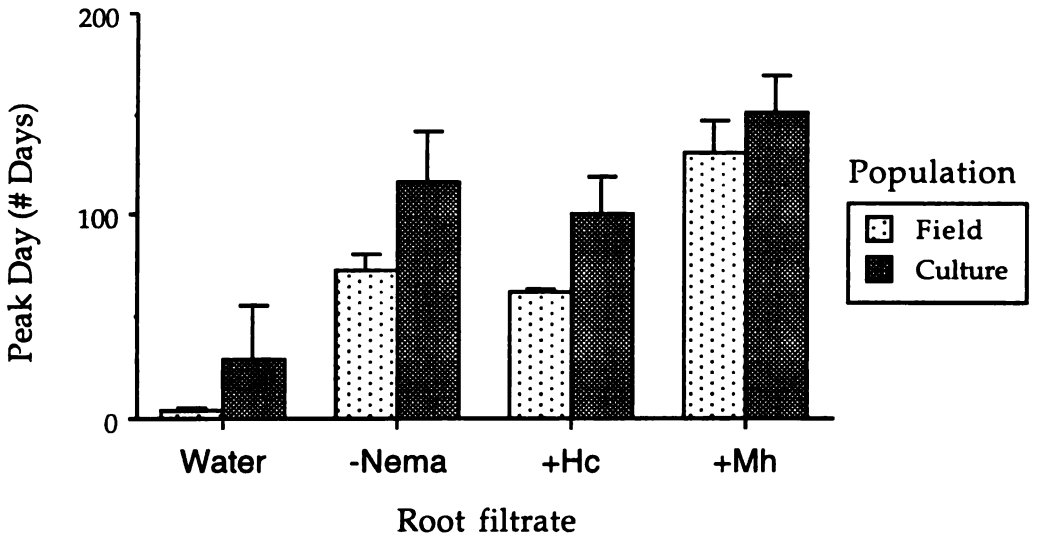

Figure 6. The number of days to reach the peak of J2 emergence from cysts at 15C, by population and root filtrate source.

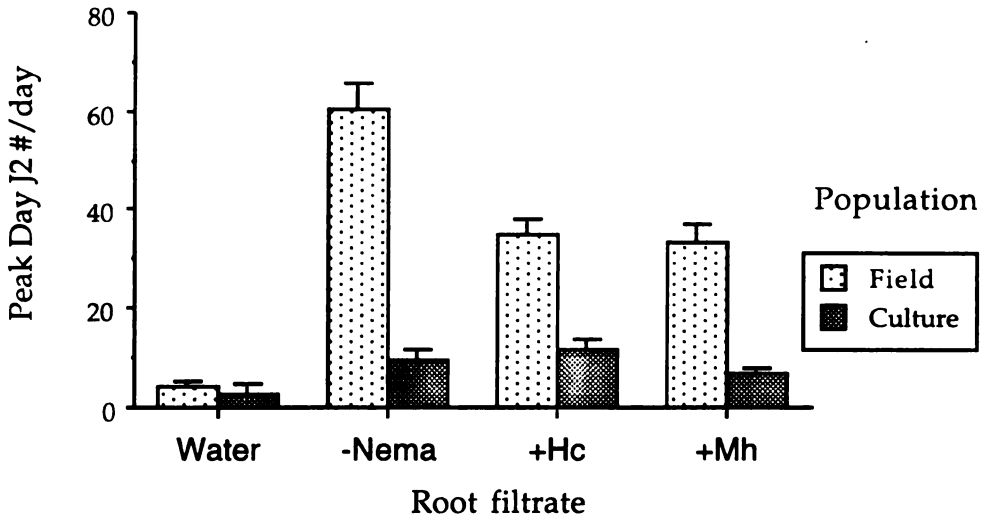

Figure 7. The number of J2s emerging on the day of peak emergence from cysts at 15C by population and root filtrate source.

EFFECT OF HOST PLANT AGE ON THE EMERGENCE OF *HETERODERA CAROTAE* FROM CYSTS

Abstract

Field collected cysts of *Heterodera carotae* (carrot cyst nematode) were held at 15 C in carrot root filtrate. A single carrot plant was used for the duration of the experiment. Each week, two new groups of cysts were added. The first group consisted of cysts washed from soil that day. The second group consisted of cysts removed from water where they had been held since the start of the experiment. This process was repeated for ten consecutive weeks. Juveniles emerging from the cysts were counted weekly, removed and the root filtrates replaced. With the exception of the groups delayed five weeks, all cysts held in water prior to their exposure to carrot root filtrates had significantly higher percentages of emergence than those maintained in soil. For eight of the ten time groups, the week of peak emergence was not significantly different between the cysts stored in soil and water environments. Cysts stored in water yielded significantly more juveniles during the week of peak emergence than cysts stored in soil. This was true for all time groups. A steady decline from 74 to 33% of possible hatch from the third to the eleventh time groups of cysts stored in water was noted. Thus, carrots older than

three weeks when first exposed to cysts, reduced hatch from those cysts if held in water.

Introduction

Heterodera carotae, carrot cyst nematode, is found in Europe, Cyprus and India as well as Michigan (Mathews, 1975; Berney and Bird, 1992). This nematode species reproduces almost exclusively on Daucus carota wild and domestic carrot (Aubert, 1985) and can cause significant alterations to plant growth and development. Symptoms include: leaf discoloration, leaf stunting, storage root stunting or malformation, excessive lateral root formation and plant death (Lamberti, 1971).

Previous laboratory and field experiments at Michigan State University have shown that the second-stage juveniles (j2s) of H. carotae in eggs within the cyst body are differentially effected by environmental and host plant conditions. The purpose of this study was to determine if the age of D. carota has any influence on the pattern, intensity or timing of second-stage juvenile emergence from eggs within cysts of H. carotae.

Materials and Methods

Field soil containing cysts of H. carotae was brought into the lab from the field research site near Sheridan, Montcalm Co. Michigan. This soil was held at 2C. Cysts were removed by modified sugar flotation-centrifugation (Jenkins,

1964). Half of the soil was processed to start the experiment. The cysts were used for the zero week delay group, with the remainder placed in distilled water and held at 2 C. Each group of cysts was composed on seven Bureau of Plant Industry dishes each containing ten cysts selected for uniformity of size and color. At the start of the experiment, a pre-germinated carrot seed was planted and overwatered to collect filtrates. Each week for ten weeks a new group of cysts from water and a new group from the soil environment were exposed to carrot root filtrate collected that week. All previous groups of cysts had the emerged second stage juveniles, (j2s) counted and removed each week, and the root filtrates replaced. All groups were maintained until two consecutive weeks passed in which the total number of j2s emerged was less than one for the group. This is illustrated in Fig. 1. At this time all cysts remaining were crushed and the remaining embryonated eggs and j2s counted.

Analysis

Comparison of twenty-one groups over twelve to twenty three weeks is a complex undertaking. Weekly mean number of j2s emerged for 5 selected groups of cysts are illustrated in Fig. 2. Three points of comparison were selected for analysis of emergence response over time and between the groups held in water and soil environments. These include: 1. week of peak emergence, 2. number of j2s emerged in week of peak emergence and 3. total percent of j2s emerged.

Results

Then effect of plant age on which week peak emergence occurs on is illustrated in Fig. 3. For time groups two and three there are no differences between cysts stored in water and cysts stored in soil. One week of delay retards peak emergence for one week. For time groups four and five there is no change from the third time group in the week of peak emergence for the cysts stored in water group, and the cysts stored in soil are one week earlier at reaching peak emergence at these time groups. The cysts from water were one week later in reaching peak emergence for each week of delay in entering cysts into the root filtrate, from time groups six to eleven. Except for time groups eight and nine, this is also true of the cysts stored in soil. The cysts stored in soil respond in a significantly longer time reaching their peak of emergence, for time groups eight and nine.

