THE LIFE HISTORY AND FEEDING HABITS OF *MESODORYLAIMUS LISSUS* THORNE, 1974

By

NESIBE NURDAN ERTEKIN

Bachelor of Science

University of Ege

Izmir, Turkey

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Thesis Approved:

Charles C Russell

Thesis Adviser

Larry J. Littlefield

Norman N. Durham

Dean of Graduate College

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INTRODUCTION

Mesodorylaimus lissus Thorne, 1974 is a member of the class Adenophorea and order Dorylaimida, equipped with a hollow, protrustive odontostyle (28).

The Dorylaimida are abundant in soils and are found around either roots of wild plants or cultivated plants as well as in bare soils. The members of the genus Mesodorylaimus live predominantly in soil but occur also in semi-aquatic biotypes. Mesodorylaimus spp. have been collected from forests, tulip fields, alfalfa fields, lake and stream soils, grass roots, oat fields, cantaloupe fields, and nursery soils (2, 4, 19, 28). However, there has been no attempt to determine the possible impacts exerted by these nematodes on their environment.

The feeding habits of this large group of nematodes are little known. They are considered as predacious species by several researchers because they possess large hollow odontostyles (8, 27); yet, the mode of prey selection of the predaceous nematodes are not well known. Dorylaimus species have been found to feed on mite eggs, oligochaet worms, rotifers, infusoria (17, 18), vegetative cells of the algae, on cysts of a protozoan, and on conidia of Cephalothecium (14, 15). Mesodorylaimus lissus was collected from the rhizosphere of wheat in Oklahoma. This nematode exhibited

parasitic feeding habit on wheat root hairs and reduced wheat seedling root weight, top weight and seedling emergence rate (25).

The objectives of this study were 1) to determine the complete life history of M. lissus from egg to adult in vitro 2) to determine the feeding habits of this nematode based on substrate suitability and substrate preference in vitro and 3) to determine the possible impacts exerted by M. lissus on the wheat and Amaranthus sp. rhizosphere and its interactions with Pythium irregulare and Bipolaris sorokiniana.

LITERATURE REVIEW

The genus Dorylaimus Dujardin, 1845 was revised by Andrassy in 1959. The new genus, Mesodorylaimus Andrassy, 1959 was proposed for those Dorylaimus spp. with a single odontostyle guide ring which exhibited sexual dimorphism in the tail (2). Mesodorylaimus lissus, with which the present study is concerned, was described by Thorne (28) in 1974.

The life history of M. lissus has not been studied. It is accepted that all molts usually occur outside the egg in other Adenophorea; thus, the first stage juvenile emerges from the egg (21). Russell (24) found that in Paratrichodorus christiei, which is the member of superfamily Dorylaimoidea, the juveniles underwent the first molt shortly after emerging from the egg, indicating that the nematode hatched while in the first juvenile stage. Ferris (9) examined the life histories of certain dorylaimids including Labronema sp. under laboratory conditions and stated that there were four molts following hatching of the egg.

Among the Adenophorean nematodes Xiphinema spp. is the most studied group because of its economic importance. The eggs of Xiphinema index required 6-7 days from oviposition to hatch and the first molt occurred 24-48 hours after hatch whereas the second, third, and forth molts occurred at approximately 6-day intervals thereafter under optimum tem-

perature conditions (23). However, Flegg (12) showed that development from egg to adult in Xiphinema spp. took at least two years under field conditions.

Flegg (11) was unable to obtain oviposition from gravid females of X. diversicaudatum and X. vuittenezi in water droplets. He conducted his embryology studies using eggs dissected from gravid females. The excised eggs showed the first cleavage between 24 and 48 hours after being removed. The time lapse between oviposition and hatching was 19-30 days. It was also shown that the odontostyle-forming cell began to lay down the replacement odontostyle about three days following the completion of the primary odontostyle. The formation of both odontostyles took 6-8 days.

The replacement odontostyle moves through the narrow lumen of the primary odontostyle extension during replacement but the time required for the migration is unknown (20). Goodey et al. (13) stated that each juvenile stage of X. diversicaudatum exhibited the primary odontostyle and one replacement whereas the third stage juveniles of some species of Dorylaimus had two replacement odontostyles. They also mentioned that after each molt a new odontostyle developed in the esophageal cell and pierced the esophageal wall while migrating into position during molting. It was observed that the odontostyle, the guiding sheath, and the lining of the odontostyle extension were shed during the last molt; however, because the other molts were not observed, the authors assumed similar processes for other

molts. Later Coomans and De Coninck (6) confirmed that this phenomenon was true for all molts when they studied the odontostyle formation in Xiphinema in detail using the juveniles fixed during the process of molting. They found that the juveniles during inter-molt periods retained two odontostyles and molting juveniles in most cases had three odontostyles. Similar observations were made on Dorylaimus juveniles and the replacement odontostyle was found situated within the esophageal wall surrounding the basal portion of the primary odontostyle while the juvenile was within the egg shell. The position of the replacement odontostyle in the second, third, and fourth stage juveniles was always posterior to the basal portion of the primary odontostyle.

The feeding habits of the Dorylaimida that are not known parasites of higher plants are little known (10, 22). It is stated that most dorylaims that are large, active nematodes possessing an unusually large well defined hollow odontostyle and powerfully muscular esophagus are predaceous (8, 10, 17, 18). Thorne and Swanger (29) observed that young plants suffering from damping-off diseases were surrounded by large numbers of common soil inhabiting dorylaims. They pointed out that it was possible that punctures made by the nematodes offered suitable openings for the entrance of the fungi responsible for damping-off. However, the specimens they had observed were in situations which suggested them to be predacious.

Thorne and Swanger (29) quoted the following remarks

from N. A. Cobb's notes on the carnivorous nature of some dorylaims: 1) Occurrence of matter from the intestine of dorylaims could have been derived only from the body contents of other animals. 2) Finding of slain animals whose intestines might have furnished the matter observed. 3) Observations of dorylaims with their odontostyles inserted into animal tissues, and failure to find a similar relationship to vegetable tissues. 4) Presence of dorylaims in the absence of suitable vegetable food e.g., in slow sand filter-beds, deep lakes, river sand etc. 5) Persistence for several years of relatively large numbers of dorylaims in bare fallow soil, thus deprived of the roots of the higher plants hitherto supposed to be one of their main sources of food. 6) Disappearance of certain dorylaims, presumably from starvation, in pure cultures of vegetable organisms. 7) Failure to discover any quantity of starch or other characteristic vegetable remains in the intestines of certain dorylaims. 8) The renette which, as in other carnivorous nematodes, was absent or difficult to see. They also reported that Dr. Cobb had observed Dorylaimus carteri feeding on the eggs of mites in a decaying narcissus bulb and D. serpentinus feeding on the body of another nematode.

The feeding habits of various species of Dorylaimus has been observed in vitro. It was found that they fed on nematode eggs, rotifers and large infusoria (17). Linford and Oliveira (18) repeatedly observed Dorylaimus spp. feeding upon other nematodes including juveniles of

Heterodera marioni. Their observations were in petri dishes of agar and Dorylaimus spp. sucked their food through hollow odontostyle. They stated that a predacious dorylaim found its prey only by chance, but when its head made contact with another nematode it responded immediately and considerable pressure was required to thrust its coarse hollow odontostyle into other nematodes. Some of these Dorylaimus spp. had been seen feeding on eggs both of H. marioni and of various free-living forms.

The first omnivorous habits of dorylaims were reported by Hollis and Fielding (16). They found that Dorylaimus ettersbergensis was able to feed on the conidia of a moniliaceous fungus probably belonging to the genus Cephalothecium and on the non-motile cysts of a small, free-living, ciliated infusorian of the genus Drepanomonas. The nematode was not observed to feed upon the fungus hyphae or the motile ciliate stage of the protozoan. D. ettersbergensis fed on parasitically on globose cells of a blue-green alga (Chroococcus sp.), green algae (Chlorella vulgaris, Tetraedron sp.), a protozoan (Drepanomonas sp.), and a fungus (Cephalothecium sp.) in water-agar cultures (14, 15). The hosts were rated for their effectiveness in promoting rate of increase and final level of nematode population in cultures and were ordered as seen above. The results demonstrated that Chroococcus sp. was the most favorable host, and feeding on fungus spores, although intensive in culture, provided the nematode supplemental food only (14).

Russell (25) recorded that Mesodorylaimus lissus collected from the rhizosphere of wheat showed an omnivorous feeding habit in vitro. The nematode fed predaceously on other nematodes and encysted amoebae; parasitically on fungal hyphae, algae and wheat epidermal cells; and microphagously in colonies of bacteria, actinomycetes and globules of a human dietary preparation. This nematode caused up to 70 per cent reduction in root weight, 46 per cent in top weight of wheat seedlings and reduction in seedling emergence rate in the greenhouse Mesodorylaimus spp. have also been collected from forests, tulip fields, alfalfa fields, lake and stream soils, grass roots, oat fields, cantaloupe fields, and nursery soils (2, 4, 19, 28).

MATERIALS AND METHODS

Embryonic Development

Gravid Mesodorylaimus lissus females were obtained from stock colonies actively feeding on Panagrellus redivivus on 0.75 per cent water agar. Individual females were picked from the colonies and placed on a 0.75 per cent water agar medium in a 100X15 mm petri dish. The females were periodically observed until egg deposition at which time the each egg was transferred to a small drop of 0.01 per cent streptomycin sulfate solution on a 25 mm square coverslip. The egg was positioned at the bottom center of the droplet in contact with the coverslip to facilitate observation and photomicrography. A drop of warm (30-32°C) 1.5 per cent water agar supercooled by stirring and approximately equal in volume to the streptomycin sulfate droplet was added. After the agar drop solidified, the coverslip was inverted onto a concave microculture slide and its edges were sealed with stopcock grease to retard evaporation.

Eggs were examined and photomicrographs were taken through the oil immersion objective of a light microscope. The number of the individual egg, developmental stage and time when the photomicrographs taken were recorded for 30 individual eggs.

An additional series of 200 eggs were examined to verify time lapses between the embryonic stages recorded in the photomicrographic series. Newly laid eggs were selected for this series from M. lissus stock colonies and transferred by capillary tube onto the surface of a 2 mm thick 0.75 per cent water agar layer in 100X15 mm petri dishes. A 13 µm thick plastic wrap film was cut by using a 8.4 cm template (26) and the surface of agar was carefully covered with this plastic wrap disc. The eggs in each petri dish were mapped and numbered to ease record keeping. Observations were made with the oil immersion light microscopy at 15 minute intervals through the blastula stage, at one hour intervals through the tadpole stage, and three hour intervals until eclosion. The date, time, the stages of eggs, and egg numbers were recorded.

Post-embryonic Development

Morphometrics

Juveniles of each of four approximate size classes were collected from the colonies of M. lissus and temporary slides were made. The juveniles were picked with a dental pulp canal file and placed into a small water drop on a glass slide. The juveniles were gently heated and relaxed over an alcohol lamp. The water drop was covered with a 22 mm square coverslip and sealed with zut. Drawings of the freshly mounted juveniles were made with the aid of camera lucida at the magnification of 800X or 2000X depending on

the size of the juvenile. Calibration was made or confirmed using a stage micrometer at each drawing date.

Eighty-nine eggs containing postembryonic juveniles were picked up from the M. lissus colonies and placed onto water agar medium. Observations were made periodically until each juvenile hatched from the egg. Upon eclosion each juvenile was picked up with a dental pulp canal file and a temporary slide was made. A drawing of each newly hatched juvenile was performed using the camera lucida. A series of 35 newly hatched juveniles were transferred to agar medium in seperate petri dishes and provided with P. redivivus for prey. Each petri dish was numbered and the date and time of juvenile hatching were recorded. These juveniles were observed through a binocular dissecting microscope until they entered premoult quiescence. The quiescent juveniles were then removed and temporary slides and drawings were made as described above.

The total length, tail length, the ratio of total length to the greatest body width (a), the ratio of total length to tail length (c), each odontostyle length, the relative positions of primary and replacement odontostyles, the esophagus length and the number of odontostyles were recorded.

Ontological Development

A drop of warm water agar was placed onto a 22 mm square coverslip. A newly hatched juvenile was transferred

into water agar droplet and two male P. redivivus were added to provide prey for the developing juveniles. Males only were used to avoid P. redivivus reproduction which would make locating the developing M. lissus juvenile more difficult. The coverslip was inverted onto a concave microculture slide and sealed with stopcock grease. Observations were made through a light microscope at 5 hour intervals until the juvenile became quiescent, during quiescent period and until molting had been completed. Each concave microculture slide was numbered and the date, time, the stages of juveniles were recorded.

Feeding Habits

Mesodorylaimus lissus, formerly considered a predaceous species, has been reported to be omnivorous in habit (25). Most omnivores have a primary feeding habit which can, to some degree, be predictive of the impact they will exert on their ecosystem. The primary feeding habit can be determined by food substrate suitability studies and/or by food preference studies.

Substrate Suitability Studies

Food substrate suitability, like host suitability, may be compared on the basis of the level of nematode reproduction supported by the respective substrates.

Studies were conducted by using the in vitro observation technique described by Russell (26) to compare

M. lissus reproduction on various food substrates. In the first study different substrates and feeding habits were compared on the basis of nematode reproduction. The substrates and feeding habits compared were: Predaceous habit on live P. redivivus, saprophagous habit on dead, air dried P. redivivus and on a human dietary compound (Similac), and no food source as a control to determine level of reproduction possible utilizing stored food reserves in the adult females.

The live P. redivivus substrate was prepared by scraping nematodes from the sides of the stock colony jar with a razor blade, transferring them to a beaker of tap water, triple washing them by suspension, settling and decanting, and finally concentrating them in a hanging dropper. A drop of concentrated live nematodes was then placed directly from the dropper onto the agar surface. The dead P. redivivus were similarly prepared except that the concentrated live nematodes were first placed on filter paper disks, allowed to air dry until 100 per cent mortality was obtained due to desiccation (approximately one hour) and transferred to the agar surface with a dental pulp canal file. About 8 milligrams of Similac was sprinkled onto the agar surface. Ten M. lissus females and one male to assure mating were extracted from in vitro stock colonies and introduced to each petri dish prior to installation of the disc of plastic wrap covering the entire agar surface.

The petri dishes were arranged vertically in a ran-

domized complete block design with 5 replications and maintained in plastic freezer bags at room temperature (22-23°C) between data collection intervals. Egg counts were accomplished by removing the petri dish cover of each unit and placing the bottom inside a petri dish cover which had been scratched to make a grid with 4 mm sectors. The grid with the *in vitro* unit installed was placed on a binocular dissecting microscope stage for counting. Because the minimum time required from oviposition to eclosion was five days, the counts were done at 5-day intervals and the number of eggs was recorded. This study was duplicated and data were subjected to analysis of variance and LSD.

In the second substrate suitability study *Pythium arrhenomanes*, *Bipolaris sorokiniana* (myceliophagous), and three isolates of Actinomycetes (microphagous) supplied by Dr. L. L. Singleton, *Rhizoctonia cerealis* (myceliophagous) obtained from Dr. K. E. Conway, wheat roots (parasitic), and *P. redivivus* (predaceous) were compared. All microorganisms were grown on PDA and wheat seeds were germinated in aerated water until the radicle reached 3 mm length.

At the time of the introduction of the candidate substrates to the *in vitro* units both agar and the plastic wrap were cut and a section was removed as described by Russell (26). All units had a section removed and were sealed in the same manner in order to maintain a uniform unit volume whether or not a seedling was introduced.

A germinated wheat seedling was placed approximately in

the middle of cut surface by inserting the radicle into the agar. Fungi, actinomycetes and nematodes were transferred to the center of the agar surface with the aid of a dental pulp canal file while the plastic disc was temporarily folded back to expose the agar surface. All units were maintained at room temperature (22-23°C) in plastic bags at 45° inclination with the film surface and the radicle pointing downward (26). They were arranged in a randomized complete block design with 5 replications. When the leaf of wheat reached 1 mm length the cut surface of all plates was sealed with low melting point wax (50:50 petroleum jelly / beeswax) and maintained as described above.

