APPROACHES TO THE CONTROL OF DITYLENCHUS DIPSACI (KHUN) FILIPJEV ON ALFALFA (MEDICAGO SATIVA L.)

By

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APPROACHES TO THE CONTROL OF *DITYLENCHUS DIPSACI* (KHUN) FILIPJEV ON ALFALFA (*MEDICAGO SATIVA* L.)

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CHAPTER I

INTRODUCTION

Graham et al. (26) described the stem nematode. Ditylenchus dipsaci (Kuhn) Filipjev, as the most economically important nematode pest of alfalfa. Each year, important losses are registered due to direct or indirect killing of alfalfa plants by this nematode (7,28). Ditylenchus dipsaci is also an important pest on many other crops and ornamentals. A wide spectrum of geographical adaptations of the alfalfa stem nematode races combined with differences in environment makes it difficult to devise universally transposable control measures.

Various control measures have been used alone or in combination against D. dipsaci. Some examples are crop rotation to non-host crops like corn or sorghum for 2 to 3 years, sanitary procedures such as cleaning farm equipment to avoid the spread of nematodes from infested areas, and chemical control, with the use of systemic nematicides applied as a foliar spray, non-incorporated surface granules or liquid injection. Chemical control has reduced D. dipsaci populations under field conditions with a resultant increase in yield at rates generally much higher than labeled rates (28,30,47,53). Some workers suggest that the primary effect carbofuran has on alfalfa yield is due to a positive physiological effect on the plant component of the alfalfa-rhizobium system rather than direct nematicidal efficacy (5). Use of resistant varieties is generally considered the most efficient method of controlling the stem nematode on alfalfa. Widespread use of existent resistant varieties is severely limited by restricted geographical adaptation of the varieties and unfavorable differential responses of stem nematode geographical races to the resistant germplasm (2,6). Field screening of alfalfa germplasm under local environmental conditions and against local stem nematode races appears to be a solution. Present technology also makes field screening difficult, primarily because of erratic inoculum distribution . Few studies have been devoted to the evaluation of some natural enemies of *D. dipsaci* as biological control agents. One of those potential biocontrol agents is a Chytridiomycetous fungus, *Catenaria anguillulae* Sorokin 1876 (54). Attacks of *C. anguillulae*, a zoosporic endoparasite, are frequently observed on *D. dipsaci* and other groups of nematodes extracted from soils or plant materials (3,6,8,20,50). Many authors consider that *C. anguillulae* has little potential as a biological control agent, probably due to its soil antagonists (8,20,35,50). Its biocontrol potential in a less competitive foliar environment has not been investigated.

The objectives of this study were, (1) to evaluate the efficacy of at plant application of carbofuran at labeled insecticidal rates for control of *D. dipsaci*; (2) to develop techniques which would facilitate local field evaluation of resistant alfalfa germplasm, and (3) to study the efficacy of *C. anguillulae* as a biological control agent in soil and foliar environments.

CHAPTER II

LITERATURE REVIEW

Historical

Ditylencus dipsaci (Kuhn 1857) Filipjev 1936, the stem nematode, was first described as *Anguillulae dipsaci* by Kuhn (40). Although the stem nematode was first reported in the United States in 1908 (44), its first attack on alfalfa *Medicago sativa* L. was not recorded before 1936 (22). *Ditylenchus dipsaci* is now widespread on alfalfa throughout the United States where it causes three to five percent loss of the alfalfa yearly crop (25). In Oklahoma, alfalfa is produced on approximately 202,347 hectares (2); nematicide trials have shown that in a given year, *D. dipsaci* can reduce alfalfa yield by more than 50%, that is by at least 3.7 metric tons/ha (47).

Symptoms

The first most common symptom observed on infected seedlings is the swelling of the cotyledonary node and a shortened appearance, especially under humid conditions (46). Under field conditions, symptoms of stem nematodes on alfalfa are patches of stunted plants with distorted leaves, swollen and discolored crown buds. These symptoms are most obvious during regrowth in early spring on heavily infested alfalfa plants (46).

Life Cycle of the Nematode

Plant parasitic species of *Ditylenchus* are generally obligate parasites of higher plants which attack both roots and above ground plant parts of numerous plant species. They are of moderate length (.8-1.4 mm) and males and females are morphologically very similar (36). The first molt occurs within the egg (36). Two postovic molts give rise to J-4 or preadult. The preadult is the primary persistent infective stage. In foliar infection the preadult enters above ground plant parts via stomata or penetrates directly into the base of stems and leaf axil. They then molt and mate (36,48,57). Under experimental conditions, the life cycle varies from 9 days (59) to 73 days (35). Under field conditions, however, the development time may vary considerably due to the influence of race-host interactions and environmental conditions (46). Thus, when we take into account the ability of the nematode to undergo anabiosis, we might say that the life cycle of *D. dipsaci* may range from 9 days (59) to more than 23 years (21).