The number of j2s emerged during the week of peak emergence shows almost complete separation of the water and soil stored groups, (Fig. 4). There is one discrete highest peak for the cysts stored in soil at time group six. This is not the case for the cysts stored in water. Here time groups three, six and seven have the same relative number of j2s during the week of peak emergence.

The percent of hatch measurement shows complete separation for the soil and water stored cysts at all time groups except interval six. For the cysts stored in soil this time group is that at which the highest percent of hatch (44%)

is reached. The water stored cysts reach their highest percent of hatch (74%) in the third time group, from which there is an irregular decline to the eleventh time group (33%).

Discussion

These experiments have shown that differences in response to carrot root filtrates occurred between H. carotae cysts held in soil and water environments. The mechanism of this difference is not clear, but more and a higher percentage of j2s emerge from cysts held in water than held in soil. The age of the carrot plant seems to be one factor in explaining the differences between the time groups. All of the groups of cysts exposed to the carrot root filtrates in the first 5 weeks of the plant's life have a single intense peak of emergence. Those exposed to the plant beginning in weeks six through ten show two less intense peaks of emergence, starting the first week after exposure to the filtrate. This is true for both soil and water stored cysts, and is illustrated in Fig. 2. How old the carrot plant is when its exudates first contact the cysts also effects the response of the cysts in total percent of hatch. The first time group reaches only 15% of possible hatch, where the soil held cysts in the sixth time group reach 44% of possible hatch and the water held cysts in time group three reach 70% of possible hatch, though each of the two later groups are exposed to the root filtrates for a shorter period of time. Starting at time group three for the

water stored cysts, a steady decline in the total percent of possible hatch occurs. This trend ends at 33% for time group eleven, the last in the experiment. A similar pattern can be seen in the number of j2s emerged in the week of peak emergence measurements.

The age of carrot plants does seem to effect H. carotae hatch from cysts, as demonstrated here, but the moisture of the cyst's environment seems to add another layer to this effect.

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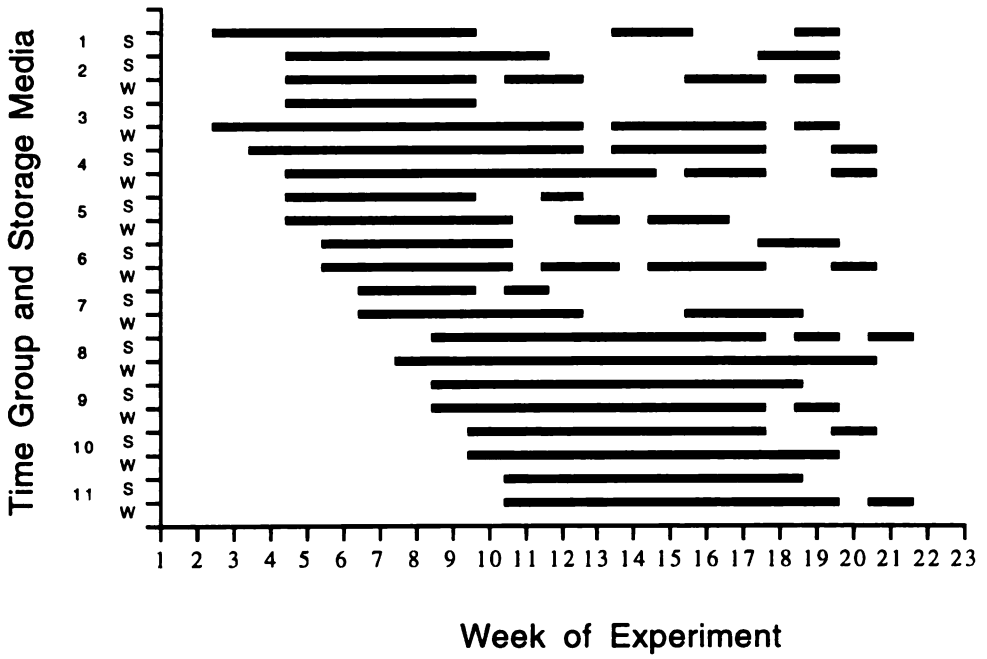

Figure 1. Weeks during which hatch occurred from Heterodera carotae cysts in eleven time groups and two different storage media.

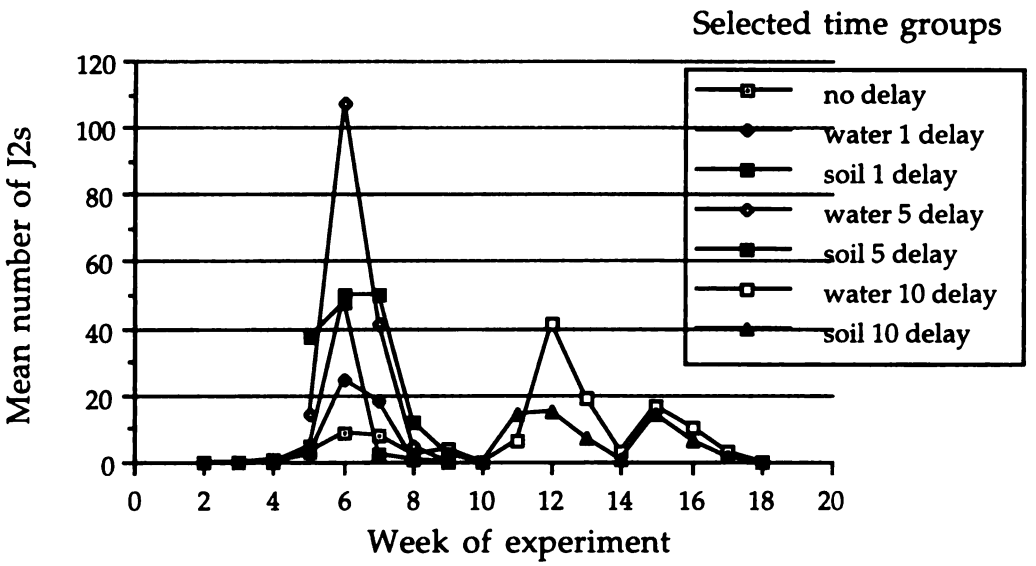

Figure 2. Mean number of J2s emerged per week vs. host plant age for selected time groups from both storage media.

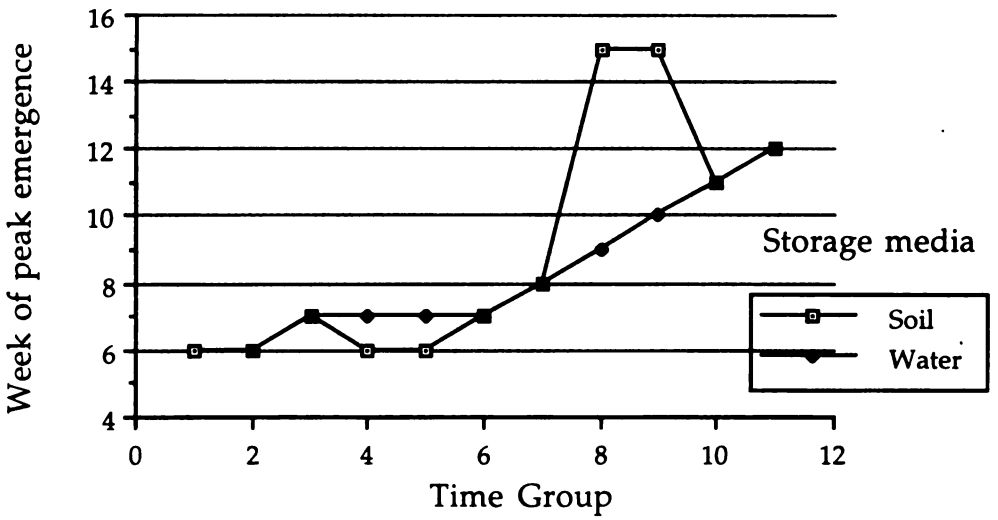

Figure 3. Peak emergence week for all time groups from both storage media.