Egg counts were performed at 5-day intervals during which eclosion was expected according to the results of embryonic development study. Egg counts were continued until agar medium dried out or any substrate became limiting. To confirm reproduction level, juveniles were extracted by modified Christie-Perry technique in which 450 ml plastic flower pots without drain holes were substituted for the funnels, and a single layer of facial tissues substituted for the muslin were used (1). The plastic wrap of each plate was removed by adding a small amount of water at the its edge prior to removal of the agar layer by scraping it onto a single layer of tissue installed on an extraction tub. After 48 hours the wire mesh and the facial tissue were removed, the sample was allowed to settle for approximately one hour, and serially decanted to a volume of

approximately 10 ml which was placed in a counting dish. Counts were made with the aid of a binocular dissecting microscope. However, the recovery of juveniles was less than expected according to the counts performed; therefore, in the following studies the plastic wrap disc as well as agar itself was placed onto tissue to assure recovery.

The third substrate suitability study was conducted including the microorganisms used in the second study and in addition the saprophagous nematodes, Diplogaster sp. and a mixed colony of Cephalobus sp. and Eucephalobus sp. (cephalobids) as prey species. The in vitro units were maintained in a randomized complete block design with 5 replications as described above. Saprophagous nematodes were obtained from soil extraction and maintained on 0.75 per cent water agar using decomposing fish as a nutrient source. The introduction of the candidate substates, maintenance of in vitro units, and counts were as described in previous studies.

Substrate Preference Studies

As previously stated, one method of determining the primary feeding habit of an omnivore is through substrate preference studies.

Until recently, members of the Dorylaimoidea which were not known to be plant parasitic were considered to be predaceous. Further, the prevailing opinion, undoubtedly based on Linford and Oliveira's early observations (18) has been

that they are rather primitive predators which locate their prey entirely by chance encounter. If predators in the Dorylaimoidea lack the advanced chemoreception capabilities found in the Secernentea no substrate (prey) preference is likely. The prey presenting the greatest obstacle value due to number and/or size should be the most frequently fed upon and no preference should be exhibited. This is not consistent with in vitro observations made during this study. Besides, Wright (31) speculated that adenophorean cuticular sense organs were all chemoreceptive and amphids contained more chemosensory units than did in secernentean nematodes.

The null hypothesis tested in the first study was: if equal numbers of candidate prey nematodes of different volumes (i.e., size or obstacle value) are presented to a predator, on the basis of chance encounter the larger prey will be fed upon most frequently. The null hypothesis in the second study was: if equal volumes of candidate prey nematodes are presented to a predator, on the basis of chance encounter each nematode species, regardless of individual size will be subject to the same number of predaceous events.

The candidate prey nematodes used in both of these studies were collected from wheat field soils. Two colonies of microphagous nematodes were established on 0.75 per cent water agar using decomposing fish as a nutrient source. One colony was a single female colony of a Diplogaster sp. The second microphage population was a mixed colony of

Cephalobus sp. and Eucephalobus sp., both genera of the family Cephalobidae. The 'cephalobid' populations were inadvertently mixed but being of equal size and habits were used as a single candidate prey choice. The Diplogaster sp. were used as a large microphagous candidate prey species and the cephalobids were the small microphagous prey species. Because of complications anticipated from endotokia matricida occurring in the very largest female Diplogaster sp. specimens, the suspensions of this nematode were screened through an 80 mesh sieve prior to morphometric characterization. The numbers of microphagous nematodes introduced as candidate prey were hand picked by using a dental pulp canal file in the nematode number study. In the nematode volume study microphage numbers were determined by aliquant. The plant parasitic nematodes, Paratylenchus sp. (small size) and Pratylenchus sp. (medium size) were extracted from field soil as needed. The Hoplolaimus sp. (large size) were extracted from a laboratory stock colony. Requisite specimens of plant parasites were hand picked by using a dental pulp canal file. The in vitro observation unit and petri dish cover grid described under food substrate suitability above were used for both number and volume studies.

Prey Nematode Number Study. Ten nematodes for each candidate species were randomly selected for this study. The specimens were picked into a water filled BPI watchglass, drawn into a dropper and concentrated by settling. The candidate prey nematodes were then transferred to the

agar surface in a single small droplet of suspension. A small droplet containing 10 M. lissus females was prepared in the same manner and added to the prey droplet prior to installation of the plastic wrap disc. Observations were made at 2 and 3 hour intervals and feeding events recorded by prey species. Only a single feeding event was recorded per grid sector to prevent duplicate recordings of a single feeding event. Observations continued until the prey nematode population became limiting and no feeding events were observed at which time the study was terminated. This study was repeated one time.

Prey Nematode Volume Study. Randomly selected specimens, 10 for each prey species, were picked using a dental pulp canal file and temporary slides were prepared for each. A camera lucida drawing of the body outline of each specimen was performed. The mean relative volume for each specimen was calculated using the formula for determination of the volume of a cylinder ($\pi R^2 H$). The mean diameter of each specimen was determined by dividing the body length into four equal parts and taking the average of the body width at the three resultant intersects. Total body length was used as height (H) in the formula. The total body volume of ten Hoplolaimus sp. (the largest nematode) was calculated and the number of each candidate prey species specimens required to constitute an approximately equal volume was calculated by proportions. The appropriate number of each prey species specimens: 10 Hoplolaimus sp., 140 Pratylenchus sp., 420

Paratylenchus sp., 488 Diplogaster sp., and 867 cephalobids was added to a BPI watchglass. The accumulated prey nematodes and 10 M. lissus females were introduced to the in vitro observation unit as described above. Observations were made at 2 and 3 hour intervals as given above. Recording the feeding events continued until the agar dried out. This study was repeated one time.

Rhizosphere Interaction Study

Mesodorylaimus lissus has been shown to have a significant impact on wheat biomass production in the greenhouse under experimental conditions described under Koch's postulates (25). The nematode does not, however, occur in monospecific populations in the rhizosphere under field conditions. Therefore, the results of a conventional pathogenicity trial, while demonstrating a pathogenic potential, cannot be considered predictive of the organisms impact on a natural rhizosphere. This is especially true of the omnivorous M. lissus which could have an impact ranging from enhancing the pathogenicity of associated fungal pathogens by providing infection courts to reducing the inoculum potential of these pathogens by feeding directly upon them. Alternatively, M. lissus might exert no influence on the host parasite relationship of a concomitant pathogenic fungus if it were feeding preferentially as a predator on other nematodes.

The objective of this study was to elucidate the effect

of three population levels of M. lissus on wheat (Triticum aestivum 'Chisholm') and Amaranthus sp. (a common genus of weed in wheat fields) and determine the nematode's interactions with concomitant infestations of Pythium irregulare and Bipolaris sorokiniana.

This study was conducted under a laboratory light bank conditions (At av. 4300 Lux light intensity for 24 hrs. per day and 26∓ 1°C). Treatments consisted of three inoculum levels of M. lissus applied alone and in combination with P. irregulare and B. sorokiniana to both wheat and Amaranthus sp. for a total of 18 treatments. Although the study was installed with six replications, weak germination of wheat seeds in the control of replications one and two made it necessary that they be deleted. Therefore, analysis of variance and LSD were conducted on the basis of a randomized complete block design with four replications.

Sterile paper towel disks were placed to cover the drain holes in 300 ml plastic pots. All pots were then filled to within 3 cm of the top with methyl bromide fumigated Lincoln fine sand (first LFS layer). Two more LFS soil layers completed the experimental unit. A two cm thick (100 grams of dry soil) second layer of LFS wetted with 14 ml sterile water (non-inoculated) or 14 ml fungal suspension (inoculated) as described below and a 0.5 cm deep (50 grams) cover soil zone were applied over first LFS layer, respectively.

All pots were inoculated with one of three inoculum

levels of M. lissus (0, 250, and 500). Inoculum of M. lissus was extracted from stock colonies by using modified Christie-Perry extraction technique as described under substrate suitability studies. Stock inoculum suspension was prepared and a requisite 1 or 2 ml extracted by aliquant to obtain 250 and 500 nematodes. The aliquant was then diluted to a uniform volume of 10 ml and evenly pippetted to the first LFS surface. Ten ml of sterile water was applied to LFS surface for the no nematode treatment. Three 'Chisholm' wheat seeds were planted at approximately 2 cm intervals by depressing them slightly into the first LFS soil surface and then covered. This resulted in a planting depth of 2.5 cm where the effect of M. lissus and its combinations with fungi on wheat were tested.

The second layer of LFS was mixed with 14 ml sterile water in double plastic freezer bags by shaking and placed over the first LFS layer where the effects of M. lissus alone was investigated. To examine the combined effects of M. lissus and B. sorokiniana, the second layer of LFS was mixed with 14 ml of spore suspension containing 15000 conidia of B. sorokiniana obtained by a surface wash of colonies grown on PDA. LFS and the spore suspension were placed in a double plastic freezer bag and mixed by shaking until uniform distrubution was obtained. The resultant inoculum level was 150 conidia per gram of LFS. To identify the combined effects of M. lissus and P. irregulare, the second layer of LFS was mixed with 0.588 grams of P. irregu-

lare which was obtained from Dr. L. L. Singleton at the level of 2549 propagules (oospores) per gram of soil and 14 ml of sterile water to maintain treatment uniformity in a double plastic freezer bag and shaken until uniform distribution was obtained. Thirty seeds of Amaranthus sp. were sprinkled over the surface of second LFS layer, due to small seed size, where the effects of M. lissus and the combinations of nematode and fungi on Amaranthus were tested.

The second layer of LFS of all pots was then covered with cover soil zone. Water was provided from the bottom by capillarity after the introduction of inocula and as needed throughout the study.

Germination rate was determined by daily seedling emergence counts. The experiment was terminated 12 days after inoculation. Plants were carefully removed from the pots and gently washed to remove soil and uniformly blot dried with sterile paper towels prior to taking fresh top and root weights.

The soil of each pot was processed by modified Christie-Perry technique previously described to determine final nematode population. Subcrown internode necrosis was rated according to 0-3 severity (16) to define the level of B. sorokiniana infection and wheat subcrown internodes were plated on a selective medium (27) for confirmation. Wheat seminal roots were plated on a Pythium selective medium (4) to confirm infection levels of P. irregulare. The below

ground parts of *Amaranthus* sp. were also plated on the appropriate *Bipolaris* or *Pythium* selective medium. The plates of *B. sorokiniana* were maintained at 23°C while those of *P. irregulare* were maintained at 15°C for a seven day incubation period. The plates of *B. sorokiniana* were read + or - indicating the presence or absence of growth. The plates of *P. irregulare* were examined daily and the infection sites (hits) were marked. At the end the seven-day period the plates where *P. irregulare* had grown were selected. Seminal root drawings were obtained by inverting the plates on a light table, covering the plate with a sheet of paper and tracing the resultant root shadow. The drawings were then measured using a map measurer.

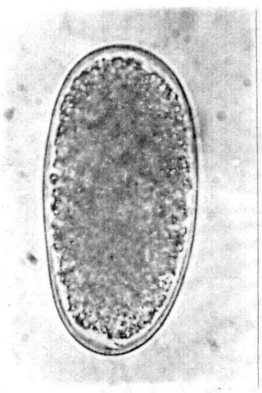
RESULTS AND DISCUSSION

Embryonic Development

Eggs were laid singly and they were oval in shape. Feeding was necessary for the oviposition, similarly Xiphinema diversicaudatum and X. vuittenezi did not lay eggs in a water droplet (10). Newly deposited eggs were always one-cell stage (Fig. 1a) and required 2-3 hours resting period before any cytoplasmic movement was observed. This phenomenon indicated that there was no embryonic development inside the uterus. The first cleavage leading to the 2-cell stage began on an average 6 hours (∓2 hrs) following oviposition (Fig. 1b). Cleavage to the 2-cell stage was completed in 2.5 hours (1.75-3 hrs). The two blastomeres were of approximately the same size and the division was transverse (Fig. 1c). The second longitudinal cleavage giving rise to the 4-cell stage started at 2.25 hours (1.5-2.5 hrs) following the first cleavage. The anterior blastomer divided before the posterior one resulting in 3-cell appearance (Fig. 1d). After 15 minutes (∓5) the posterior blastomer divided and 4-cell stage was completed in 1.25 hours (∓0.25 hrs) following the initiation of the second cleavage (Fig. 1e).

The 8-cell stage, the result of the longitudinal

Figure 1. Embryonic development of Mesodorylaimus lissus from one-cell stage to blastula stage.
a= One-cell stage
b= Initiation of first cleavage
c= Two-cell stage
d= Three-cell stage
e= Four-cell stage
f= Eigth-cell stage
g= Sixteen-cell stage
h= Morula stage
i= Blastula stage



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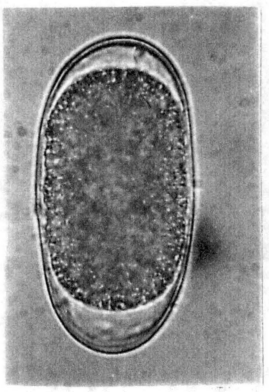

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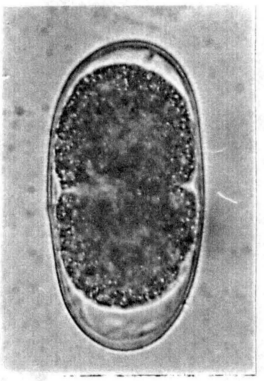

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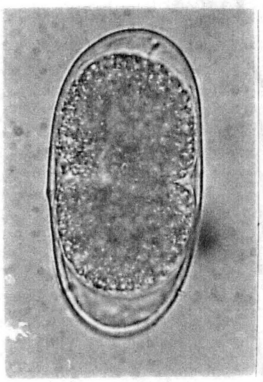

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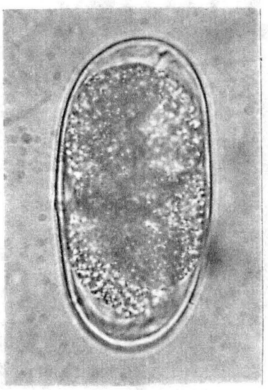

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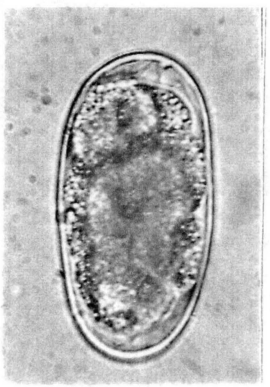

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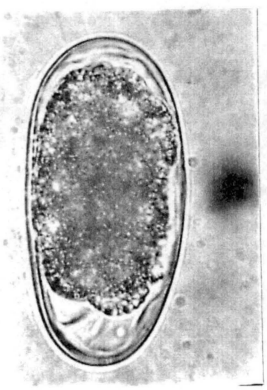

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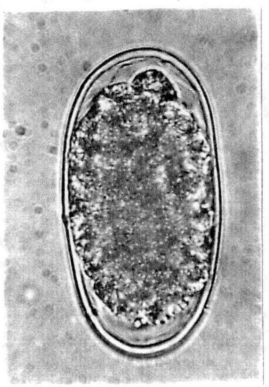

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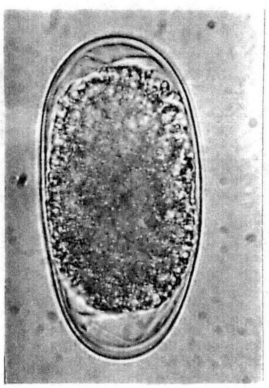


i

simultaneous divisions of the four cells was accomplished in 3.25 hours (2.5-4 hrs) immediately after the 4-cell stage (Fig. 1f). After completion of the 8-cell stage, the individual cells continued revolving until following cleavage leading to the 16-cell stage occurred. The completion of cleavage to the 16-cell stage was observed in 3 hours (1.5-5 hrs) (Fig. 1g). During this division the cleavage pattern could not be determined.

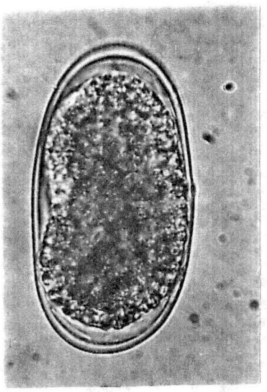
The morula stage followed 16-cell stage completed in 2.5 hours (2-3 hours) (Fig. 1h). The morula stage lasted 10 hours (7-12.5 hrs).

Blastula stage where the embryo was in the form of a hollow ball with all blastomeres arranged in a single layer around a central blastocoel continued for 20.5 hours (19-22.75 hrs) (Fig. 1i). During this stage cleavages continued and the blastomeres diminished in size.