Control Measures

Various control measures are used alone or in combination against *D. dipsaci* (2, 5, 19, 25, 47, 48, 53).

- Crop rotation to non-host crops like sorghum or corn for 2 to 3 years can reduce the nematode populations to a point where alfalfa can be economically grown.
- Sanitary procedures such as cleaning farm equipment will prevent or decrease the spread of nematodes from infested areas; the ability of the nematodes to undergo anabiosis allows them to dry out in plant tissue on equipment. In this dormant state, they can survive until favorable moisture permits their return to activity.
- Chemical control. Soil fumigation started in the mid 1800's for the control of insects. It was soon discovered that the same chemicals used as insecticides, also had nematicidal effects. The first obstacle against the widespread application of nematicides was their high cost, even on high-priced crops (51,57). Cheaper nematicides of the carbamate and phosphoric groups proved not to be the solution because of their high mammalian toxicity. High toxicity of contact nematicides, poor circulation of fumigant nematicides in heavy soils together with the low value of alfalfa forage have severely limited the clearance of many nematicides for use on that crop (57). Only 2 nematicides, carbofuran (2,3-dihydro-2, 2-dimethyl-7-benzofuranyl methylcarbamate) and telone C-17 (74% 1,3-dichloropropane, 16.5% chloropicrin) are labeled for use on alfalfa. Carbofuran, however, is labeled at rates below those that have been effective for nematode control

(7,8,47). The efficacy of the labeled insecticidal rates against D. dipsaci needs to be determined.

- Resistant Varieties. The use of resistant varieties is generally considered the most efficient method of controlling D. dipsaci. Large numbers of resistant cultivars have been developed (46), but the cultivars are not useful across all alfalfa growing areas. Great variations have been encountered in D. dipsaci races from one region to another. Geographical populations often respond differently to a given resistant variety; the variety in turn has its own cultural requirements, especially dormancy, which will make it poorly adapted to some areas (9,48). With adaptation so important, it becomes easy to understand why many cultivars selected for resistance under growth chamber and greenhouse testing, perform poorly under more arduous field conditions. Another obstacle encountered by plant breeders and nematologists is the difficulty of obtaining qualitative determination of resistance through symptomatology exhibited by alfalfa plants in their early stages of development (13). McBurney (42) found a total of 20% of the alfalfa plants sampled either showed symptoms but no nematodes in their tissue, or had nematodes without symptoms. Histochemical differences in cell walls of nematode-infected and noninfected alfalfa plants could not be observed by Krusberg (38,39). Many studies indicated that the same types of tissue damages were observed on both resistant and susceptible alfalfa. It was noted however, that the rate and degree of damage were lower with the resistant plants. Reed found that resistant cultivar infested with Ditylenchus formed smaller cavities, fewer cells with dense cytoplasm and very heavy lignification surrounded the cavities (46).

Reproducible field screening techniques would partially solve some of these problems. Not only is seedling symptomatology difficult to interpret, but field evaluation on the basis of nematode population (or number of nematodes per unit of plant tissue) is difficult to reproduce due to the great variation in field soil populations (variable initial inoculum). It seems likely that development of artificial inoculation techniques for field conditions would permit observations of cultural adaptation of candidate cultivars while producing duplicatable nematode population data. This would be achieved by superimposing greater inoculum uniformity which will in turn reduce the number of plants escaping infection and confusing the results of screening trials. The development of technology for field germplasm evaluation includes a set of procedures, the first of which is the extraction of the inoculum.

Inoculum Extraction

Various extraction procedures are used to recover nematodes from soil or plant materials, but they can be all compared on the basis of nematode emergence from the substrate and quantitative extraction of inoculum. The rate of nematodes extraction is important when a high inoculum level is needed in a short period of time for field inoculation. Faster emergence of nematodes from forage produces more vigorous, infective inoculum and more plant material can be processed to still yield more inoculum per unit time for use on the field. The quantitative extraction helps to determine as accurately as possible the nematode population within the substrate. The determination of such population is useful for evaluating resistance of germplasm based on nematodes per gram of plant tissue. This is an indication of the inoculum reproductive potential in the germplasm.

The efficacy of nematode extraction from plant material is influenced by the nematode and the nature of the plant tissue. Most of the methods used today to extract nematodes from plant tissue are based essentially on the principles of wetting, aeration, sieving and flotation (4,15,51,52,56). Those methods have been improved by many authors by one or more of the following elements: aeration, temperature, maceration, sugar-flotation and centrifugation (12,14,18,23,32).