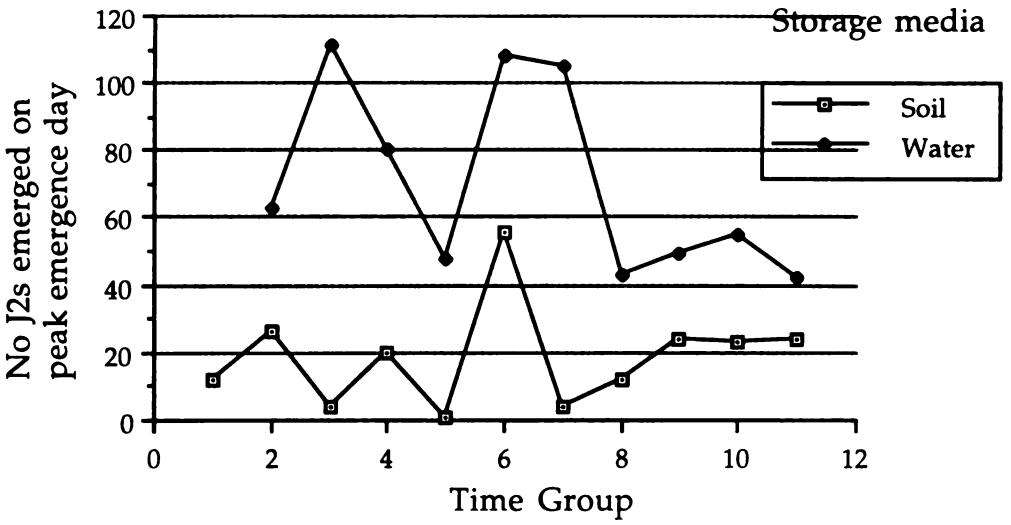

Figure 4. Number of J2s emerged on peak emergence day for all time groups from both storage media.

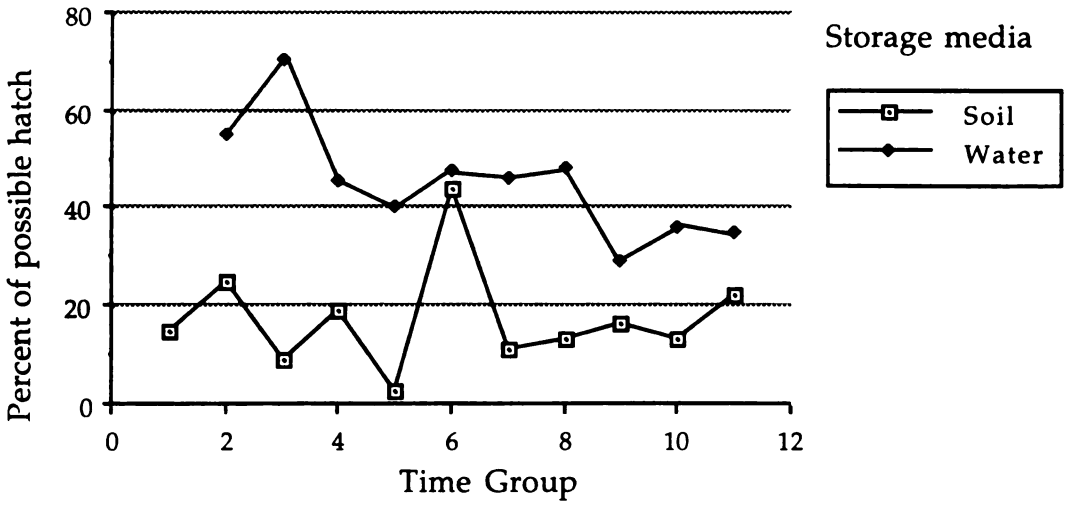

Figure 5. Total percent of possible hatch achieved over the duration of the experiment for all time groups from both storage media.

INFLUENCE OF PLANT SEQUENCE AND NEMATICIDES ON *HETERODERA CAROTAE* POPULATIONS AND CARROT PRODUCTION IN MICHIGAN

Abstract

A series of alternative plant sequences were maintained for one or two years between crops of carrots to measure the impact on populations of *Heterodera carotae*, the carrot cyst nematode. Onions, corn, sudax (cut and un-cut) and fallow were the one year alternatives. The two year alternatives were onions followed by onions, corn, sudax (cut and un-cut), fallow or oats. One year of a monocot plant increased carrot storage organ weight and resulted in a decrease in nematode related symptoms in subsequent carrot plantings compared to sequences including dicots, fallow or continuous carrots. Fewer differences were found in subsequent plantings after two year plant sequences. Population densities of *H. carotae* associated with the alternative plants closely follow the storage organ weights, with significant differences in the population size and changes in the population size by plant chosen. The impact of the previous year's plant choice was still reflected in the carrot cyst nematode population at maturity of the subsequent carrot crop. None of the three nematicides (Telone II, Vorlex or Vydate) evaluated for three years provided significant control of *H. carotae* or resulted

in a significant reduction in nematode related symptoms on subsequent carrot plants, when compared to the untreated controls. When the nematicides are compared to the alternative plant sequences there is no significant differences between the nematicides and the one or two year alternative plant sequences in total carrot weight produced or percent of good carrots. Similarly there is no significant difference between the nematicide treatments and the alternative plant sequences in the reduction or control of H. carotae populations.

Introduction

Heterodera carotae (Jones, 1950), the carrot cyst nematode, is widely distributed throughout the commercial carrot growing regions of Europe and Michigan. (Mathews, 1975; Berney and Bird, 1992). In combination with Meloidogyne hapla, the northern root-knot nematode, H. carotae represents a very serious challenge to commercial carrot production in Michigan under the current status of the production system. Different plant sequences have been observed to have differential impacts upon the populations of the carrot cyst nematode. The impacts of these plant sequences were compared with selected nematicide treatments to determine if any of the current production practices are significantly better choices for carrot cyst nematode management.

Materials and Methods

Two Year Sequences. During the carrot harvest in late summer in the year prior to the start of these studies, fields at the William Bolthouse farm at Sheridan, Montcalm Co. Michigan, were sampled to locate an area of as nearly uniform as possible H. carotae population density. An area of approximately 1 hectare with two population gradients (increasing from North to South and increasing from West to East) was chosen. The spring of the following year the entire area was prepared by moldboard plowing. Thirty six sample areas for the plant sequences were laid out in a latin square. Adjacent to this seventy two sample areas were laid out for the nematicide tests. Each sample area was 1m by 6.5m running North to South. Plants included carrot, onion, sudax, (cut and un-cut) corn and fallow. All of the nematicide test areas were planted to carrots. All carrot plantings were three rows of carrots (cv. chancellor), the onion sampling areas were composed of two rows of onion plants, the sudax areas were planted to .5 kg of hybrid sorghum/sudangrass broadcast and incorporated. The cut sudax areas were harvested at bloom and the cut material placed on the soil surface. The un-cut areas were allowed to reach senescence naturally. Corn areas were planted to 2 rows of Pioneer field corn spaced 70 cm. apart. The fallow areas were kept plant free by hand weeding, cultivation and herbicide application. All sampling areas were maintained weed free by herbicide and hand weeding. All plants were maintained through their normal growing season.

Both soil and root samples were taken weekly until carrot harvest from the plant sequence areas. The nematicide sampling areas were sample 4 times during the season, including pre-treatment and harvest. All plant sequence sampling areas were sampled through the fall winter and spring on an alternate week basis. The following spring all of the alternate plant sampling areas were prepared by rototiller and planted to carrot (cv. chancellor) as the previous season. These areas were sampled (both roots and soil) on a weekly basis through the growing season. In September, all of the carrots in the center 3.25 m of the center row of each area were harvested weighed counted and graded.