Gastrulation was characterized by convex dorsal and concave ventral surfaces. The early gastrula stage (Fig. 2a) lasted for 2.25 hours (1-3 hrs). The middle gastrula stage (Fig. 2b) continued for 7.25 hours (5-8 hrs). The late gastrula stage (Fig. 2c) was the longest period among the gastrula stages and terminated by the formation of tadpole stage in 12.5 hours (11-13 hrs).

Tadpole stage began when the anterior end was hyaline and broader than the dark colored posterior end (Fig. 2d). At that time the embryo was vermiform and began slow rhythmic movement which continued for 2 hours (Fig. 2e and

Figure 2. Embryonic development of *Mesodorylaimus lissus* from early gastrula stage to eclosion.
a= Early gastrula stage
b= Middle gastrula stage
c= Late gastrula stage
d= Early tadpole stage
e= Tadpole stage
f= Tadpole stage (moving)
g= First stage juvenile with two odontostyles
h= First stage juvenile
i= Eclosion (Note empty chorion near the juvenile)



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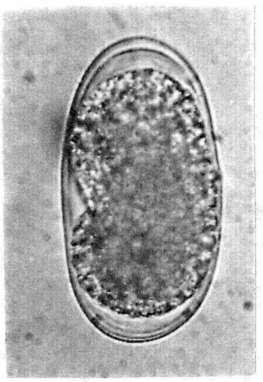

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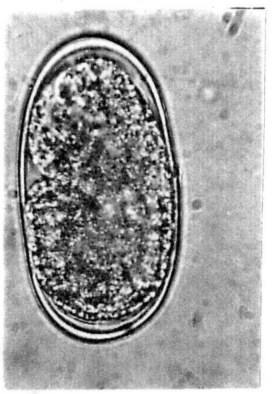

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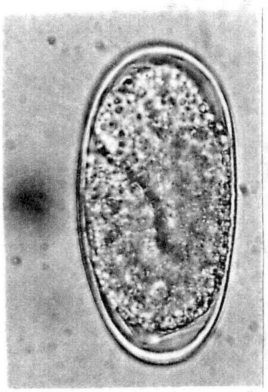

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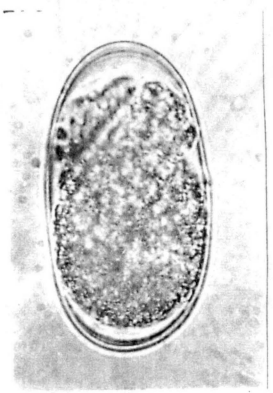

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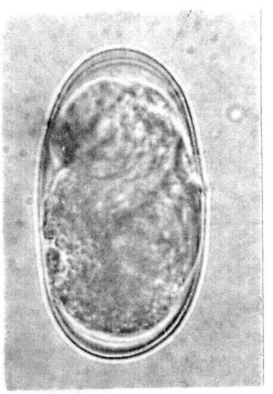

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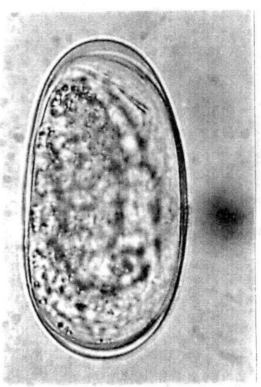

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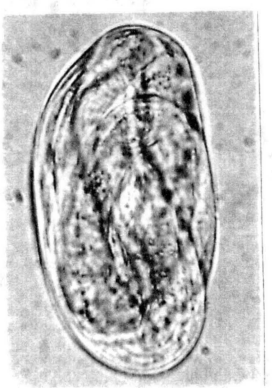

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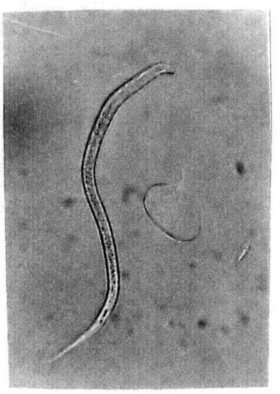


i

2f). Tadpole stage was completed in 23.75 hours (23-24 hrs). At this time the primary odontostyle and the lumen of esophagus could be seen.

When the primary odontostyle was first observed in place the first juvenile stage was considered to have commenced (Fig. 2g). The primary odontostyle fully developed approximately 10 hours (∓2 hrs) after its formation. The replacement odontostyle was formed about 12 hours (∓2 hrs) after the formation of the primary odontostyle and moved up close to the posterior end of the primary odontostyle. The juvenile became very active after the replacement odontostyle had completed its migration thrusting its odontostyle into the chorion. The chorion gradually stretched and became pliable as the juvenile crawled inside (Fig. 2h). Eclosion by the first stage juvenile occurred 52 hours (48-57.5 hrs) after the first juvenile stage had developed (Fig. 2i). Some of the other members of the superfamily Dorylaimoidea such as Paratrichodorus christiei (24), Labronema sp. (8), and Xiphinema spp. (23) are also known to hatch as a first stage juvenile.

The period from oviposition to eclosion was 5 days 19 hours (5 days 3.5 hrs-6 days 13 hrs). The embryonic development was completed from oviposition to first stage juvenile in 4 days 19.5 hours (3 days 3 hrs-4 days 4 hrs). The time required between oviposition to eclosion was relatively shorter than of X. diversicaudatum and longer than of P. christiei.

Post-embryonic Development

Morphometrics and Ontological Development

Freshly prepared temporary slides were used for morphometric illustrations in order to avoid any effect of fixation on the body measurements.

Juvenile stages can be distinguished from each other by differences in total body and esophagus lengths (Table 1). Total body length ranged from 376.9 to 514.14 µm in J1 (first juvenile stage); from 522.3 to 588.6 µm in J2 (second juvenile stage); from 595.9 to 828.75 µm in J3 (third juvenile stage); and from 829.28-1180.77 µm in J4 (forth juvenile stage) based on 42, 20, 37, and 29 individuals, respectively. Average total body lengths were 459.19, 559.29, 749.22, 983.18 µm for J1, J2, J3, and J4, respectively. Between J1 to J2 a juvenile gained 19.1 per cent of its final total length where it was 36.3 per cent (J2-J3) and 44.6 per cent (J3-J4) which indicated that the greatest growth occurred between J3 to J4 stages followed by J2-J3 and the lowest increase was between J1-J2.

Esophagus length, which was not as dependable as total body length in terms of identification of postembryonic developmental stages, overlapped among the stages (Table 1). However, based on the average, esophagus length could be meaningful and it relatively increased as a juvenile grew. It was 47.29 (35.86-54.50), 56.89 (51.30-67.70), 71.28 (54.80-91.54), and 93.83 µm (76.20-116.66) in J1,J2, J3, and

J4, respectively. As in total body length, the greatest increase in esophagus length, based on average, was in the period between J3 and J4.

"A" values (total body length/the greatest body width) did not drastically vary among J1, J2, and J3 (Table 1). In J1 was 26.62 (20.19-34.50), in J2 was 27.75 (24.21-31.54), and in J3 was 29.39 (25.03-36.69) µm. However, it sharply changed in J4 reaching 34.25 (28.38-42.79) µm possibly because a juvenile became more slender during this developmental stage.

"C" values (total body length / tail length) ranged from 5.02 to 7.63, 6.09 to 7.95, 6.70 to 10.48 µm and on an average 6.748, 6.580, 7.570, and 8.510 µm in J1, J2, J3, and J4 (Table 1).

Primary odontostyle length excluding the extension showed differences among the developmental stages. The length of the primary odontostyle reduced in premolting quiescent period where 2 replacement odontostyles could be found. The average length of primary odontostyle was 6.34 (5.05-7.07) in J1; 7.54 (5.90-7.70) in J2; 8.73 (6.21-11.90) in J3; and 10.35 (8.52-12.63) µm in J4 (Table 1).

Apparently, the length of the replacement odontostyle and distance between it and the primary odontostyle were the most variable since the replacement odontostyle was formed in the esophagus and migrated up while it was developing (28); therefore, it was not measured until it fully developed. The replacement odontostyle length was approxi-

Table 1. Mesaurement of *Mesodorylaimus lissus* juveniles in μm.

Characters	J1 (n=42)		J2 (n=20)		J3 (n=37)		J4 (n=28)	
	mean	range	mean	range	mean	range	mean	range
Total length	459 19	376.90-514 14	559 29	522.3-588 60	749.22	595.90-828.75	983.18	829.28-1180.77
Esophagus length	47.29	35.86-54.5	56.89	51.30-67.7	71.28	54.80-91.54	93.83	76.20-116.66
a (Total length/ body width)	26.62	20.19-34.5	27.75	24.21-31.54	29.39	25.03-36.69	34.25	28.38-42.79
c (Total length/ tail length)	6 75	5.02-7.63	6.58	6.09-7.95	7.57	6.04-7.95	8.51	6.70-10.48
Primary odontostylet length	6.34	5.05-7.07	7.54	5.90-7.70	8.73	6.21-11.90	10.35	8.52-12.63
Replacement odontostylet length	6.52	5.13-7.07	7.26	6.10-7.70	8.57	5.69-10.50	10.31	8.00-12.63

mately equal to that of the primary unless a juvenile was in premolting period where the replacement odontostyle was longer than the primary and ready to replace the primary one. The average length of replacement odontostyle was 6.52 (5.13-7.07) µm in J1, 7.26 (6.10-7.70) µm in J2, 8.57 (5.69-10.50) µm in J3, and 10.31 (8.00-12.63) µm in J4 (Table 1).

The stylet orifice is one of the primary characteristics separating the Adenophorea from the Secernentea. Observations in this study indicated that while the primary odontostyle orifice was always in the expected dorsal position the orifice of the replacement odontostyle was variable in position. This suggests a rotation of the replacement odontostyle during migration. Further, as the replacement odontostyle itself was sometimes dorsal and sometimes ventral in relation to the esophageal lumen a helical migration route might be inferred. These observations were incidental to the objectives of the study and need to be confirmed by further studies.

The first juvenile stage began inside the chorion and as indicated before lasted 52 hours inside the chorion. The primary and replacement odontostyles were close together at least 24 hours (∓5 hrs) before eclosion. Premolting quiescent was observed approximately 24 hours after eclosion and the first molt was completed in about 36 hours (∓12 hrs). Prior to the first molt there were only two odontostyles, primary and replacement. After the completion of the first molt the J2 became active and fed upon Panagrellus redivius

in agar plates. Six days (∓6 hrs) after the first molt the J2 became quiescent and possessed 3 odontostyles. The molting process, including quiescent period, took approximately 30 hours (∓6 hrs). The juvenile did not feed during molting process.

The J3 became active and began feeding upon prey nematodes after the completion of the second molt. The J3 became quiescent 10 days (∓2 days) after the second molt and feeding was terminated. The molting process was identical to that observed in the first and second molts. As in other molting processes feeding resumed after the completion of molting. The period from J4 to adult was 12 days (∓2 days) for females and 10 days (∓2 days) for males. At the end of J4 stage, prior to molting the genital organs of both J4 females and J4 males were easily observed through a binocular dissecting microscope. There was no sexual dimorphism apparent in earlier juvenile stages.

The completion of postembryonic development of M. lissus from eclosion to adult required 28 days (∓1 day) under the laboratory conditions (23-24°C) in agar plates on the prey nematode P. redivivus. The complete life cycle of Labronema sp. from egg to adult was 3-4 months under laboratory conditions (8) and the longest juvenile stages were J3 and J4 (1 month) which is also true for M. lissus. The shortest stage was the J1 in Xiphinema index (24-28 hours) (23), in Labronema sp., (10-12 days) (8) and in M. lissus. It was also reported that Paratrichodorus christiei first juvenile

underwent the first molt shortly after eclosion (24). This may imply that first juveniles do not or are not able to feed. The first juveniles of M. lissus were never observed feeding on prey nematodes although they were in close proximity. The first feeding activity observed was immediately followed completion of the molt to the J2.

Even though the life history of M. lissus has been determined by this in vitro study further studies should be performed to determine the influence of biotic and abiotic factors of the soil environment on life cycle duration. The time between oviposition to adult of X. index ranges from 22 to 27 days under greenhouse conditions (23), but requires at least two years under field conditions (11).

Feeding Habits

Substrate Suitability Studies

First Study. The mean egg numbers produced by M. lissus females feeding in vitro on live P. redivivus, air dried P. redivivus, human dietary compound (Similac), and the control (no food) in the two studies (9 replications) are combined and presented in Figure 3. The data obtained from these two studies were subjected to analysis of variance and LSD separately. The results of both studies were identical. The nematode was observed feeding predaceously on live P. redivivus (Fig. 4a), and saprophagously on dead, air dried P. redivivus and within granules of Similac (Fig. 4b). Although a few eggs were produced as a result of feeding on

Figure 3. Reproduction level of Mesodorylaimus lissus by substrate.
Figure represents the mean number of egg produced in two studies (9 replications)

Reproduction Level of

Mesodorylaimus lissus by Substrate

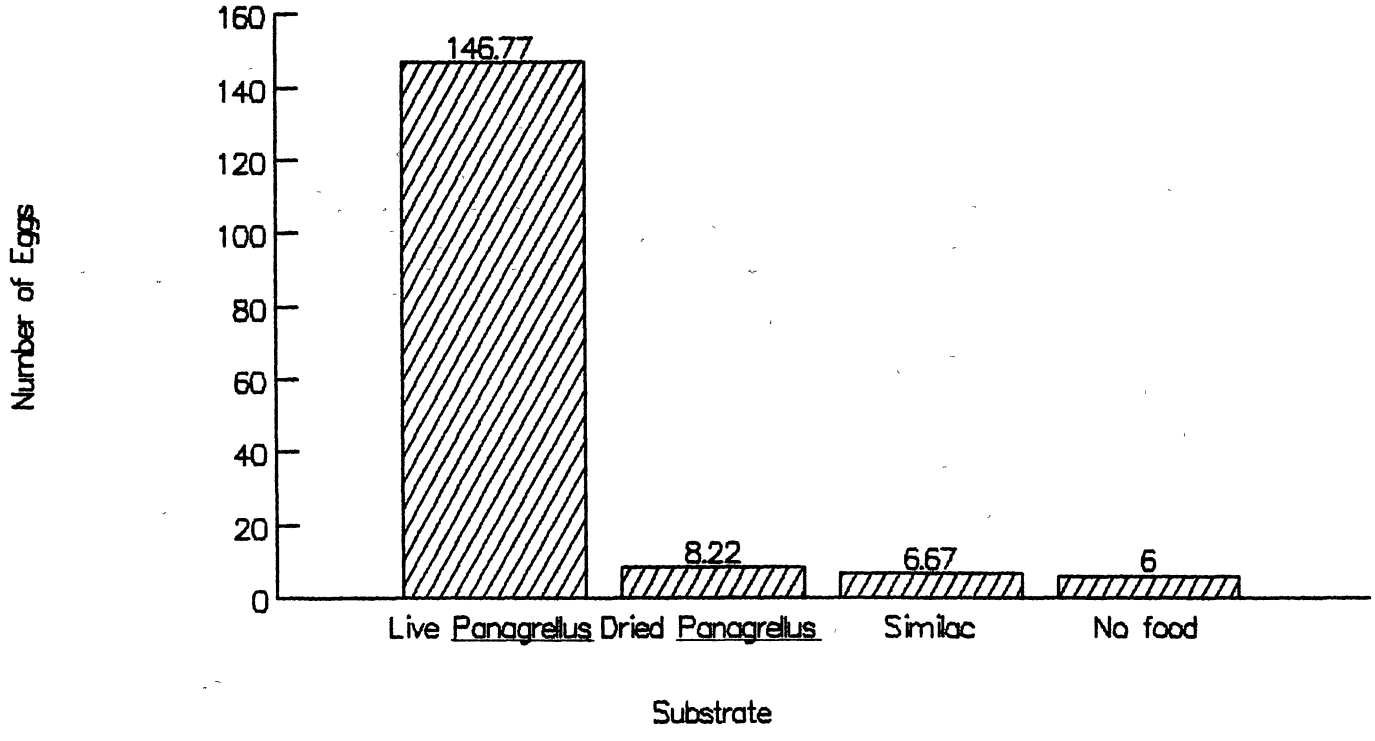
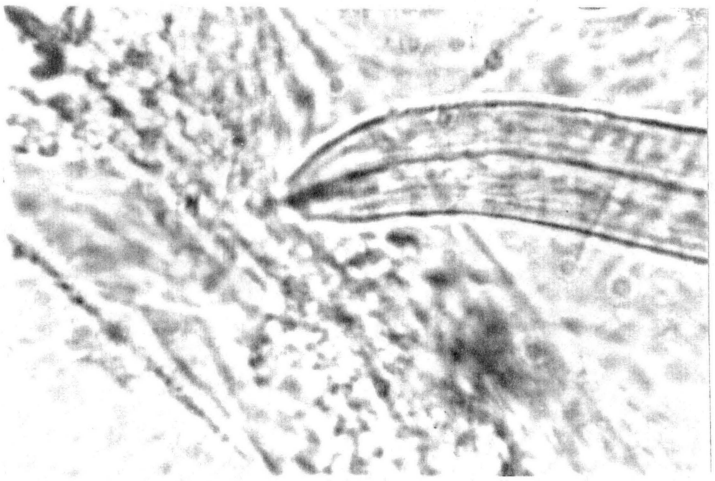