Three Year Sequences. In the spring of the second year of the research reported on here, an area adjacent to the two year studies but starting 10 m to the east was laid out to replicated sampling areas of seven alternative plants. These were the same as the first year with the addition of oats. All of the area planted to the three year study had been planted to onions the previous year. All materials and methods were the same as for the two year studies.

Nematicides For each of the three years of the study, a different area adjacent to the alternative plant sampling areas was laid out for nematicide areas. The first year this was to the north, the second to the west and the third to the south. Thus the plant sequences for these areas were 1st year - commercial carrots / nematicide carrots, 2nd year - commercial carrots / commercial onions / nematicide carrots

and third year - commercial carrots / commercial onions / field corn / nematicide carrots. A variety of nematicides were applied in a replicated fashion in each of these years only three and the untreated controls are reported here: Telone II (36 gal/acre pre plant), Vorlex (20 gal/acre pre plant) and Vydate (Vydate 2L, 4 lbs / acre at planting). All treatments were replicated 5 to 10 times each depending upon the year. All areas were laid out and maintained in the same way as the alternate plant sampling areas in the year in which carrots were grown in them.

Results

Two Year Sequences. **Plant factors** No significant differences between plant types were found in the total yield of carrots in kg (Table 1) The percentage of #1 (marketable) carrots does show significant differences between plant types. Grass plantings are 1 group and continuous carrots and onions in a second and fallow areas in a group by themselves and intermediate between the other two. This same pattern is presented in greater contrast in the percent of forked carrots. In the percent of stubby carrots, only 2 groups are found, with the fallow areas falling into the second group with the onion and carrot plantings.

Nematode Populations No significant differences among the sampling areas were found in the pre-plant soil assessment of plant parasitic nematodes. At the end of the first season, only the continuous carrot sampling areas had a significantly

higher nematode population as measured by density of cysts in the soil. At the end of the second season the fallow and onion sampling areas had significantly higher carrot cyst densities than the other plant sequences.

Three Year Sequences **Plant factors** The data for total carrot yield in kg (Table 2) shows two distinct groups, with carrot/onion/ carrot/carrot producing the distinctly lowest yield and carrot/onion/corn/carrot producing the highest yield, all other treatments produced intermediate yields, not significantly different from either of the extremes. No significant differences were detected in the % of #1 carrots. In the % of forked carrots, only the carrot/onion/carrot /carrot had a significantly higher percentage than any of the other treatments, and in the % of stubby carrots, no significant differences were found.

Nematode Populations No significant differences were found in H. carotae cyst densities or M. hapla j2 counts prior to the planting of the rotation crops at the start of the second year. In the middle of the second year differences began to appear between the treatments. Both the onion and carrot sequences had significantly more H. carotae cysts than the corn, fallow and sudax (not cut). At the end of the second year, The H. carotae cyst densities were significantly greater in the carrot sequences than all of the others and the densities from the sudax (not cut) sequences were also higher than the corn sequences. This pattern was also followed in the H. carotae j2 densities showed an identical pattern. At

the start of the third year, the H. carotae cyst and j2 densities were essentially unchanged in pattern from the previous fall. The soil estimates from the mid year samples in the third year show both (cut and not cut) sudax sequences had greater H. carotae cyst densities than the oat, carrot or fallow sequences. Changes over the second year indicate that the carrot sequences had a significantly higher rate of increase in cyst density than all other sequences. When this comparison is extended from the start of the second year to the end of the third year, the carrot sequences have the lowest level of increase of all the sequences, by a significant margin. The comparison over the third year indicates that the oat sequences have a significantly higher increase in H. carotae cyst density than the other sequences.

Nematicide Sequences **Plant factors** The first year nematicide sequences show no significant differences between the three selected nematicides and the no nematicide control in the plant factors measured. This pattern continued through the subsequent two years of the study (Table 3).

Nematode Populations No significant differences in H. carotae populations as measured were found among the three selected nematicide sequences, or between them and the no nematicide control for the three years of this study.

Conclusions

Plant / Plant Sequences The total yield of carrots was relatively unaffected by the choice of plants in the 2 year

sequences. The effects upon plant factors are reflected in quality. These differences illustrate the inadvisability of a carrot/onion/carrot or carrot/fallow/carrot rotation as these rotations are as detrimental to plant quality as continuous carrot production. There appears to be little to chose between the three plant sequences, with the sudax as a statistically insignificant best choice. The suppressive effects of 2 year rotations upon the rate of population growth of H. carotae as compared to the rate of growth under continuous carrot production are reversed in the first year of carrot growth. In fact, the rate of growth in this first year of carrot growth after the growth of non-host crops exceeds the change in population over two years of continuous carrot growth.

The differences in the 3 year plant sequences are harder to distinguish. The carrot/onion/carrot/carrot and carrot/onion/oat/carrot appear to be distinctly poor choices. Both of these sequences produced significantly more unmarketable carrots, and the oat sequence produced the significantly highest rate of H. carotae increase. Otherwise there appears to be little to distinguish between the remaining 3 year sequences.

Plant / Nematicide Sequences The nematicides followed through the three years of this study were did not result in significantly lower populations of Heterodera carotae or the symptoms of this nematode on carrot plants.

Comparison of the plant/nematicide sequences with the plant/plant sequences is fraught with difficulties. There was not a planned randomization of plant/plant sequences with plant/nematicide sequences. Thus even though the materials and methods are almost complete in overlap, and the sampler / sampling error is consistent between these two studies, they are independent and direct comparison is difficult.

Limited conclusions can be drawn from comparisons of studies conducted in the same year. In the first year of the 2 year plant/plant sequences reported here, end of season nematode populations measured as H. carotae cysts per 100 cm3 of soil were reduced for 4 of the 6 plant sequences. This was not true for any of the nematicides applied to the carrots grown that year.

In the second year of the two year plant / plant sequence study the nematicide sequences of the same year held the nematode populations essentially unchanged for the season, but the H. carotae populations in the plant / plant sequences all increased at least 100% and all were significantly higher than in the plant nematicide sequences.

In the second year of the three year plant / plant sequences H. carotae populations were held stable in the plant / plant sequences where carrots were not grown, and the same was true in the plant / nematicide sequences of the same year. Population levels were similar in both studies throughout this year, except in the controls.

In the third year of the three year plant / plant sequences, all of the plant / plant sequences showed population increases over the season similar to the plant / nematicide sequences except for the onion / carrot / carrot sequence at -55% and the onion / oat / carrot sequence at +71%.There were no other significant differences between the plant / nematicide sequences (including the controls) or between the plant / plant sequences and the plant / nematicide sequences.

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Table 1. Influence of two-year plant sequences on carrot yields.

Crop Rotations 1987/1988/1989	Carrots		Carrots	
	Yield (lb)	#1 (%)	Forked (%)	Stubby (%)
Carrot/Carrot/Carrot	16.3 a	52 a	27 a	23 a
Carrot/Onion/Carrot	13.8 a	43 a	27 a	31 a
Carrot/Sudax-c/Carrot	16.0 a	77 bc	10 c	13 b
Carrot/Sudax-nc/Carrot	18.1 a	83 c	10 c	12 b
Carrot/Corn/Carrot	16.0 a	79 bc	8 c	13 b
Carrot/Fallow/Carrot	15.7 a	58 ab	17 b	25 a

Table 2. Influence of three-year plant sequences on carrot yield and quality.

Crop Rotations 1987/1988/1989/1990	Carrots		Carrots	
	Yield	#1 (%)	Forked (%)	Stubby (%)
Carrot/Onion/Carrot/Carrot	19.8 a	64	12 b	24 a
Carrot/Onion/Onion/Carrot	20.7 ab	85	4 a	14 a
Carrot/Onion/Corn/Carrot	23.9 b	87	4 a	9 a
Carrot/Onion/Sudax-c/Carrot	21.2 ab	88	1 a	16 a
Carrot/Onion/Sudax-nc/Carrot	22.6 ab	78	3 a	15 a
Carrot/Onion/Oats/Carrot	21.9 ab	75	5 ab	14 a
Carrot/Onion/Fallow/Carrot	23.1 ab	82	4 ab	14 a
Carrot/Carrot/Tomato/Carrot	23.5 b	73	8 b	17 a

Table 3. Results from Plant/Nematicide Sequence Sampling Year One and Three.

Treatment	Cyst PI	Cyst PF	PF-PI	Total wt of carrots	No. 1 % carrots
Year 1					
Telone II	42.4 a	100.6 a	58.2 a	17.7 a	71.5 a
Vorlex	53.2 a	156.4 a	103.2 a	17.0 a	57.2 a
Vydate	61.6 a	84.2 a	22.6 a	17.0 a	70.6 a
Check	65.2 a	156.0 a	90.8 a	16.0 a	75.7 a
Year 3					
Telone II	54.3 a	59.8 a	5.5 a	19.9 a	77.0 a
Vorlex	46.6 a	65.0 a	18.3 a	26.2 a	73.0 a
Vydate	56.0 a	47.0 a	-9.0 a	26.3 a	65.0 a
Check	88.6 a	51.5 a	12.8 a	26.7 a	53.0 b

SUMMARY

Daucus carotae, wild and cultivated carrot, was the only host of *Heterodera carotae* found in the study. Whether or not a carrot root system was infested with *H. carotae* or *Meloidogyne hapla* influences the effect of the root filtrates in stimulating hatch of *H. carotae* from cysts. Field population cysts responded less intensely to exudates from plants infested with these two nematode species. The age of carrot plants does influence the hatch of *Heterodera carotae* eggs within cysts. The carrot must be at least three weeks old to stimulate hatch. Cysts exposed to root filtrates through the lifetime of the carrot produce a lower percent of possible hatch than those exposed to root filtrates starting later in the life of the carrot. Cysts held in water respond differently than those held in soil, prior to the entry of those cysts into the experiment. Plant growth sequences effect *H. carotae* populations and carrot crop yields in subsequent years. One year of any tested monocot was superior to one year of any tested dicot for suppression of *H. carotae* effects on commercial carrot production. *Heterodera carotae* populations stabilize after two years of continuous carrot planting. The interruption of planting nonhost plants causes boom and bust cycles of *H. carotae* populations.

SECTION FOUR

Environmental Response

INTRODUCTION

Nematodes, like all poikilothermic organisms develop under a temperature controlled rate, not a strict time rate. Similarly moisture conditions can advance, retard or even prevent development. Temperature is key in the development rate of nematodes, and relatively little studied, in part because of the temperature variation over short vertical distances in soil. Since hatching is the first step in plant infestation, and since it is a discrete and measurable step, investigations on the temperature effect upon hatch of Heterodera carotae second stage juveniles from eggs within cysts were initiated.

EFFECT OF TEMPERATURE ON THE EMERGENCE OF *HETERODERA CAROTAE* FROM CYSTS

Abstract

Cysts of two populations of *Heterodera carotae* (carrot cyst nematode) were held at 5, 10, 15, 20 and 25 C in carrot root filtrates for at least two hundred days. The filtrates were collected from carrots grown in sterile sand. Cysts were selected from two populations one each field and greenhouse. No significant emergence of second stage juveniles occurred at 25 C. The total percent hatch at 5 and 20 C was significantly less than at 10 and 15 C. The two populations differed significantly in their response to temperature.

Introduction

Heterodera carotae (carrot cyst nematode) is found in Europe, Cypress and India as well as Michigan (Mathews, 1975; Berney and Bird, 1992). This species reproduces almost exclusively on *Daucus carota* (domestic and wild carrot) (Aubert, 1985). Significant results of carrot cyst nematode infestation occur to carrot plants. These range from leaf discoloration to root proliferation, storage root reduction to plant death (Lamberti, 1971).

Greco (1981) reported up to 74% hatch from cysts held in root diffusate at 10C. Cysts held outside from september to February had 90% hatch. Previous field and laboratory observations indicated that there were significant difference in the response of eggs within cysts to external stimuli between a greenhouse research culture population of Heterodera carotae (carrot cyst nematode) maintained for several years in a Michigan State University Nematology Program greenhouse, and a population of H. carotae collected from a field research site at Sheridan, Montcalm Co. Michigan. The Michigan greenhouse population (MGP) of carrot cyst nematode had been maintained in a Greenhouse where temperatures ranging from 7 to 40C were recorded on a seasonal basis, carrots were grown in the same soil for several years without other plants or fallow periods. The Michigan Field Population (MFP) of carrot cyst nematode was collected from a commercial carrot production field where surface soil temperatures during the growing season were observed to exceed 40C on a sunny day. In this field the soil temperature at 20 cm below the surface never exceeded 20 C during three growing seasons. Further, at a soil depth of 90 cm, the temperature never fell below 0 C in 2 consecutive winters. This variation in temperature did not prevent reproduction in both the field and greenhouse. MCP cysts did not respond as expected to controlled temperature hatching experiments. These findings led to the series of experiments reported here.

The objective of this research was to determine the influence of temperature on the hatch (or emergence) of juveniles from cysts of these two populations.

Materials and Methods

Cysts of the two populations of carrot cyst nematode were maintained at five temperatures: 5, 10, 15, 20 and 25 C in carrot root filtrate in Bureau of Plant Industry (BPI) dishes. Daucus carota used for the collection of root filtrates in these experiments were grown in the greenhouse. The initial filtrate and the material used for each change of filtrate were collected from the same plant for all cysts of both populations and all temperatures. All treatments were maintained in the dark at constant temperature in controlled environment chambers. For each temperature, seven BPI dishes, each with 10 cysts chosen for uniform size and color were suspended in 2.0 ml of carrot root filtrate. Each set of seven BPI dishes was held in a glass petri dish lined with a moist filter paper.

The terms emergence and hatch are used interchangeably in this paper. For the purposes of this paper, the terms hatch and emergence are used to indicate the process during which second-stage juveniles (j2s) of H. carotae leave the egg, and remove themselves beyond the cyst body. In carrot cyst nematode, hatch and emergence are sequential with no appreciable delay separating them. On very few occasions were j2s observed uncoiled within the cyst body.

MFP cysts used here were collected in the spring. The number of j2s hatching from cysts were counted daily, and removed from the BPI dishes on a weekly basis. The root filtrates were replaced weekly. All experiments were maintained for at least 200 days. All experiments were repeated once. Experiments were terminated when the total number of j2s emerging during two consecutive weeks was less than one per seven BPI dishes. At this time, all remaining cysts were crushed and the residual j2s and embryonated eggs counted. This was used to calculate the percent total emergence and other measures.

Analysis

There was no hatch of H. carotae at 25 C, based upon the criteria that less than 1% of potential hatch represents no hatch. For this reason only data from the four remaining temperatures will be discussed here. Analysis of an extended series of counts from such a large number of treatments makes analysis very complicated. Figure 1 illustrates the pattern of emergence from MFP at four temperatures. This figure clearly illustrates that the 10C and 15C temperatures are very different in emergence pattern from the 5C and 20C temperatures. To understand the differences between the emergence patterns of the two populations at the four temperatures, six points of mean comparison were used: The mean number of days to reach 10% emergence, mean number of days to 50% emergence, mean number of days to 90% emergence,

mean total percent emergence, mean number of days to peak emergence and mean number of j2s on peak emergence day.

Results

Rate of Emergence. The rate of emergence as measured at the 10% point of both MFP and MGP increased with increasing temperature (Fig. 2). At 10C the MGP population took fewer days to reach 10% emerged than the MFP population. The results were similar for the 50% emergence. Increasing temperature resulted in fewer days to reach the 50% emergence point. At 10C MGP population took significantly more ($p=.09$) days to reach 50% emergence (Fig. 3).