Figure 4. *Mesodorylaimus lissus* feeding upon
a= *Panagrellus redivivus*
b= Similac
c= *P. arrhenomanes* zoosporangia



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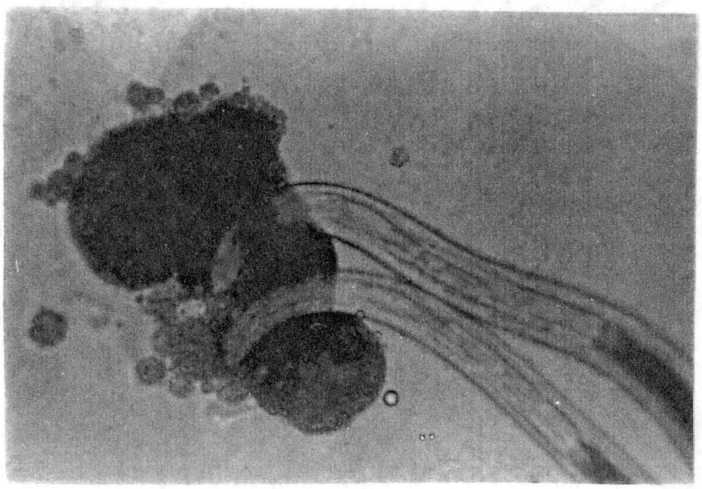

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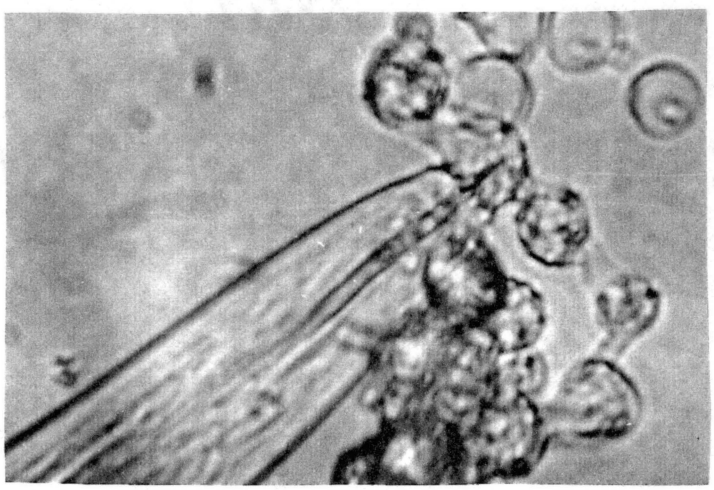


c

dead prey nematodes or Similac there were no statistically significant differences between the control and either of them. However, egg production supported by live prey was significantly different from the control and the other substrates used. This suggests that the primary feeding habit of M. lissus is predaceous and that the other substrates utilized through its secondary omnivorous feeding habits contribute to the perpetuation of the species at a subsistence or maintenance level for individual survival. Therefore, dead prey nematode and Similac were not included in further food substrate suitability studies.

Second Study. Suitability of Rhizoctonia cerealis, Pythium arrhenomanes, Bipolaris sorokiniana, three isolates of Actinomycetes: L. 39-4, A. 20-5, and A. 32-5, wheat roots, and P. redivivus for M. lissus reproduction was compared in a second study. The data are presented in Table 2. Data from each count were analyzed separately. The first egg count was on the fifth day, the second count was on the tenth day. The final population count on the fifteenth day was based on the number of juveniles extracted upon completion of the study. Yet, as explained in Materials and Methods the recovery of juveniles was far less than expected apparently due to extraction efficiency; therefore, the final population reflected the relative number but not the actual number of juveniles. However, those numbers were still meaningful if not comparable to the number of eggs produced since each count was analyzed separately. The egg

Table 2. The reproduction level of *Mesodorylaimus lissus* on different food substrates.

Substrates	Average egg number** counts		Final population (juvenile count)
	5th day	10th day	15th day
No food (control)	0.1	0.8	0.5
Panagrellus redivivus	61.2	112.3	48.4
Pythium arrhenomanes	24.1	20.9	7.5
Wheat roots	10.4	20.8	10.0
Rhizoctonia cerealis	1.1	0.6	1.2
Bipolaris sorokiniana	0.4	0.3	1.4
Actinomycetes L. 39-4	1.0	0.1	0.5
A. 20-5	0.0	0.0	0.0
A. 32-5	0.0	0.0	0.0
LSD	11.71	19.7	8.53

**Averages are means of ten replications.

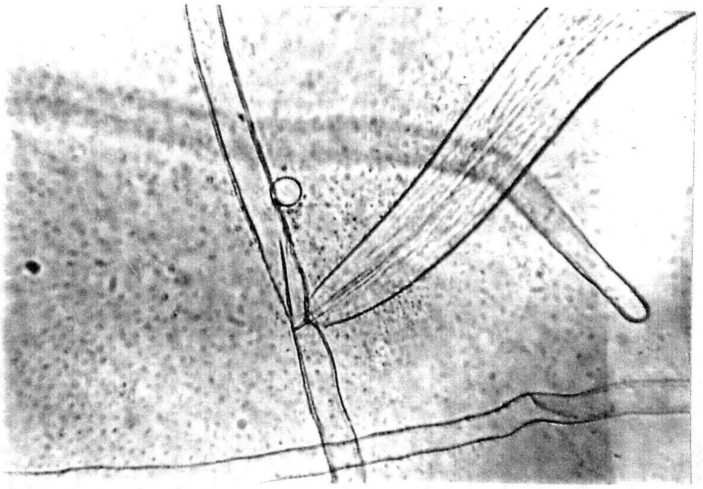
numbers; however, did represent the actual number of eggs laid between the counts inasmuch as the time required from oviposition to eclosion was about 5 days.

Mesodorylaimus lissus was observed feeding upon all substrates but not all of them were suitable in terms of reproduction. As expected from the results of the first study P. redivivus was the optimum substrate for M. lissus. The reproduction level obtained on P. redivivus was significantly higher than the control and other substrates ($p=0.05$) in both egg and juvenile counts.

The female M. lissus frequently observed feeding upon P. arrhenomanes zoosporangia (Fig. 4c) and wheat roots (Fig. 5a) exhibited dark colored intestines suggesting a viable nutrient acquisition. The nematodes fed on the hyphae of R. cerealis and B. sorokiniana (Fig. 5b,c) and on Actinomycetes but their intestines became transparent indicating a depletion of stored food reserves. This observation was confirmed by the few or no eggs produced on these substrates.

Within the five days of introduction to the substrates, M. lissus produced the highest number of eggs on P. redivivus followed by P. arrhenomanes and finally by the others. According to the statistical analysis P. redivivus was significantly different from each of the rest by producing 61.2 eggs. Reproduction on P. arrhenomanes (24.1) was significantly higher than B. sorokiniana (0.4), R. cerealis (1.1), Actinomycetes (1.0), wheat roots (10.4), and the control

Figure 5. *Mesodorylaimus lissus* feeding upon
a= Wheat root hairs
b= *R. cerealis* hyphae
c= *B. sorokiniana* hyphae



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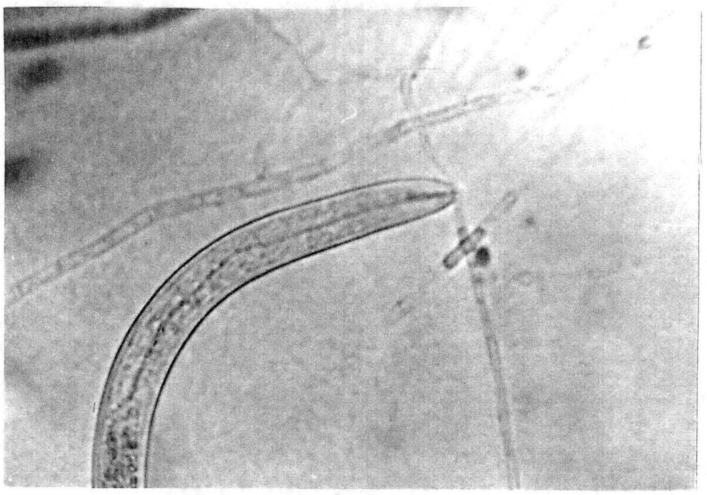

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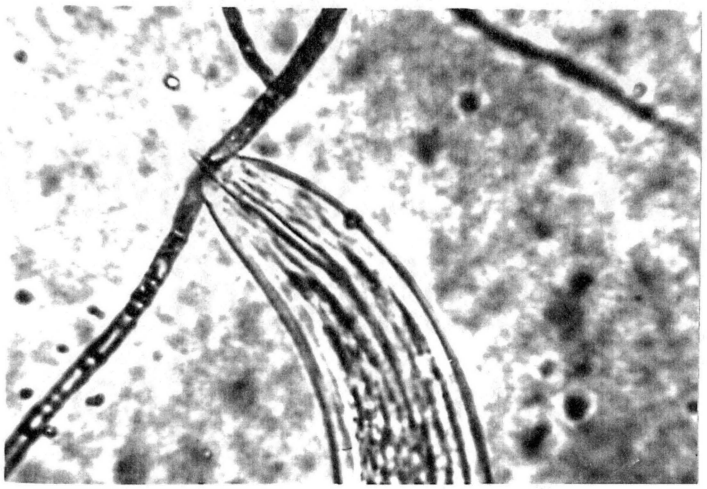


c

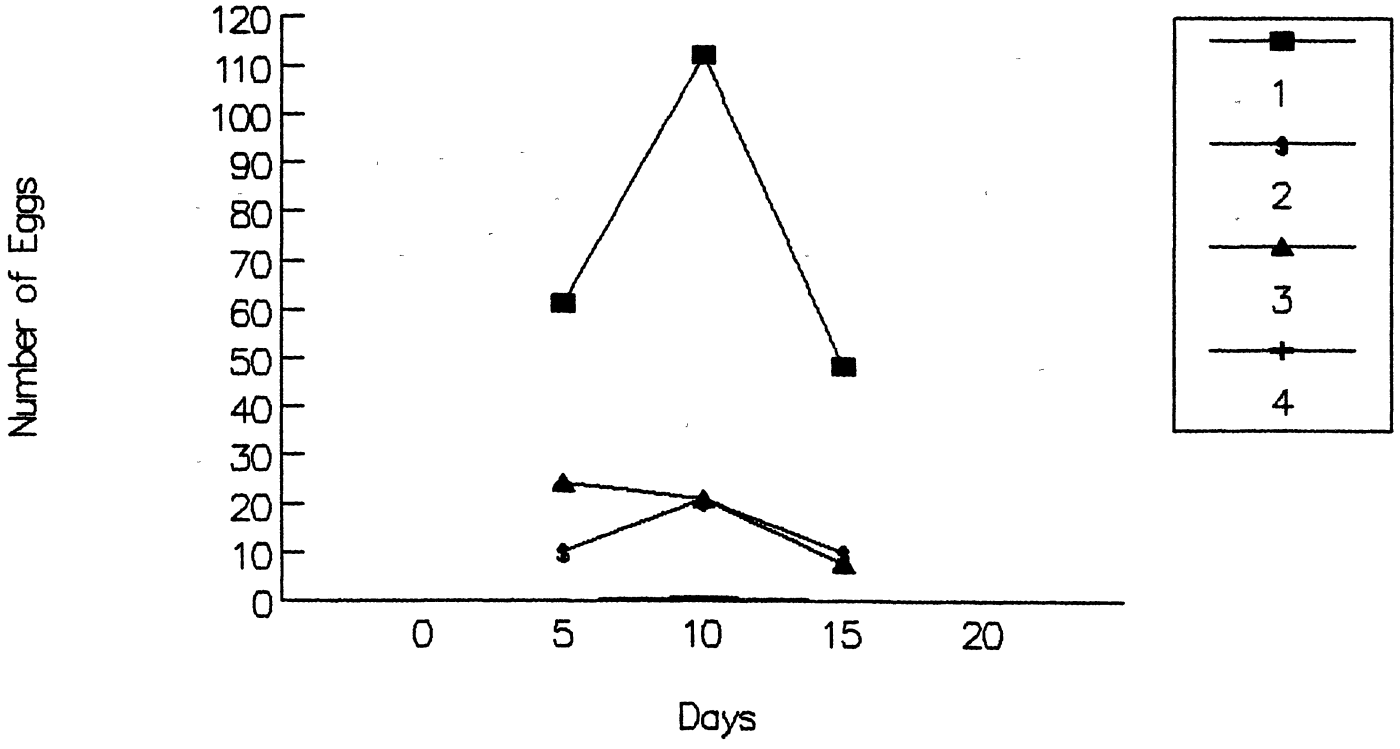
(0.1) between which there was no statistically significant difference.

On the tenth day count the number of eggs was 112.3, 20.9, 20.8, 0.8, 0.6, 0.3, 0.1, and 0 on P. redivivus, P. arrhenomanes, wheat roots, the control, R. cerealis, B. sorokiniana, L. 39-4 (Actinomycetes), and A. 20-5 and A. 32-5 (Actinomycetes), respectively. Based on analysis of variance and LSD (p=0.05), again P. redivivus supported greater reproduction than the other. The reproductive level was not significantly different between P. arrhenomanes and wheat roots both of which significantly differed from and supplied better food source than the control, Actinomycetes, R. cerealis, and B. sorokiniana. There was no significant difference between the control, Actinomycetes, R. cerealis, and B. sorokiniana. The result of the juvenile count is consistent with that for eggs. The reproduction level of M. lissus based on the egg production and juvenile count on P. redivivus, P. arrhenomanes, wheat roots, and the control is presented in Figure 6. The other substrates were deleted from the figure due to the low level of reproduction they supported. The reproduction level was the highest on P. redivivus and the lowest in the control. The reproductive level on P. arrhenomanes declined through the second count whereas it increased on wheat roots. The decrease in the juvenile count actually resulted from the loss of juveniles during extraction process which was on the fifteenth day. Therefore, the slope between the tenth and

Figure 6. Reproduction level of Mesodorylaimus lissus on some selected substrates based on the number of eggs produced at the 5th and 10th days and the number of juveniles at the 15th day.
1= Panagrellus redivivus
2= Wheat roots
3= Pythium arrhenomanes
4= No food (control)
Note: The other substrates included in this study have been deleted due to the low level of reproduction obtained.

Reproduction Level of M. lissus

on Selected Substrates



fifteenth days should not be interpreted as a further decline in reproductive level. The previous study was duplicated and the results of the average number of eggs in three counts and final population are given in Table 3. In the first count performed on the fifth day following of the initiation of the study there was no statistically significant difference ($p=0.05$) between the reproduction levels of M. lissus on P. redivivus and wheat roots. But both were significantly higher than the remaining substrates. However, in both tenth and fifteenth day counts the reproduction level on P. redivivus was significantly higher ($p=0.05$) than the rest including wheat roots. There were no significant differences between the remaining substrates. According to the final population P. redivivus supported significantly higher M. lissus reproduction than the remaining substrates. There were no significant differences on the reproduction level between wheat roots, P. arrhenomanes, and R. cerealis. However, the reproduction level on wheat roots was significantly higher than the control, three isolates of Actinomycetes, B. sorokiniana while there was no significant differences between those substrates, P. arrhenomanes and R. cerealis.

Figure 7 represents the reproduction level of M. lissus on P. redivivus, B. sorokiniana, P. arrhenomanes, R. cerealis and wheat roots. The fifth, tenth, and fifteenth day counts were based on the number of eggs produced between these periods and the twentieth day count was the final population

Table 3. The reproduction level of *Mesodorylaimus lissus* on different food substrates.