The times required to reach 90% emergence show a distinct temperature response pattern in both populations. MFP takes significantly fewer days to reach 90% emergence at 10 or 20C than at 5 or 15C (Fig. 4). For MGP, the response at 20C is significantly faster than at the other three temperatures. Comparison of the two populations shows that at 10 C, MGP is significantly slower to reach the 90% emergence point.

Total % Emergence. MFP had a significantly higher total percent emergence at 10C and 15C than at 5C and 20C (Fig. 5). MGP showed no response to temperature at this measurement. The differences between the two populations are apparent in the significantly higher total percent emergence of MGP over the MFP at 5 and 20C.

Days to Peak Emergence. The days to peak emergence shows the same general trend in both populations that the other non-

cumulative factors show (Fig. 6), a decrease in the number of days to reach a given point with increasing temperature. For both populations 20C shows the fewest days to reach the peak of emergence. The differences between the two populations show that MGP requires more days to reach the peak at 5C, 10C and 15C.

J2 Number on Peak Emergence Day. The results from MFP indicate that the 5C and 20C temperatures are alike but distinct from the 10C and 15C, which are also significantly different from each other (Fig. 7). MGP results show no significant differences between temperatures. Contrasting these two populations shows that at both 10C and 15C there were significantly more j2s emerged on the peak day for MFP than for MGP.

Conclusions and Discussion

MFP shows a significant temperature response for all six of the measurements used in the analysis. This population had the highest total percent emergence and the smallest number of days to 10% emergence at 15C. Conversely at 10C this population had the highest j2 number on peak emergence day. At 20C this population had the most rapid arrival at 50% and 90% hatch as well as peak emergence. MFP was slowest to respond in all non-cumulative measures at 5C.

MGP showed no significant differences in peak j2 number and total percent emergence by temperature. For the 50% and 90% emergence measurements as well as the days to peak

emergence, 20C was significantly lower than the other temperatures.

A comparison of the two populations shows that for total percent emergence, MFP is significantly lower at 5C and 20C than MGP, only because MGP does not respond to differences in temperature in this measurement, where MFP does. A similar pattern appears in the between population comparison of measurements at 3 temperatures of j2s on peak emergence day (Fig. 7).

Overall comparison of the two populations shows that MGP responds less to temperature differences and experiences less intense emergence response than MFP.

Though speed of arrival at percent based measurements was most rapid at 20C for both populations, the total percent emergence and number of j2s on day of peak emergence were at 15C and 10C respectively for both populations. Thus dependent upon measure used to determine it, the optimal temperature for the hatching of H. carotae is either 10C or 15C.

The differences illustrated here separate these two populations. The underlying mechanisms are not known. There are two major possibilities. The first of these is that genetic selection has occurred differentially in the field and greenhouse. The second possibility is that MGP has been conditioned to a different thermal response than MFP.

Further studies conducted concurrently with those reported on here indicate that the relationships between temperature, population and hatching response to carrot root

exudates are not the same when the carrot plant from which the exudates are collected is infested with H. carotae or Meloidogyne hapla (Northern Root-knot nematode).

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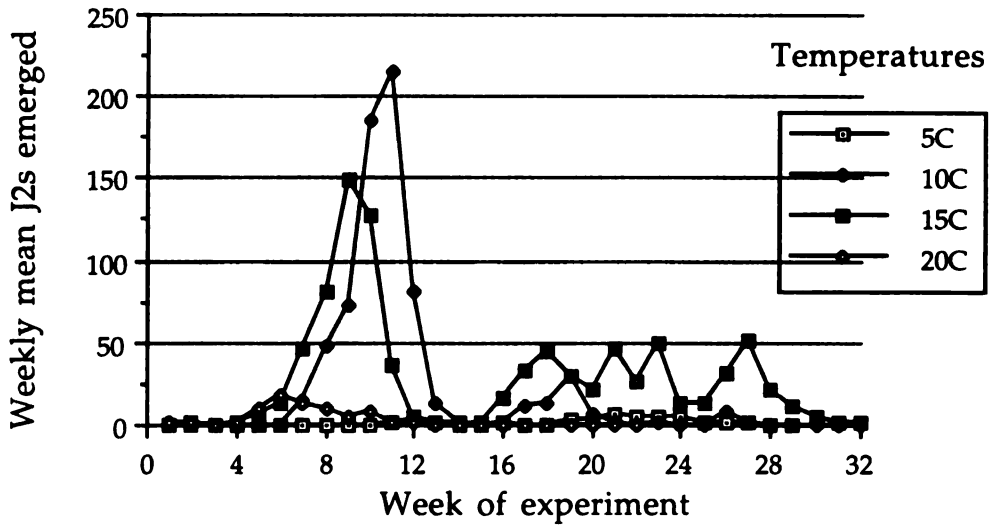

Figure 1. Mean weekly sums of J2s emerged from field population cysts held at four temperatures.

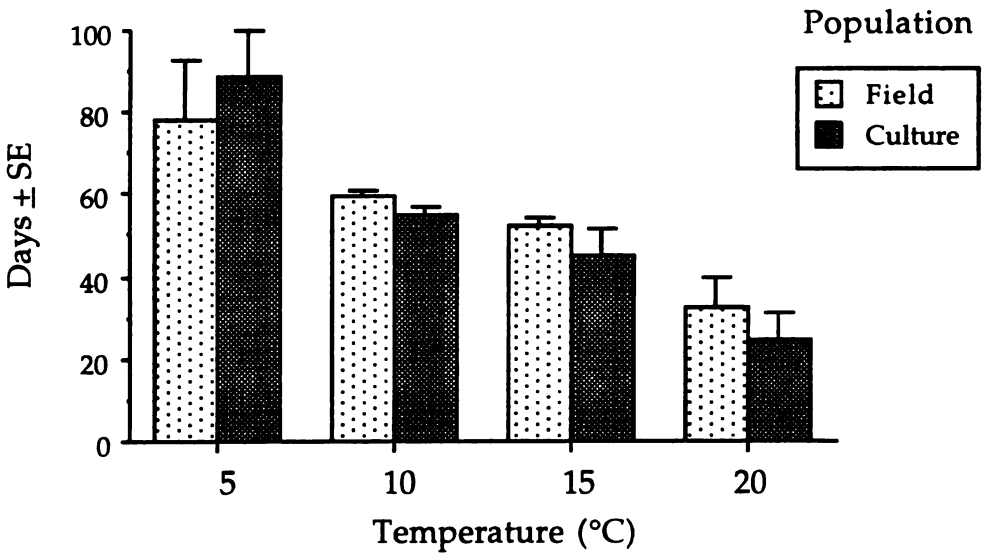

Figure 2. Mean number of days to reach 10% hatch for two populations of Heterodera carotae at four temperatures.

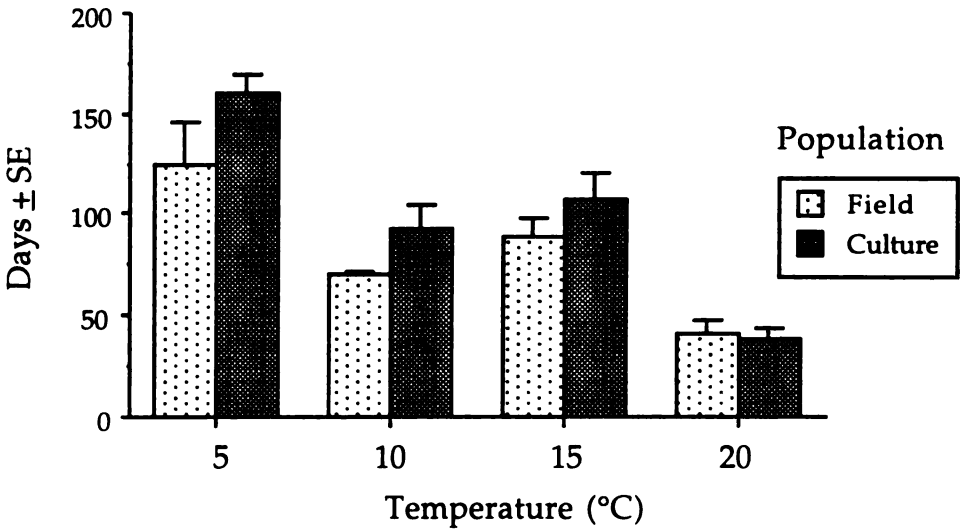

Figure 3. Mean number of days to reach 50% of hatch for two populations of Heterodera carotae at four temperatures.