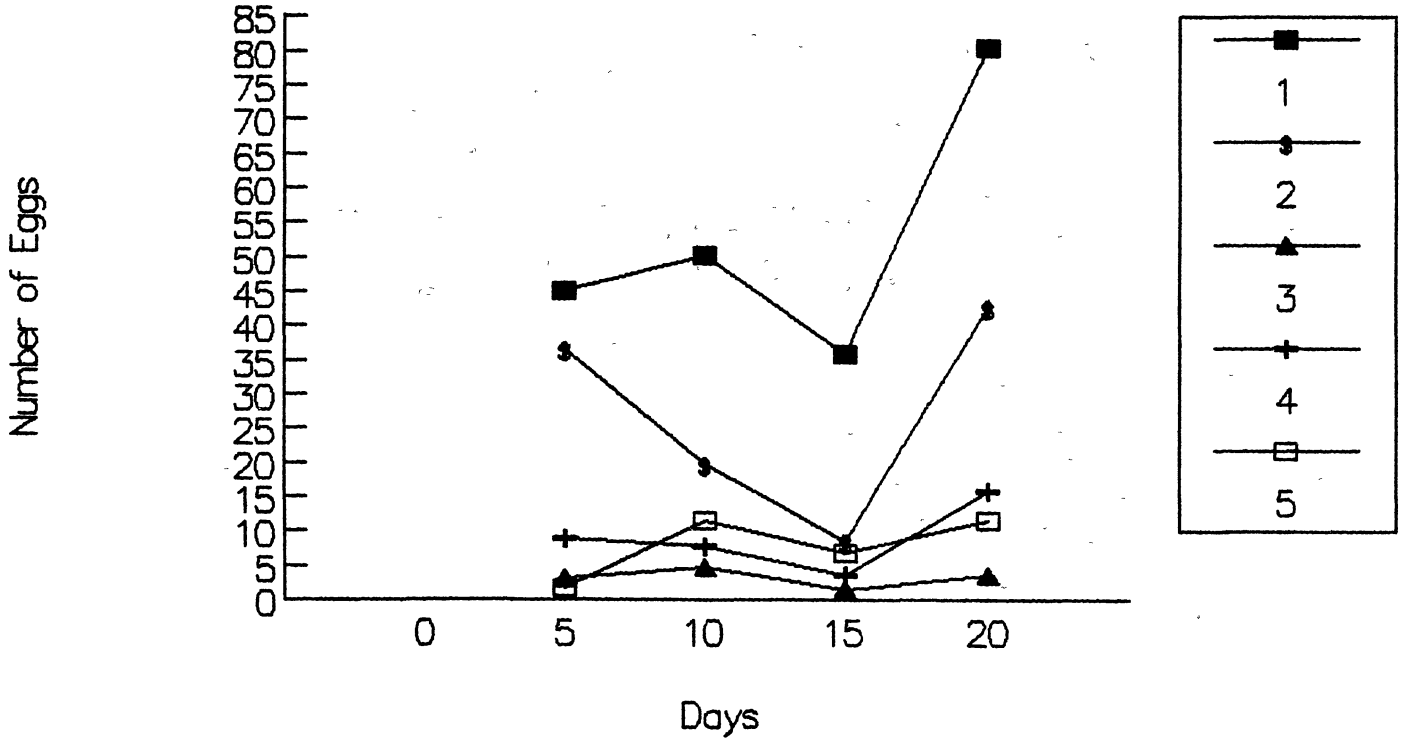
Substrates	Average egg numbers*			Final population
	5th day	10th day	15th day	20th day
No food (control)	4.6	4.2	1.4	4.2
Panagrellus redivivus	45.0	50.2	36.0	80.4
Wheat roots	36.6	19.8	8.4	42.6
Pythium arrhenomanes	9.0	7.8	3.6	15.8
Rhizoctonia cerealis	1.6	11.4	6.8	11.4
Bipolaris sorokiniana	3.0	4.8	1.4	3.6
Actinomycetes				
L. 39-4	0.0	0.0	0.0	0.0
A. 20-5	2.4	2.4	1.0	3.2
A. 32-5	6.0	3.6	1.8	7.4
LSD	22.94	25.86	12.62	34.84

*Averages are means of five replications.

Figure 7. Reproduction level of Mesodorylaimus lissus on some selected substrates based on the number of eggs produced at the 5th, 10th, and 15th days and the number of juveniles at the 20th day.
1= Panagrellus redivivus
2= Wheat roots
3= Bipolaris sorokiniana
4= Pythium arrhenomanes
5= Rhizoctonia cerealis

Reproduction Level of M. lissus

on Selected Substrates



according to the number of juveniles obtained by extraction.

The egg production of M. lissus showed a decline on wheat roots and P. arrhenomanes through the tenth day at which time it increased on P. redivivus, B. sorokiniana, and R. cerealis. Nevertheless, the egg production declined on fifteenth day on all substrates. Because the biology of M. lissus has not been studied the reproductive capability of an individual at different ages is not known. The M. lissus females used in these studies were randomly selected from the stock colonies and were undoubtedly of different ages. In addition, females obviously aged throughout the study. This may explain the fluctuation of reproduction levels on the same substrates between these duplicated studies.

The data obtained from second study confirmed that the primary feeding habit of M. lissus is predaceous. However, the studies also confirmed that it can successfully feed parasitically upon wheat roots if not primarily and preferably. Russell (25) reported that M. lissus feeds upon wheat roots and can cause significant reduction on biomass production of wheat. Among the fungi P. arrhenomanes is the most viable substrate on zoosporangia of which M. lissus feeds readily.

Third Study. The results of first and second studies suggest that the primary feeding habit of M. lissus is predaceous; therefore, some saprophagous nematodes, Diplogaster sp., the mixture of Cephalobus sp. and Eucephalobus sp. were included to this study in addition to the substrates used in

the second study.

The results of this substrate suitability study are given in Table 4. As in the other substrate suitability studies, counts were performed at the fifth, tenth, and fifteenth day on the basis of eggs produced and at the twentieth day the study was terminated by juvenile extraction.

In the first count the reproduction level of M. lissus on P. redivivus was significantly different from each of the other substrates by producing average 30.20 eggs. The nematode produced average 15.60 eggs on cephalobids which was significantly lower than the number of eggs produced on P. redivivus, but significantly higher than the others. The number of eggs produced was 7.40 on Diplogaster sp. and 1.80 on P. arrhenomanes but there was no statistically significant difference between them. However, the reproduction level on Diplogaster sp. was significantly different than the control, three isolates of Actinomycetes, wheat roots, and R. cerealis while there was no difference between P. arrhenomanes and the latter on the reproduction level.

In the second count the highest egg number (143.8) was obtained on P. redivivus which significantly differed from each of the remaining substrates. It was followed by Diplogaster sp. (47.4) and cephalobids (39.2) in terms of supporting M. lissus reproduction which were significantly different from each other and each of the other substrates. Although the number of eggs found in the Actinomycetes "A. 32-5" was relatively high and there was no significant

Table 4. The reproduction level of *Mesodorylaimus lissus* on different food substrates.

Substrates	Average egg numbers*			Final population
	5th day	10th day	15th day	20th day
No food (control)	0.00	1.00	1.00	0.00
Panagrellus redivivus	30.20	143.00	131.60	62.60
Diplogaster sp.	7.40	47.40	47.20	30.60
Cephalobids	15.60	39.20	26.00	26.40
Wheat roots	1.00	11.20	6.40	0.80
Pythium arrhenomanes	1.80	5.60	3.20	0.60
Rhizoctonia cerealis	0.00	0.00	0.00	0.00
Bipolaris sorokiniana	0.40	2.20	1.80	1.60
Actinomycetes				
L. 39-4	2.00	1.20	3.40	1.40
A. 20-5	3.00	3.00	6.20	0.00
A. 32-5	0.00	11.20	11.80	3.60
LSD	5.92	6.76	15.1	13.08

*Averages are means of five replications.

difference between this and wheat roots, the high number of eggs may have been due to bacterial contamination in two of the replications which might have supplied an additional food source. There was also low egg production on the other Actinomycetes and P. arrhenomanes while no egg production was found on the control and R. cerealis. However, there was no statistically significant difference between either of them.

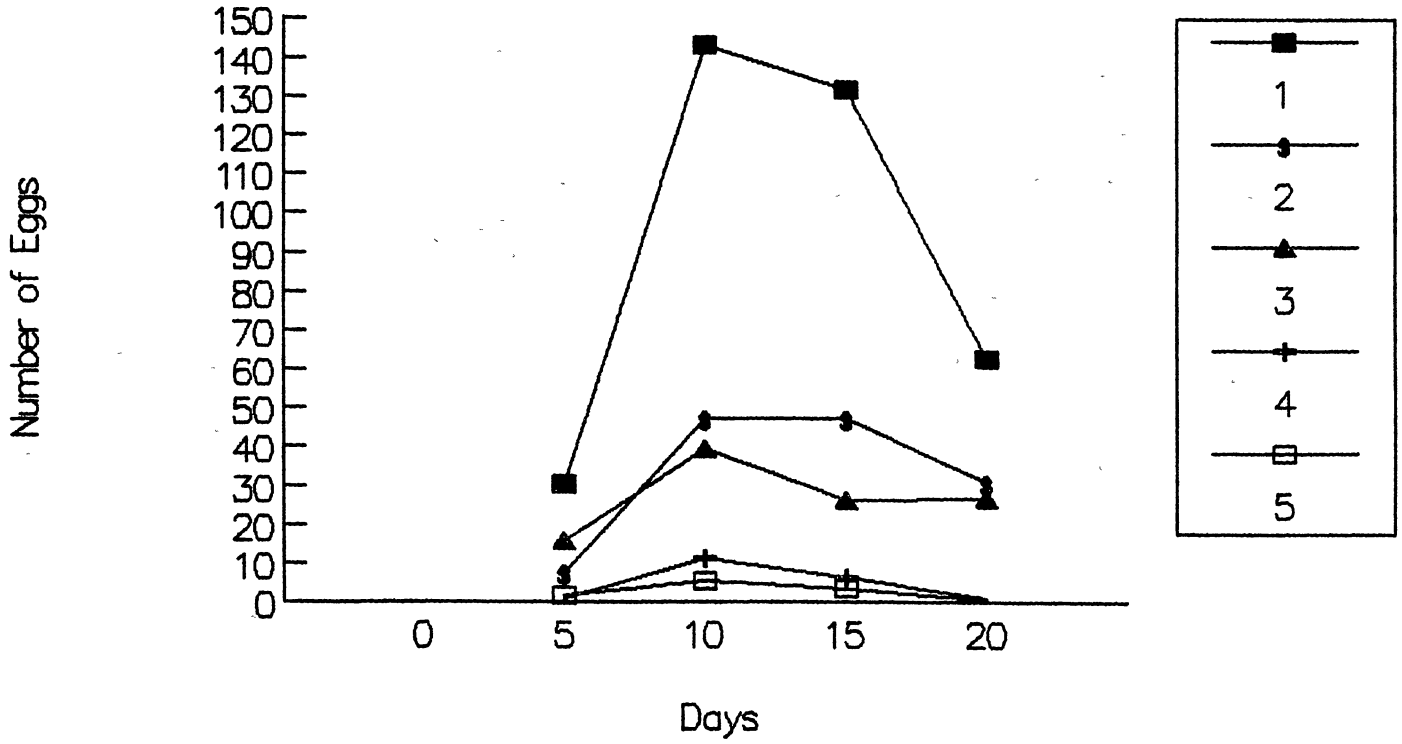
The result of the third count was similar. The final population count based on juveniles extracted showed almost the same results. The highest juvenile recovery was on P. redivivus (62.6) which was significantly different from the rest. There was no significant difference between saprophagous nematodes and between the other substrates. Although, the in vitro unit was extracted including the plastic wrap, the recovery of juveniles was still lower than expected. This might be explained by death of some juveniles following eclosion or unsuitability of the extraction technique. Thus, the extraction technique which is a soil extraction technique should be improved or modified for use in extraction of agar substrates.

The reproduction level of M. lissus on prey nematodes, wheat roots, and P. arrhenomanes is given in Figure 8. It can be easily seen from the figure that prey nematodes significantly supported M. lissus reproduction indicating the primary feeding habit of this nematode was predacious. On the other hand, its secondary omnivorous feeding habits were

Figure 8. Reproduction level of *Mesodorylaimus lissus* on some selected substrates based on the number of eggs produced at the 5th, 10th, and 15th days and the number of juveniles at the 20th day.
1= *Panagrellus redivivus*
2= *Diplogaster* sp.
3= Cephalobids
4= Wheat roots
5= *Pythium arrhenomanes*

Reproduction Level of M. lissus

on Selected Substrates



parasitic on wheat roots and myceliophagous on P. arrhenomanes which served as supplementary food sources.

The conclusions can be drawn from the result of all food substrate suitability studies are: 1) The primary feeding habit of M. lissus seemed to be predacous in vitro 2) It can feed parasitically upon wheat roots and myceliophagously upon P. arrhenomanes zoosporangia and B. sorokiniana 3) It may feed myceliophagously upon R. cerealis hyphae, and microphagously upon actinomycetes but the reproduction level could be very low.

Substrate Preference Studies

The results of substrate suitability studies showed that the primary feeding habit of M. lissus was predacous. Linford and Oliveira (18) stated that predacious dorylaims found their prey only by chance, but they immediately responded when their heads made contact with another nematode. Therefore, this study was carried out to elucidate if there was any substrate preference on the basis of equal numbers and equal volumes of candidate prey nematodes provided for M. lissus. Candidate prey nematodes used in this study were: the plant parasitic nematodes Hoplolaimus sp., Pratylenchus sp., and Paratylenchus sp., and the saprophagous nematodes Diplogaster sp., Cephalobus sp. and Eucephalobus sp., the last two genera were presented in combination (cephalobids). The rank of the prey nematodes based on their size from the biggest to the smallest was

Hoplolaimus sp., Pratylenchus sp., Paratylenchus sp., Diplogaster sp., and cephalobids.

Prey Nematode Number Study. Ten of each candidate prey nematodes and 10 female M. lissus were placed in an in vitro observation unit and observations were made 2 or 3 hour intervals through a binocular dissecting microscope.

The predacious events recorded by prey nematodes and the feeding event frequency by observation period are given in Figure 9a and 9b, respectively.

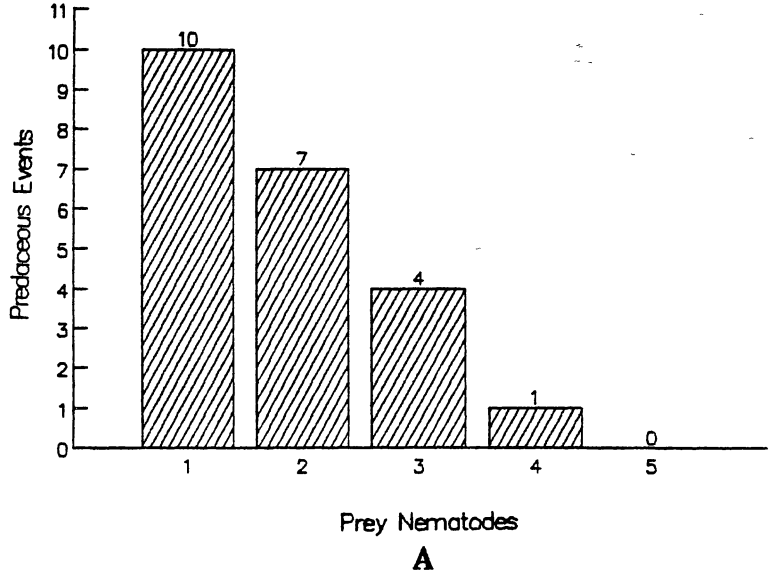
According to the predaceous events Diplogaster sp. was the most frequently attacked followed by cephalobids. Both nematodes were also suitable prey in the substrate suitability study. Of the plant parasitic nematodes tested Pratylenchus sp. appeared to be most readily attacked by M. lissus. Although Hoplolaimus sp. was attacked infrequently no successful feeding was observed, possibly due to physical or chemical resistance found in some plant parasitic nematodes (6, 7).

The null hypothesis established for number study was: if equal numbers of candidate prey nematodes of different volumes are presented to a predator, on the basis of chance encounter the larger prey will be fed upon most frequently. The rank of feeding frequency in the present study was Diplogaster sp., cephalobids, Pratylenchus sp. and Paratylenchus sp. When compared to the rank based on the size Diplogaster sp. and cephalobids were the two smallest nematodes whereas they were the most frequently attacked.

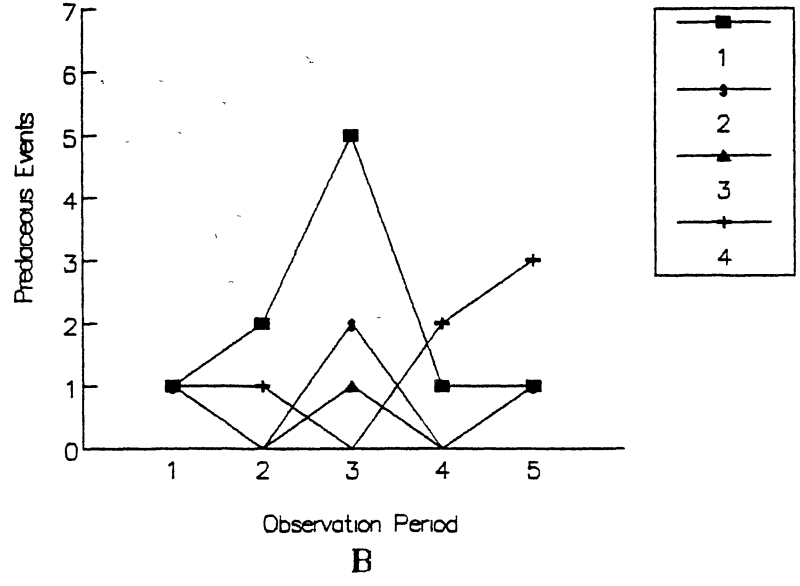
Figure 9. Feeding preference of *Mesodorylaimus lissus* on equal numbers of prey nematodes.
A= Predacious events recorded by prey nematodes
1= *Diplogaster* sp.
2= Cephalobids
3= *Pratylenchus* sp.
4= *Paratylenchus* sp.
5= *Hoplolaimus* sp.

B= Feeding event frequency by observation period
1= *Diplogaster* sp.
2= *Pratylenchus* sp.
3= *Paratylenchus* sp.
4= Cephalobids
Note: *Hoplolaimus* sp. is deleted due to no observed feeding

Feeding Preference of M.lissus Provided With Equal Numbers of Prey Nematodes



A



B

The biggest prey, Hoplolaimus sp. was attacked infrequently even if M. lissus never fed successfully. Further, M. lissus frequently fed upon Diplogaster sp. at first and when Diplogaster sp. number available for feeding declined it began to feed more frequently upon cephalobids. Therefore, the null hypothesis must be rejected. It must be concluded that M. lissus found its prey not by chance but a positive and selective capability was indicated in its prey selection.

Prey Nematode Volume Study. Equal volume of the prey nematodes as described in Materials and Methods were introduced to the in vitro observation units. This study was repeated one time. The number of candidate prey nematodes which constituted an equal volume was: 10 Hoplolaimus sp. as the biggest candidate prey nematode, 140 Pratylenchus sp., 420 Paratylenchus sp., 488 Diplogaster sp., and 867 cephalobids.

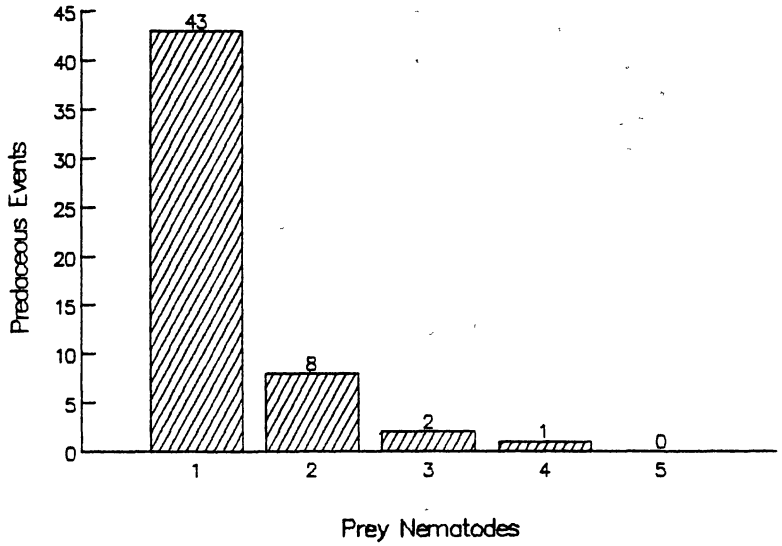
The predacious events by prey nematodes and the feeding event frequency by observation period are given in Figure 10a and 10b, respectively. As is seen from the figures Diplogaster sp. was the highly preferred prey nematode followed by Pratylenchus sp. The other prey nematodes being fed upon were cephalobids and Paratylenchus sp., respectively. There was no observation of M. lissus attempting to devour Hoplolaimus sp., possibly due to the presence of high numbers of preferred prey nematodes.

The null hypothesis for volume study was: if equal

Figure 10. Feeding preference of *Mesodorylaimus lissus* on equal volumes of prey nematodes.
A= Predacious events recorded by prey nematodes
1= *Diplogaster* sp.
2= *Pratylenchus* sp.
3= Cephalobids
4= *Paratylenchus* sp.
5= *Hoplolaimus* sp.

B= Feeding event frequency by observation period
1= *Diplogaster* sp.
2= *Pratylenchus* sp.
3= Cephalobids
4= *Paratylenchus* sp.
Note: *Hoplolaimus* sp. is deleted due to no observed feeding

Feeding Preference of M.lissus Provided With Equal Volumes of Prey Nematodes



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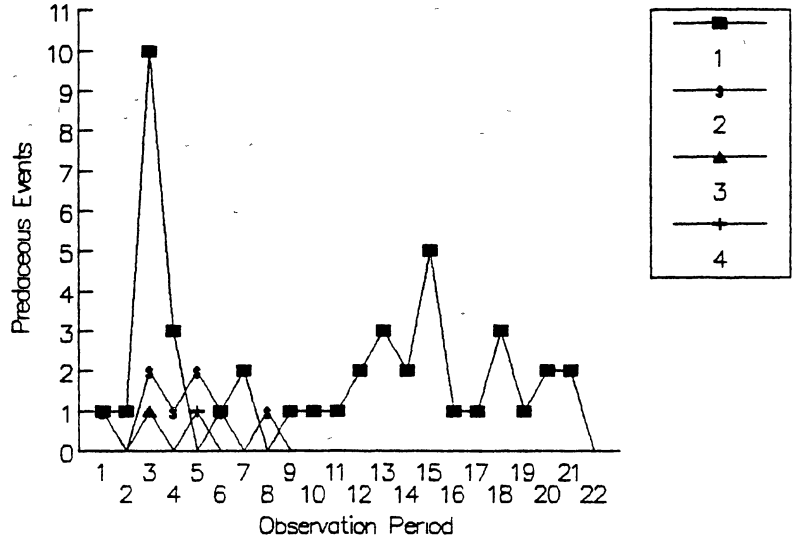


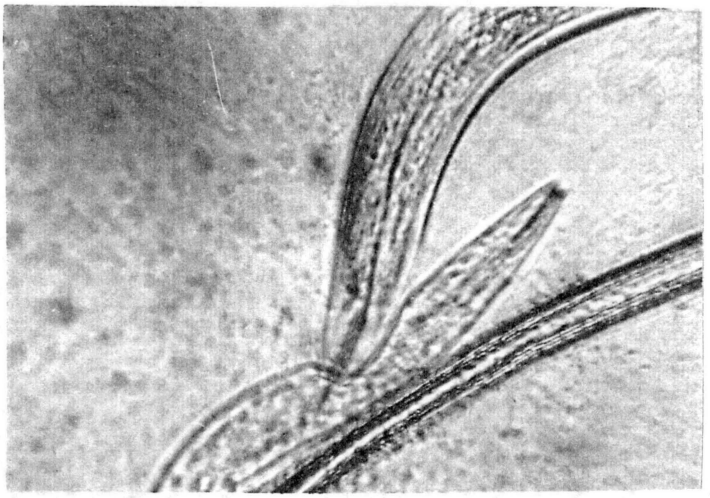
B

volumes of candidate prey nematodes are presented to a predator, on the basis of chance encounter each nematode species, regardless of individual size will be subjected to the same number of predaceous events. However, the data showed that the predaceous event numbers by prey nematodes was not the same. Therefore, the null hypothesis was rejected and the same conclusion derived above was drawn from this study.

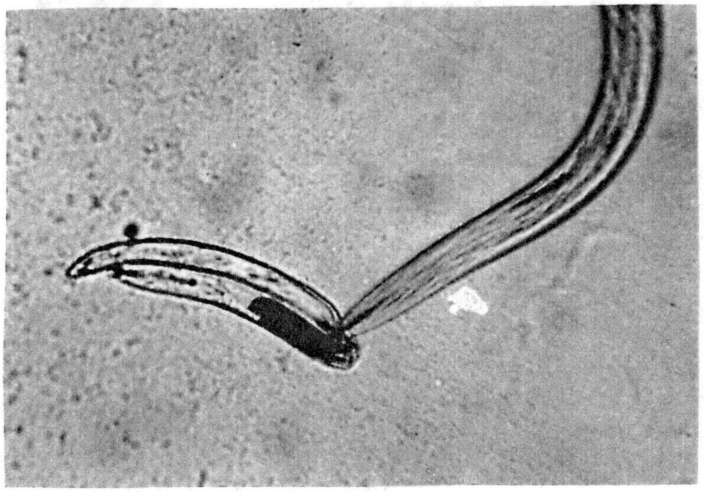
When the feeding preference of M. lissus in number and volume studies was compared, the preference order was highly consistent in both studies. The reason M. lissus did not feed upon cephalobids in the volume study as frequently as in the number study was probably due to the number of the most preferred prey nematode, Diplogaster sp. always being available due to its high population. This might indicate that cephalobids became acceptable only when the number of Diplogaster sp. declined; however, the acceptance level of Pratylenchus sp. and Paratylenchus sp. was relative stable. In conclusion, M. lissus fed preferentially upon Diplogaster sp., cephalobids and/or Pratylenchus sp., (Fig. 11a, b, c) less preferentially upon Paratylenchus sp., and little or not at all on Hoplolaimus sp.

Although the number study was a single run and there was only a single repetition of the volume study, the results in terms of total predacious events were highly conclusive. The results of these studies leave little doubt that M. lissus found its prey not by chance but that a

Figure 11. *Mesodorylaimus lissus* feeding upon
a= *Diplogaster* sp.
b= *Pratylenchus* sp.
c= Cephalobids



a



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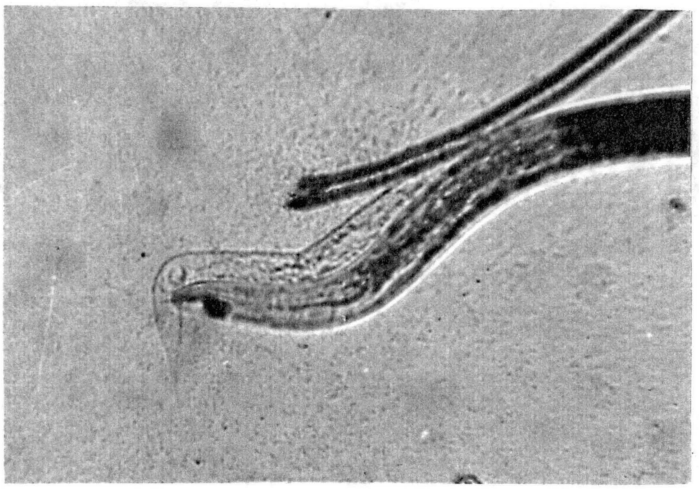


c

positive and selective orientation capability was evidenced in its prey selection.

Rhizosphere Interaction Study

Feeding habits of most dorylaims are considered as predacious while some species are omnivorous (6, 7, 9, 13, 14, 15, 17, 18, 22, 25, 28, 30). Furthermore, Mesodorylaimus spp. have been collected around higher plant roots as well as in bare soils (2, 3, 19, 29). Mesodorylaimus lissus has been shown to have a significant impact on wheat biomass production and seedling emergence rate in a greenhouse study (25). However, the nematode does not occur in monospesific populations in the rhizosphere under field conditions. Thus, the results of a conventional pathogenicity trial, while demonstrating a pathogenic potential, cannot be considered predictive of the organisms impact on a natural rhizosphere. This is especially true of the omnivorous M. lissus which could have an impact ranging from enhancing the pathogenicity of associated fungal pathogens by providing infection courts as speculated by Thorne and Swanger (30) to reducing the inoculum potential of these pathogens by feeding directly upon them. Alternatively, M. lissus might exert no influence on the host parasite relationship of a concomitant pathogenic fungus if it were feeding preferentially as a predator on other nematodes. In the previous substrate suitability studies it was found that the primary feeding habit of M. lissus was predacious. Its

secondary omnivorous feeding habits were found to be parasitic upon wheat root hairs and basal epidermal cells and myceliophagous upon B. sorokiniana and P. irregulare.

In light of the previous studies and literature an additional study was conducted under laboratory conditions to elucidate the impact of M. lissus populations in a soil rhizosphere environment. The effects of M. lissus at three population levels previously encountered in a natural wheat rhizosphere were evaluated on wheat and amaranth on the basis of seedling emergence and host root and top biomass production. These nematode population levels were also studied in combination with the pathogenic soil fungi B. sorokiniana and P. irregulare in an attempt to elucidate any rhizosphere interactions which might occur between the potentially pathogenic organisms. In these treatments data on fungal disease severity were also recorded. All treatment combinations were included in a single randomization but will be presented by organism or organism combinations to facilitate discussion.

Effects of M. lissus Alone

On Wheat

Seedling Emergence. The effect of M. lissus alone on emergence of wheat at the levels of 0 (control), 250, and 500 individuals per pot is given in Figure 12a. The first emergent seedling was observed on the third day after planting.

Figure 12. Effect of *Mesodorylaimus lissus* on 'Chisholm' wheat.
A= Effect of *M. lissus* on seedling emergence of wheat
0= No nematode
250= The 250 inoculum level of *M. lissus*
500= The 500 inoculum level of *M. lissus*

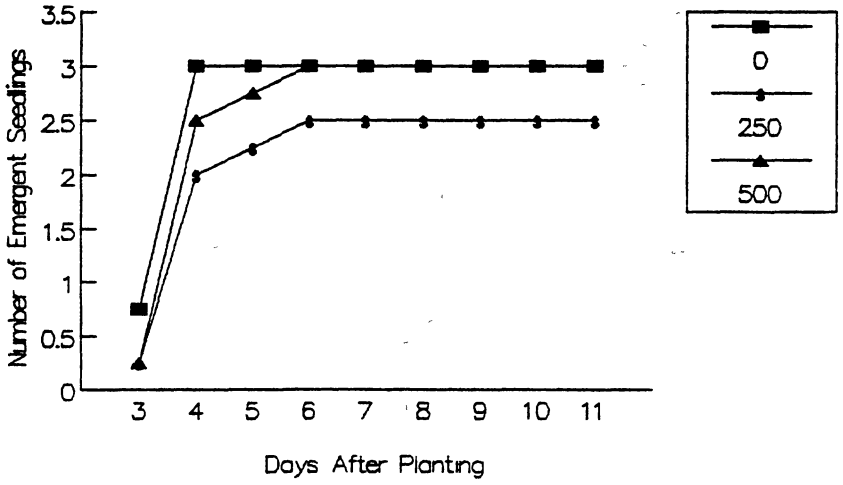
B= Effect of *M. lissus* on biomass production of wheat
0= No nematode
250= The 250 inoculum level of *M. lissus*
500= The 500 inoculum level of *M. lissus*

Effect of Mesodorylaimus lissus

on 'Chisholm' Wheat

Effect of Mesodorylaimus lissus

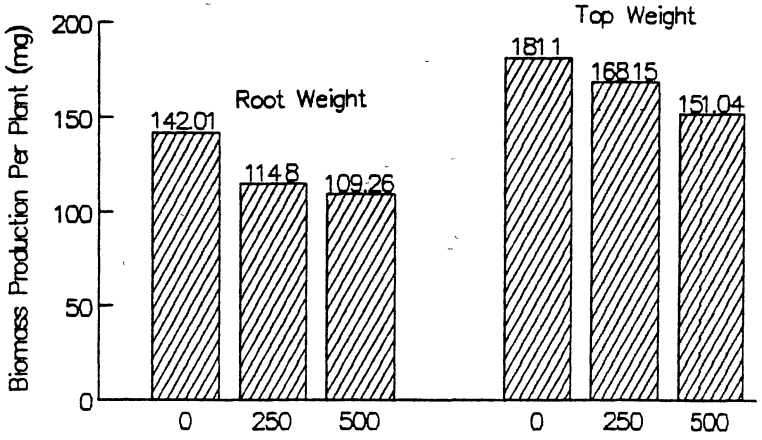
on Seedling Emergence of Wheat



A

Effect of Mesodorylaimus lissus

on Biomass Production of Wheat



B

Although there was no statistically significant difference (p=0.05) between the control and the 250 level of M. lissus, this inoculum level caused a biologically significant reduction in seedling emergence and total emergence. The 500 inoculum level did not reduce total emergence but emergence was slightly delayed. The reduction in damage at the higher level may be a result of competition for feeding sites. The substrate restriction might also cause M. lissus to cannibalize at this population level or Catenaria sp. epidemiology may be enhanced by higher nematode population density.