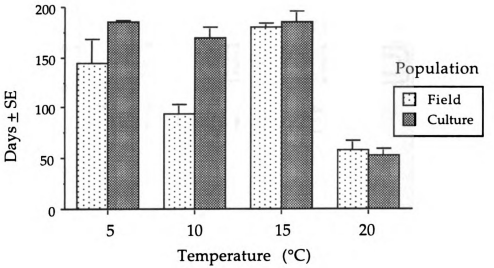

Figure 4. Mean days to reach 90% hatch for two populations of Heterodera carotae at four temperatures.

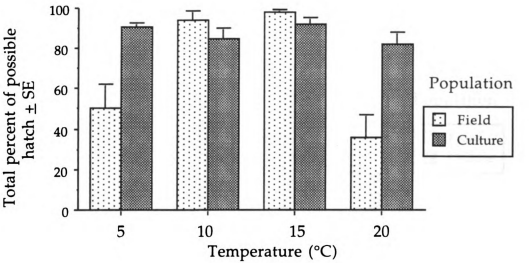

Figure 5. Total percent of possible hatch for two populations of Heterodera carotae at four temperatures.

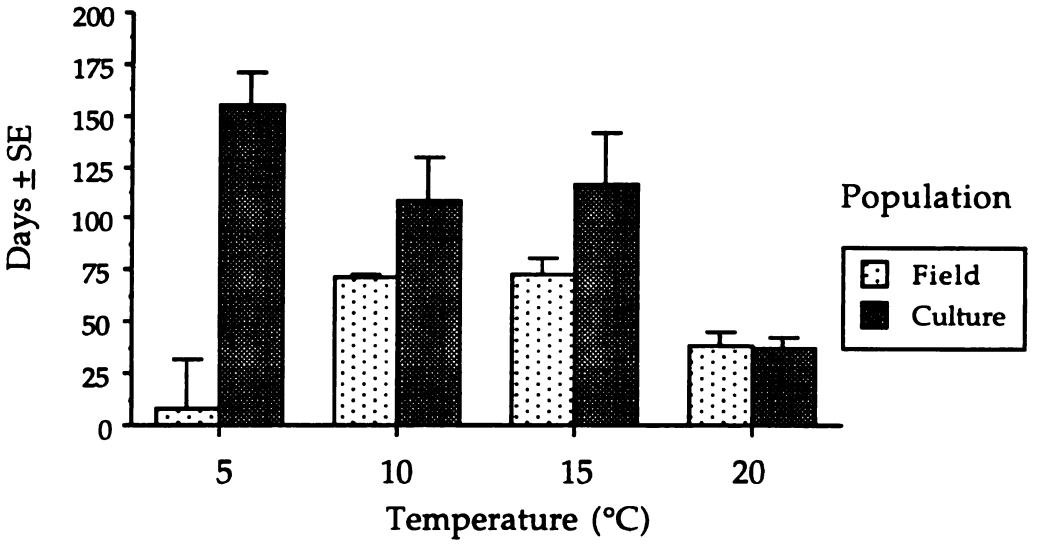

Figure 6. Days to reach peak hatch for two populations of Heterodera carotae at four temperatures.

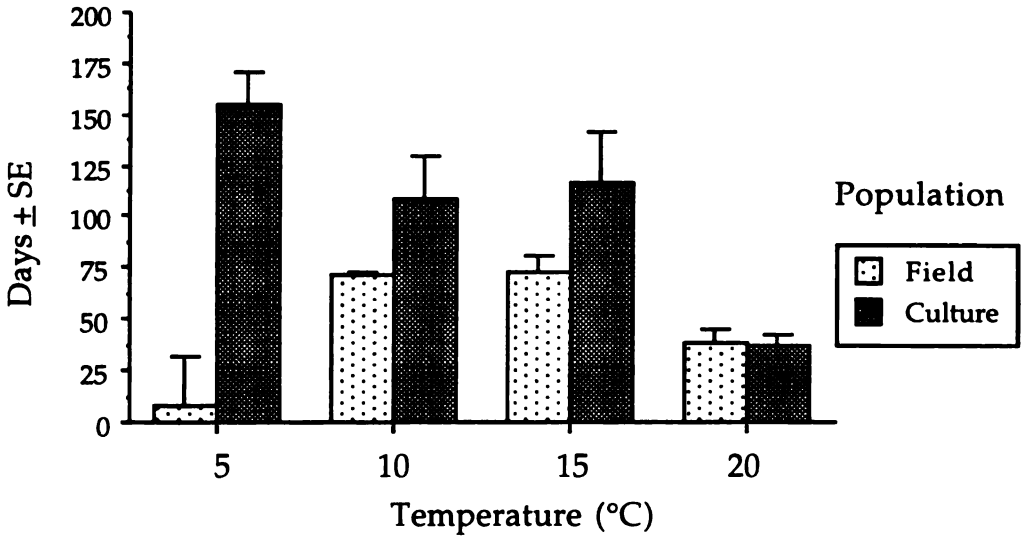

Figure 7. Number of J2s emerging on day of peak hatch for two populations of Heterodera carotae at four temperatures.

SUMMARY

Heterodera carotae eggs within cysts do not hatch at 25C. Hatch at 5 and 20C is much less as a percent of total possible than at 10 or 15C. The total number of second stage juveniles emerging on the day of peak hatch was also much higher at 10 and 15C than at 5 or 20C. The peak hatch is most intense at 10C, but occurs over a longer period at 15C.

SECTION FIVE

Summary

SUMMARY

In the Michigan carrot nematode survey, Heterodera carotae was detected in every histosol carrot growing region of Michigan. In every field where H. carotae was detected, Meloidogyne hapla, the northern root-knot nematode was also detected. Soil sampling for Heterodera carotae was found to be highly objective dependent as to proper method. The modified timed sampling procedure used in the survey was found to be an effective compromise between the aggregate sample and a long series of discrete samples. In vertical distribution soil sampling conducted in one carrot field, H. carotae was found in all soil profiles where carrot roots were present, but the majority of the population was in the top 30 cm of soil, where most of the carrot roots were.

In the series of ecological studies conducted, the known host range of H. carotae in MI was not expanded beyond Daucus carotae, the wild and cultivated carrot. Infestation of carrot roots with H. carotae or M. hapla reduced the intensity and altered the timing of the emergence of H. carotae from cysts. The age of the host carrot plant effects the hatch of H. carotae from cysts. The plant must be at least three weeks old to stimulate hatch. Plants older than five weeks stimulate a greater percent of total hatch. Older plants may

stimulate two discrete peaks of hatch activity. The sequence of plants grown in soil containing H. carotae has an effect on that population, and through this effect, on subsequent carrot crops. Monocots have the most impact of any plant type grown for one year. All plant types grown for two years have more effect than any one year of plant growth, on subsequent carrot growth. Heterodera carotae populations stabilize after two years of continuous carrot planting. Planting non-host plant types after one year of carrot growth, leads to wide swings in H. carotae populations.

Heterodera carotae eggs in cysts hatch most readily at 10C, but hatch for a longer time at 15C. Lesser degrees of total hatch occur at 5 and 20C. No hatch occurs at 25C.