Biomass Production. Average 12-day fresh root and top weights are presented in Figure 12b. Although the presence of M. lissus at the 250 and 500 levels did not cause a statistically significant (p=0.05) reduction in root weight, it yielded only 80 per cent and 77 per cent of the control root weight, respectively, which was of biological interest. No significant reduction was observed in top weight; however, as in root weight, top weight also slightly declined in the presence of M. lissus by producing 92 per cent and 83 per cent of the control, respectively. These results suggested that M. lissus fed upon wheat root hairs and basal epidermal cells as found in previous substrate suitability studies and caused dysfunction of roots. It is known that plants can withstand higher levels of nematode population and produce economically acceptable level of yield in the absence of environmental stress. The highest nematode-induced crop

losses are found where plants are lacking in sufficient water or nutrient supply. It should be noted that the present study was carried out under optimum conditions and plants were so watered that there was no drought stress. This may explain why the nematode population did not cause a significant biomass reduction, and does not necessarily mean that there was no feeding upon root hairs and basal epidermal cells.

Effects of M. lissus alone

On Amaranthus sp.

Seedling Emergence. The effect of M. lissus on the emergence of Amaranthus sp. at the inoculum levels of 0 (control), 250, and 500 is given in Figure 13a. As seen from the figure the number of emergent seedlings at the level of 250 was the lowest throughout the study. However, there was no statistically significant difference ($p=0.05$) between the control and either of levels until the sixth day. After this time a significant reduction in seedling emergence was obtained at the 250 level of M. lissus. Emergence at the 500 level was not significantly different from the control, but the numbers of emergent seedling were numerically intermediate between the control and the 250 inoculum rate. Both nematode population levels caused a delay in emergence rate and a reduction in total emergence. Only the 250 inoculum level yielded a statistically significant reduction ($p=0.05$) in total emergence.

Figure 13. Effect of *Mesodorylaimus lissus* on *Amaranthus* sp.
A= Effect of *M. lissus* on seedling emergence of *Amaranthus* sp.
0= No nematode
250= The 250 inoculum level of *M. lissus*
500= The 500 inoculum level of *M. lissus*

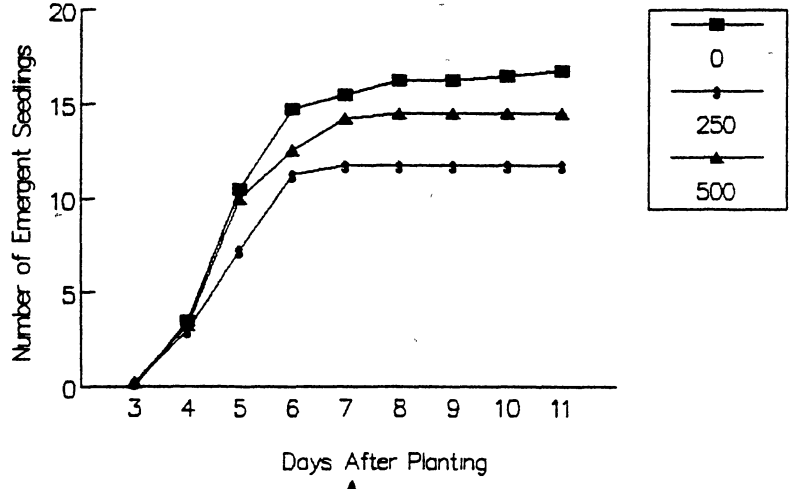
B= Effect of *M. lissus* on biomass production of *Amaranthus* sp.
0= No nematode
250= The 250 inoculum level of *M. lissus*
500= The 500 inoculum level of *M. lissus*

Effect of Mesodorylaimus lissus

on Amaranthus sp.

Effect of Mesodorylaimus lissus

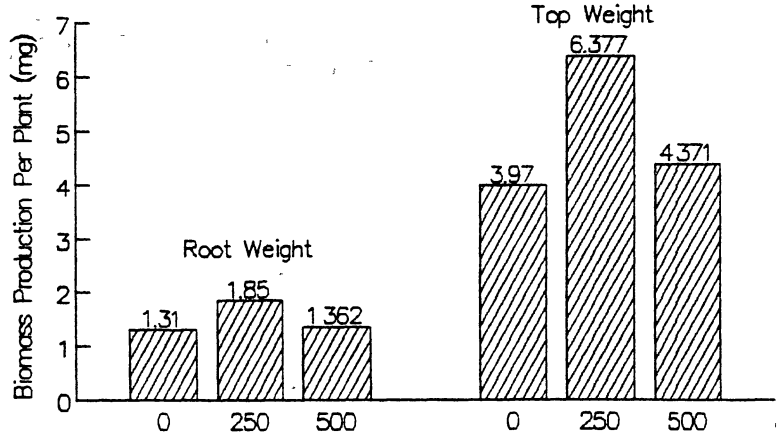
on Seedling Emergence of Amaranthus sp.



A

Effect of Mesodorylaimus lissus

on Biomass Production of Amaranthus sp.



B

Biomass Production. The biomass production of Amaranthus sp. in the presence of three levels of M. lissus is given in Figure 13b. Although there was a slight increase in root and top weights at the levels of 250 and 500 over the control, there was no statistically significant difference between the control and either of the levels in terms of either root or top weights. This unexpected increase in the biomass production might be attributed to the irritation of M. lissus feeding which may have been stimulatory under the optimum conditions of the experiment. It should be noted that the biomass production was given on a per plant basis. Total emergence in the 250 and 500 levels was 29.8 and 13.4 per cent less than the total emergence in control, respectively. Therefore, this unexpected increase can also be a result of the reduced competition among the plants in the 250 and 500 inoculum rates.

Combined Effects of M. lissus and B. sorokiniana

On Wheat

Seedling Emergence. The combined effects of B. sorokiniana and M. lissus at three levels are given in Figure 14a. All fungus-nematode combinations tested reduced the rate of seedling emergence. Total emergence was reduced by all treatments but only B. sorokiniana alone provided a significant reduction ($p=0.05$) in total emergence. The combination of the 250 level of M. lissus and B. sorokiniana; however, resulted in numerical reduction in seedling emergence.

Biomass Production. The biomass production of wheat plants in the combination with the 0, 250, and 500 inoculum levels of M. lissus and B. sorokiniana is given by root and top weights in Figure 14b.

All treatments, Bipolaris sorokiniana alone (the 0 level of M. lissus) and the 250 and 500 levels of M. lissus plus B. sorokiniana significantly reduced ($p=0.05$) the root weight compared to the control. All treatments reduced the top weight but only B. sorokiniana alone produced a significant reduction.

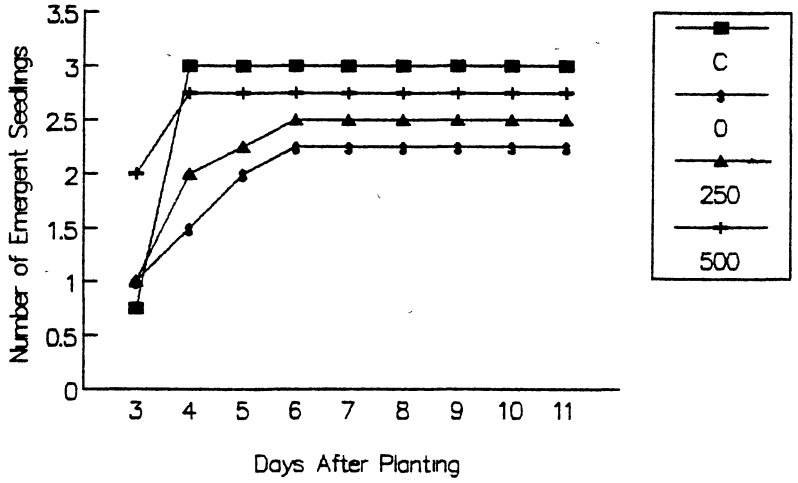
The tentative conclusions drawn from these results are highly speculative due to the duration and the optimum conditions of the experiment and far more data will be required to make a definitive interpretation. However, data suggested that B. sorokiniana attacked the germinating seedlings reducing the emergence rate and total emergence. In the meantime M. lissus began feeding preferentially on B. sorokiniana. The 500 level of M. lissus were numerous enough to reduce the soil inoculum potential of B. sorokiniana by day 4. The 250 level reduced inoculum potential of B. sorokiniana more slowly and B. sorokiniana had the greatest effect on emergence. The contrast in the biomass data may be due to M. lissus starting to feed upon the wheat root hairs and basal epidermal cells after the free soil B. sorokiniana hyphae has been consumed. The relationship between M. lissus and B. sorokiniana can be speculated to be competitively inhibitory on the basis of these data.

Figure 14. Effect of *Mesodorylaimus lissus* in combination with *Bipolaris sorokiniana* on 'Chisholm' wheat.
A= Effect of *M. lissus* + *B. sorokiniana* on seedling emergence of wheat.
c= Control(No nematode, no fungus)
0= *B. sorokiniana* alone
250= *B. sorokiniana* + *M. lissus* at 250 level
500= *B. sorokiniana* + *M. lissus* at 500 level

B= Effect of *M. lissus* + *B. sorokiniana* on biomass production of wheat.
Control= No nematode, no fungus
0= *B. sorokiniana* alone
250= *B. sorokiniana* + *M. lissus* at 250 level
500= *B. sorokiniana* + *M. lissus* at 500 level

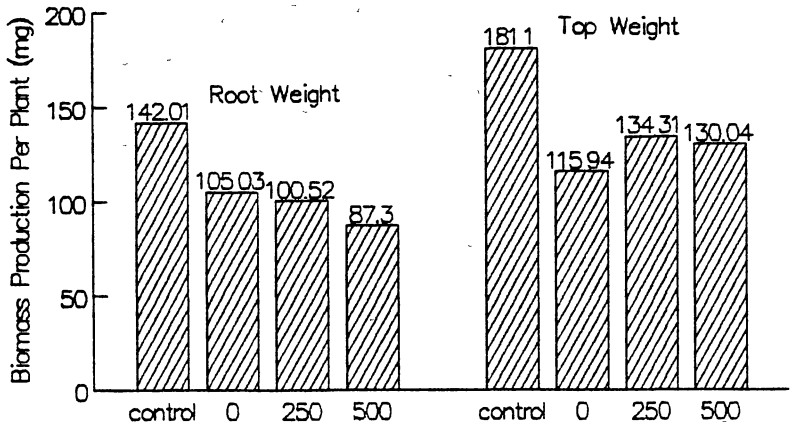
Effect of M. lissus + B. sorokiniana on 'Chisholm' Wheat

Effect of M. lissus + B. sorokiniana on Seedling Emergence of Wheat



A

Effect of M. lissus + B. sorokiniana on Biomass Production of Wheat



B

Disease severity of B. sorokiniana. The disease severity of B. sorokiniana on the subcrown internodes rated, based on 0-3 severity scale (16) is given in Table 5.

Table 5. Disease severity on wheat subcrown internodes caused by Bipolaris sorokiniana in combination with Mesodorylaimus lissus.

Level of M. lissus	Disease severity[a]
0	3[b]
250	2.9
500	2.47

[a]0= Healty 1= Slightly 2= Moderate 3= Severe
[b]Means of four replications

The 500 inoculum level of M. lissus plus B. sorokiniana reduced the disease severity of B. sorokiniana compared to the B. sorokiniana alone treatment. There was no significant difference (p=0.05) between B. sorokiniana alone and the 250 M. lissus plus fungus. In all petri dishes, where the subcrown internodes exhibiting necrosis were plated, B. sorokiniana growth was observed. There was no B. sorokiniana growth on the subcrown internodes taken from control pots. These results confirmed that M. lissus feeding upon B. sorokiniana hyphae reduced the inoculum potential of this fungus and resulted in a slightly reduced disease severity. These results; however, do raise the question of whether or not M. lissus preferred B. sorokiniana to wheat root hairs

in the rhizosphere. This can be clarified by additional *in vitro* host preference studies.

Combined Effects of *M. lissus* and *B. sorokiniana*

On *Amaranthus* sp.

Seedling Emergence. The combined effects of *M. lissus* at the 0, 250, and 500 levels and *B. sorokiniana* compared to the control on the seedling emergence of *Amaranthus* sp. are given in Figure 15a.

All treatments showed a slight reduction in seedling emergence rate and seedling vigor compared to the control. Total emergence was slightly reduced in all treatments with *B. sorokiniana* alone having the lowest total emergence.

Biomass Production. The biomass production of *Amaranthus* sp. on the basis of root and top weights is given in Figure 15b. All treatments increased amaranth seedling root and top weight compared to the control. But the only significant increase was in the top weight produced by the 500 level plus *B. sorokiniana*.

The results of this study were not sufficient to be interpreted perhaps due to the shortness of the duration of the experiment.

Disease Severity of B. sorokiniana. The below ground parts of *Amaranthus* sp. from all treatments were plated onto a selective medium (27). All plates except control produced a low level of *B. sorokiniana* growth. The effects of the

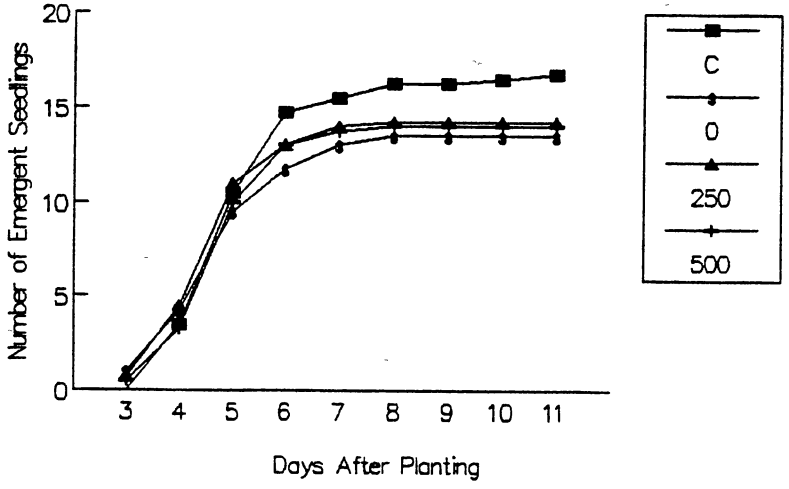
Figure 15. Effect of *Mesodorylaimus lissus* in combination with *Bipolaris sorokiniana* on *Amaranthus* sp.