APPENDIX A

THE USE OF SELECTED PRIMERS, PCR AND GEL ELECTROPHORESIS ON MITOCHONDRIAL DNA TO DIFFERENTIATE POPULATIONS OF *HETERODERA CAROTAE*

Abstract

Individual *Heterodera carotae* second-stage juveniles of selected populations were crushed, and subject to polymerase chain reaction (PCR) using selected primer pairs. The *H. carotae* populations included four from Michigan and one each from Italy and North Ireland. The primer pairs included: CO2 .105 - CO2 .215, C2F3 - 1106, ND4F3 - ND4R2, C2F3 - 860, N5R2 - SRF3 and C3F1 - C3R1. The resulting PCR products were subject to gel electrophoresis. The banding patterns were compared for similarities and differences. Only the ND4F3 - ND4R2 primer pair yielded a series of banding patterns sufficient to differentiate the European from the North American populations of *H. carotae*.

Introduction

To date the only reports of *Heterodera carotae*, carrot cyst nematode, in North America have come from Michigan. The origin of this species is presumed to be the same as for its most prominent host; *Daucus carota*, wild and cultivated carrot. This plant species originated in Iran/Afghanistan,

and is now cultivated throughout the world. First reported from England, H. carotae has since been reported from Europe, Cyprus and India, as well as Michigan. When or how this species entered Michigan is unknown. A Michigan (MI) field research population of H. carotae has shown considerable differences in response to environmental and host stimuli from the responses reported in the European literature. These differences and the presumed geographical isolation of MI from the nearest reported location of H. carotae motivated attempts to determine the similarity of the MI populations of H. carotae to European populations of this species.

A wide variety of biochemical and genetic techniques have been used to differentiate between species and among populations of a given species. Comparison of Nucleotide sequence is probably the most definitive of these. A short cut on the way to Nucleotide sequencing may be the comparison of polymerase chain reaction (PCR) products from different species or populations with a series of primer pairs. That technique was chosen here, as samples were prepared for Nucleotide sequencing.

Materials and Methods

Three Heterodera carotae populations were selected from research sites located in Michigan, Sheridan, Grant and Imlay City. In addition, a greenhouse research population was maintained at Michigan State University, was also selected. The Italian samples were provided by N. Greco from a carrot

field near Zapponeta, Italy. A population from northern Ireland was available from T. O. Powers of the University of Nebraska.

All nematode populations were extracted from soil and cysts were collected. Individual cysts were crushed and single second stage juveniles then selected. Each juvenile was processed separately. Specific regions of Mitochondrial DNA were selected for comparison by the selected primer sets. Primer sets included: CO2 .105 - CO2 .215, C2F3 - 1106, ND4F3 - ND4R2, C2F3 - 860, N5R2 - SRF3, and C3F1 - C3R1. Negative controls consisting of the mixture of all components, except a crushed nematode, and brought up to volume, were run with each amplification to detect contamination. Reaction profiles were: modified fast start, 94C 1 min., 45C 1 min., 72C 2 min. for 40 cycles. All reactions were run on a Perkins Elmer Cetus DNA Thermal Cycler. The resulting amplifications were subject to gel electrophoresis. All gels were composed of 200 ml of 2% agarose. The running buffer was 1% TDE. The charge was 94 volts from a BRL 250 power supply. Running time varied from 2 to 3 hours. The stain was 10 ul of 2.5 mg/ml Ethylene Bromide. Staining time was 10 minutes. A DNA "ladder" was included with all gels to help estimate the size of DNA fragments in each band. All gels were then photographed under UV light.

Results

All primer sets produced strong bands of amplified fragments of targeted DNA. CO2 .105 - CO2 .215, C3F1 - C3R1, C2F3 - 860, C2F3 - 1106 and C3F1 - C3R1 all failed to produce a consistent pattern of "accessory" bands resulting from non-target amplifications within each population. Only the ND4F3 - ND4R2 primer set yielded a consistent banding pattern of non-target amplifications. Repeated trials with this primer set yielded consistent banding patterns for each of four populations. A diagrammatic representation of these banding patterns is presented in Figure 1. The Sheridan and culture populations from MI share six bands in common. The MI populations share four bands with the Italian population, at 30, 40, 47 and 76. By contrast, the MI populations share only two bands, 46, and 76 with the Northern Ireland population. The Irish and Italian populations share six bands at 25, 28, 36, 47, 53 and 76. In contrast, the MI populations from Sheridan and the culture population have no bands different from each other. The MI populations have seven non-shared bands with the Italian population. The MI populations have 10 bands not in common with the North Ireland population. The Italian and North Irish populations have seven bands not in common (Table 1).

An arbitrary index comparing bands shared with bands not shared can be used to give a relative measure of relatedness.

The index value could range from 0 to 1, and was determined by the following equation:

$$\text{index value} = \frac{(2 \times \text{no. of common bands})}{\text{total no. of bonds from each population}}$$

This approach applied here indicates that the Sheridan and culture populations from MI are the same. The Italian and North Irish populations have a commonality index of 0.63. By the same measure the Italian population has a commonality with the MI populations of 0.53. The comparison showing the least relatedness is between the MI populations and the North Irish population where the commonality is only 0.29 (Table 1).

Discussion and Conclusions

The banding pattern resulting from the primer pair ND4F3 - ND4R2 could be used to distinguish european from MI populations of H. carotae. This same method was useful in distinguishing the Italian from Irish populations. Though a genetic basis is present for these differences, it is difficult to put any absolute measure to these differences. Subsequent nucleotide sequence work by Sui and Powers in 1992 revealed from 1 to 5% nucleotide variation from the MI populations for the North Ireland and Italian populations. No variation was detected among the MI populations. These findings support the findings presented here and provide a numerical measure or the differences described.

Table 1. Comparison of band similarities and differences for ND4F3-ND4R2 electrophoresis gel.

Population	Common bands	Different bands	Commonality index[1]
Sheridan X Culture	6	0	1.00
Sheridan or Culture X Italy	4	7	0.53
Sheridan or Culture X Ireland	2	10	0.29
Italy X Ireland	6	7	0.63

[1]Formula for commonality index: (2 x no. of common bands)/(2 x no. of common bands + no. of different bands).

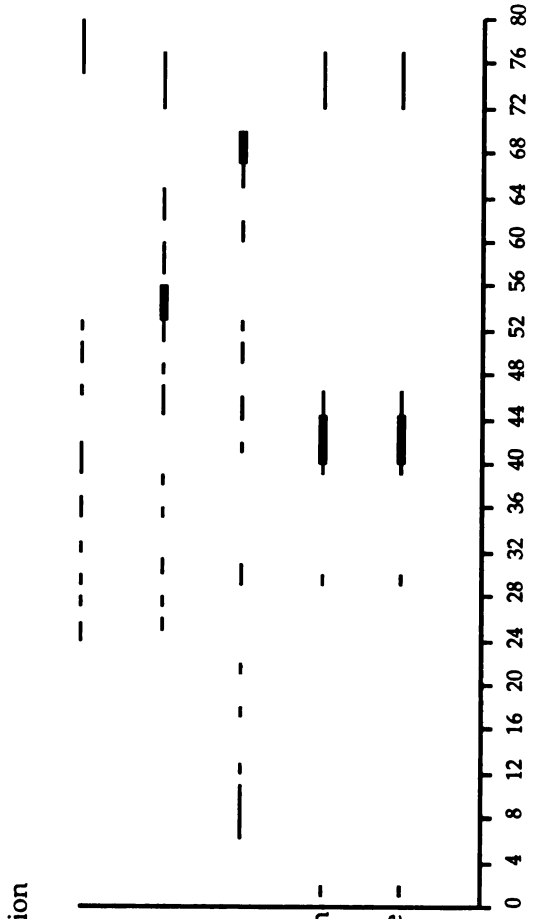

Figure 1. Diagram of Gel Electrophoresis results (to scale) for four populations of Heterodera carotae with the ND4F3-ND4R2 primer set.