A= Effect of *M. lissus* + *B. sorokiniana* on seedling emergence of *Amaranthus* sp.
C= Control (No nematode, no fungus)
0= *B. sorokiniana* alone
250= *B. sorokiniana* + *M. lissus* at 250 level
500= *B. sorokiniana* + *M. lissus* at 500 level

B= Effect of *M. lissus* + *B. sorokiniana* on biomass production of *Amaranthus* sp.
Control= No nematode, no fungus
0= *B. sorokiniana* alone
250= *B. sorokiniana* + *M. lissus* at 250 level
500= *B. sorokiniana* + *M. lissus* at 500 level

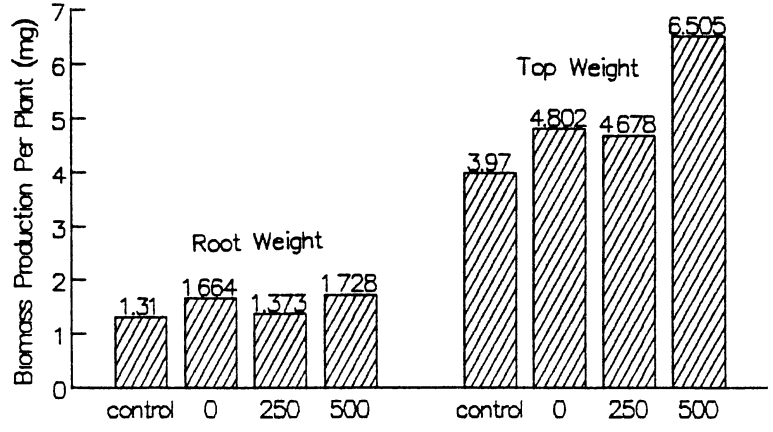
Effect of M. lissus + B. sorokiniana on Amaranthus sp.

Effect of M. lissus + B. sorokiniana on Seedling Emergence of Amaranthus sp.



A

Effect of M. lissus + B. sorokiniana on Biomass Production of Amaranthus sp.



B

fungus on the amaranth root system were not sufficiently distinctive to allow a severity rating.

Combined Effects of M. lissus and P. irregulare

On Wheat

Seedling Emergence. The effects of P. irregulare and M. lissus at three levels are given in Figure 16a. The P. irregulare, alone treatment produced a slight delay in emergence through day 5 but none of the treatments reduced the total emergence.

Biomass Production. The biomass production of wheat in the combinations with the 0, 250, and 500 inoculum levels of M. lissus and P. irregulare compared to the control plants by root and top weights is given in Figure 16b. Although a significant decrease ($p=0.05$) in the root weight was observed at both combinations of the 250 and 500 levels with P. irregulare, their interaction appears to be competitive/inhibitory.

There was no observed significant difference between the control and the combinations in the top weight. However, the reduction caused by M. lissus 250 level plus P. irregulare was slightly less than additive (20.8%) compared to the reduction caused by either of them alone (7.1% and 14.8%, respectively). this may indicate a positive interaction due to infection if one considers there was an apparent reduction in P. irregulare inoculum

Figure 16. Effect of *Mesodorylaimus lissus* in combination with *Pythium irregulare* on 'Chisholm' wheat.
A= Effect of *M. lissus* + *P. irregulare* on seedling emergence of wheat.
C= Control(No nematode, no fungus)
0= *P. irregulare* alone
250= *P. irregulare* + *M. lissus* at 250 level
500= *P. irregulare* + *M. lissus* at 500 level

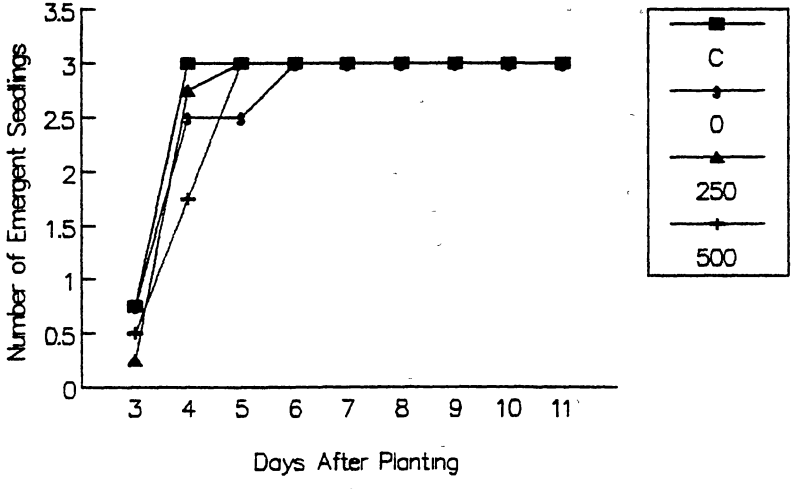
B= Effect of *M. lissus* + *P. irregulare* on biomass production of wheat.
Control= No nematode, no fungus
0= *P. irregulare* alone
250= *P. irregulare* + *M. lissus* at 250 level
500= *P. irregulare* + *M. lissus* at 500 level

Effect of M. lissus + P. irregulare

on 'Chisholm' Wheat

Effect of M. lissus + P. irregulare

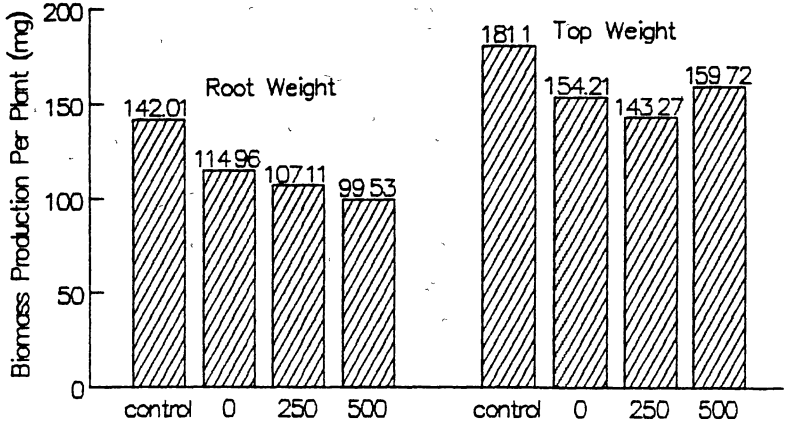
on Seedling Emergence of Wheat



A

Effect of M. lissus + P. irregulare

on Biomass Production of Wheat



B

potential due to M. lissus feeding. The P. irregulare combination with 500 level of M. lissus; however, reduced the damage to top weight compared to the damage caused by each pathogen alone. This can be explained by a competitive or inhibitory interaction between these organisms at the higher level of M. lissus. This interaction may be a result of both the nematode feeding on P. irregulare and competing for infection sites.

Disease Severity of P. irregulare. The seminal roots of wheat plants were plated onto a Pythium selective medium (4) to define the disease severity. The number of hits per 10 cm of seminal roots is given in Table 6. There was no significant difference between the fungus alone and its combinations with M. lissus. However, 75 per cent increase in hits at the 250 nematode level could be a result of infection court provision by the nematode.

Table 6. Disease severity on wheat seminal roots caused by Pythium irregulare in the presence of Mesodorylaims lissus.

Level of M. lissus	Disease severity[a]
0	0.24[b]
250	1.0
500	0.68

[a]Number of hits per 10 cm of seminal roots
[b]The average of four replications

Combined Effects of M. lissus and P. irregulare

On Amaranthus sp.

Seedling Emergence. Figure 17a represents the effects of P. irregulare alone and its combinations with the 250 and 500 inoculum levels of M. lissus on the seedling emergence of Amaranthus sp. Although all treatments showed a slight reduction in seedling emergence, there was no statistically significant difference ($p=0.05$) between the control and the other treatments throughout the study. Mesodorylaimus lissus alone at the 250 level and P. irregulare alone; however, caused a greater reduction in seedling emergence than did their combination indicating that P. irregulare was attacked first reducing its damage. Perhaps amaranth was nonpreferred substrate and M. lissus started feeding upon it so late that only a stimulatory effect was observed due to the short duration of the study.

Biomass Production. Biomass production of Amaranthus sp. in the combinations of the 0, 250, and 500 inoculum level of M. lissus and P. irregulare is given in Figure 17b. All treatments increased amaranth seedling root and top weight compared to the control. But the only significant increase was in the top weight produced by the 500 level plus P. irregulare. The increase in the biomass production was previously attributed to the irritation of M. lissus stimulatory feeding under the optimum conditions of the experiment and the reduced competition among the plants in the 250

Figure 17. Effect of *Mesodorylaimus lissus* in combination with *Pythium irregulare* on *Amaranthus* sp.
A= Effect of *M. lissus* + *P. irregulare* on seedling emergence of *Amaranthus* sp.
C= Control (No nematode, no fungus)
0= *P. irregulare* alone
250= *P. irregulare* + *M. lissus* at 250 level
500= *P. irregulare* + *M. lissus* at 500 level

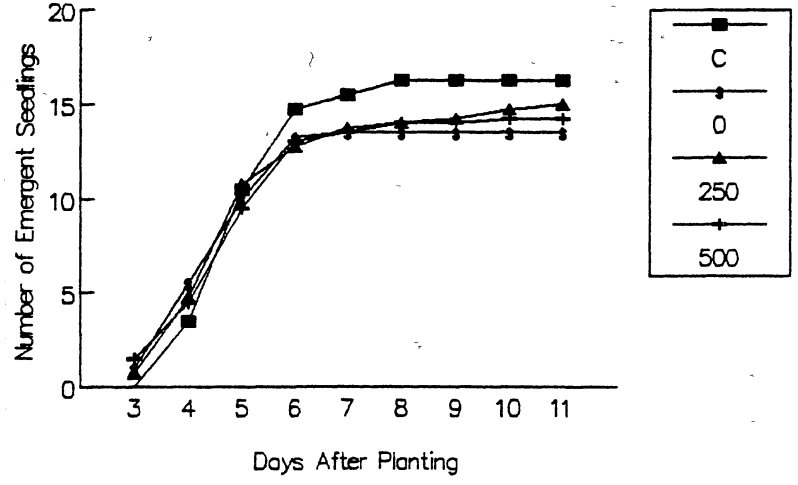
B= Effect of *M. lissus* + *P. irregulare* on biomass production of *Amaranthus* sp.
Control= No nematode, no fungus
0= *P. irregulare* alone
250= *P. irregulare* + *M. lissus* at 250 level
500= *P. irregulare* + *M. lissus* at 500 level

Effect of M. lissus + P. irregulare

on Amaranthus sp.

Effect of M. lissus + P. irregulare

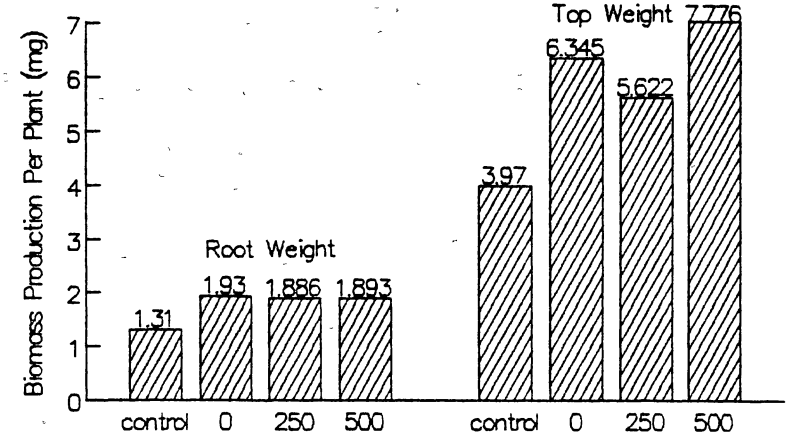
on Seedling Emergence of Amaranthus sp.



A

Effect of M. lissus + P. irregulare

on Biomass Production of Amaranthus sp.



B

and 500 inoculum rates. Besides, the duration of the study was not long enough to interpret these data. However, in light of the previous studies it can be speculated that M. lissus fed preferentially upon the fungus. This could be determined by future in vitro substrate preference studies.

Disease Severity of P. irregulare. The below ground parts of Amaranthus sp. were plated onto a Pythium selective medium (4) in order to define the disease severity of P. irregulare. There was no fungal growth in any plates. Thus, no interpretation was possible.

The data obtained from the rhizosphere interaction study showed that 1) M. lissus impacted emergence and biomass production, however, the impact was minimized by the optimum moisture conditions and short duration of the study. Therefore, further studies should be carried out at different levels of water stress in order to find the effect of M. lissus on the seedling emergence and the biomass production under drought stress 2) Amaranthus sp. was not a suitable or preferable host for M. lissus although the nematode did feed upon it. In vitro substrate suitability and preference studies should be conducted to confirm this finding 3) There was competitive inhibitory relationship between M. lissus and B. sorokiniana. It seemed that B. sorokiniana was a preferred substrate to wheat roots. This also needs to be clarified by in vitro substrate preference studies 4) The relationship between M. lissus and

P. irregulare was predominantly competitive inhibitory. The substrate preference of M. lissus should be studied comparing this fungus and wheat roots.

This study was conducted under the optimum laboratory conditions and was not repeated. Russell (25) found significant reductions in wheat seedling emergence, root weight and top weight in the presence of M. lissus by in a greenhouse study. On the contrary, there was no significant reduction in seedling emergence or biomass production of wheat in this study. This can be explained by the difference between the conditions and wheat variety used in both studies. Therefore, the present study should be repeated under diverse greenhouse conditions to confirm the findings mentioned above.

SUMMARY AND CONCLUSION

The life history, feeding habits, and rhizosphere interactions of Mesodorylaimus lissus were studied. The life cycle from oviposition to eclosion required an average of 5 days and 19.25 hours (5 days 3.5 hours - 6 days 13.25 hours) and from enclosion to adult 28 days (∓1 days) under in vitro laboratory conditions. The first molt occurred 36 hours (∓12 hrs) after eclosion. The period between first molt to second molt was 6 days(∓6 hrs); between second molt to third molt was 12 days (∓1 days).

It was found that the primary feeding habit of M. lissus was predacous; however, it fed parasitically upon wheat roots, myceliophagously upon Pythium arrhenomanes, Bipolaris sorokiniana, and Rhizoctonia cerealis, microphagously upon Actinomycetes, and saprophagously upon dead Panagrellus redivivus and a human dietary compound (Similac). The substrates in order of suitability for the reproduction of M. lissus were as follows: 1) Prey nematodes, P. redivivus, Diplogaster sp., and cephalobids 2) Wheat 3) P. arrhenomanes 4) B. sorokiniana 5) R. cerealis 6) Actinomycetes. Prey nematodes and wheat root hairs and basal epidermal cells were relatively adequate substrate while the remainder supported M. lissus reproduction only at a maintenance level.

Linford and Oliveira (18) concluded that the predacious nematodes found their prey by chance; in this study, it was clearly indicated that M. lissus found its prey not by chance but by an orientation capability which facilitated a definitive preference in prey selection.

Mesodorylaimus lissus is a common soil inhabiting nematode and has been collected from the rhizosphere of higher plants (2, 3, 19, 25, 28). The possible impacts exerted by M. lissus on its environment have not been studied. This study elucidated that M. lissus might delay seedling emergence and biomass production of wheat and Amaranthus plants. The relationship between M. lissus and fungi was predominantly competitively inhibitory. The only additive relation was found at the combination of M. lissus 250 level with P. irregulare on the top weight of wheat plants. The combined effects of the nematode and the fungi on the Amaranthus sp. rhizosphere were not definitive due in part to the short duration of the study.

Recommendations for Future Studies

The position and migration of the replacement odontostyle in dorylaims have not been adequately studied. During this study it was observed that the replacement odontostyle changed its position dorsoventrally during its migration, suggesting it moved up by a helical route. This should be studied in detail.

The life history of M. lissus was studied under in

vitro laboratory conditions; therefore, further studies should be performed to determine the influence of biotic and abiotic factors of the soil environment on its life cycle duration.

Feeding habits of this nematode based on substrate preference should be extended to identify its preference for all possible combinations of potential rhizosphere interactants.

The rhizosphere interaction study was repeated one time under optimum conditions. Therefore, a study should be conducted to elucidate the possible combined effects of *M. lissus* and fungi under diverse conditions such as under different levels of water stress.

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VITA 2

Nesibe Nurdan Ertekin

Candidate for the Degree of

Master of Science

Thesis: THE LIFE HISTORY AND FEEDING HABITS OF MESODORYLAIMUS LISSUS THORNE, 1974

Major Field: Plant Pathology

Biographical:

Personal Data: Born in Corum, Turkey, February 21, 1953, the daughter of Mr. and, Mrs. Ergin.

Education: Received Bachelor of Science Degree in Plant Protection from University of Ege in 1976; completed requirements for the Master of Science degree at Oklahoma State University in July, 1990.

Professional Experience: Researcher, Plant Protection and Research Institute Diyarbakir, Turkey, 1976-1988.

Professional Organizations: Turkish Phytopathological Society, Turkish Entomological Society, and Society of Nematologist.