Inoculum Storage and Viability

Wide scale field inoculations attempted at Oklahoma State University have not been successful due to significant and rapid infection of the inoculum by parasitic fungi following extraction and prior to their application as field inoculum. Platzer and Brown were unsuccessful in their attempt to protect suspension of Romanomermis against C. anguillulae infection by using relatively high concentrations of fungicides (45). The efficacy of some other fungicides to protect stored D. dipsaci against this fungus requires further investigation. The condition of any inoculum that would be protected by the fungicide suspension needs to be studied. The infectivity of the nematodes toward alfalfa plants must not be altered or impaired by eventual detrimental effects of the fungicides on the inoculum. Infectivity tests of the inoculum surviving in different fungicide concentrations and time periods could be compared to determine if the nematodes can be stored in fungicide suspensions for any period of time and still be used as inoculum.

Biological control

Although some natural agents have the potential of being good biocontrol agents, few studies have been conducted to assess or evaluate the efficacy of those natural agents in controlling D. dipsaci. Our routine observations of D. dipsaci and other genera of nematodes extracted from soil, roots, or aerial plant parts, have shown that significant number of nematodes were infected by various types of nematode destroying fungi, a few days after storage on the laboratory bench. Zoosporic fungi were the most commonly observed, but fungi with trapping or constricting rings were also present.

Russell (48) reported D. dipsaci being destroyed by Arthrobotrys anconia Drech., a constricting ring nematode-trapping fungus, and an apparently undescribed Dactyella sp. that produces adhesive knobs and stalked non-constricting rings. The same author (48), along with other workers (3,8,16,20,24,50) reported C. anguillulae, a zoosporic fungus, attacking D. dipsaci and/or many other plant parasitic nematodes. Catenaria anguillulae which belongs to the Chytridiomycetes, is the most commonly reported endoparasitic fungus attacking nematodes. Infection is most often observed, when nematodes are kept for a time in water in the laboratory (3,8). Sorokine (54) is credited with the name C. anguillulae, but the fungus is probably the one described in 1874 by Villot as a fresh water alga (58). Many authors (6,16,37) now believe that the name C. anguillulae encompasses 3 or 4 species due to the great variation in size, shape, and structure of the zoospores and sporangia that are reported. Catenaria anguillulae has limited hyphal growth; Couch (16) and Butler et al. (10) described the thallus as branched or unbranched nonseptate or sparingly septate hypha with rhizoids. Both zoospores and zoosporangia vary greatly in size according to the medium from 3.8 - 5.4 um and 3 - 36 x 12 - 71 um (16). Zoospores exhibit chemotaxic ability; they are preferentially attracted to nematode body orifices where there is the greatest concentration of exudates (8,20). When the uniflagellate zoospore reaches the nematode it usually encysts near natural openings (anus, vulva, buccal cavity) (3,8,20,50). A germ tube forms from the encysted spore and penetrates through the orifices or directly through the cuticle. An infection thallus develops lengthwise inside the nematode and produces septa-divided swellings that become sporangia at maturity (3). Life cycle duration has been found to be between 25 and 58 hours on nematodes (20) and 48 hours on agar medium (50). _Catenaria_ is omnivorous in habit; it has been found to parasitize rotifers, tardigrades many free living and plant parasitic nematodes in addition to a wide variety of dead organic matter (8,16,20,50). _Catenaria_ _anguillulae_ would probably be considered a facultative parasite. Esser et. al. (20) have found that differences in infectivity exist between isolates of presumably the same fungus. Sayre and Keely (50) attempted to use soil application of a single isolate of _C_. _anguillulae_ as biological control agent of _D_. _dipsaci_ on onion. They did obtain a reduction in the incidence of onion bloat with the application of massive numbers of zoospores to the soil. They concluded, however, that their isolate of the fungus "was not a very good biological control agent."

There are many factors that may have contributed to their dissatisfaction with the efficacy of the fungus as a biological control agent. Several authors (6,20,37,50) have found that C. anguillulae is favored by high pH (6.7-9.0) and low salt concentrations. Ho and Hickman (29) found that the infective zoospores of a related fungus were reduced in number due to their physical contact with glass beads. Presumably sand grains would have a comparative effect. Further, in a soil environment, the zoospores are exposed to fungistatic, antibiotic and fungilytic processes as noted by Sayre and Keely (50). The possibility that they may have been working with weakly infective isolate in a soil environment may also have adversely affected their findings. Finally they, like other workers (6,8,16,20,50) used zoospores which are apparently not polyplanetic and therefore are not persistent (50). Because of this, Sayre and Keely (50) suggested that knowledge of the factors governing the release of zoospores and efficacy of resting sporangia as inoculum were requisite for use of the organism as a biological control agent.

Many of the above conditions which mitigate against the use of C. anguillulae as a biological control agent in the soil are more favorable in a foliar environment. It would appear that application of the life cycle stage that survives desiccation to the foliage of a plant might result in the release of zoospores upon the availability of free moisture. This would coincide with the activity of D. dipsaci on alfalfa shoots. Thus the nematodes and chemotactic zoospores would be confined in an essentially two dimensional environment on the plant surface.

CHAPTER III

MATERIALS AND METHODS

Chemical Control

A chemical control trial was installed April 23, 1986 on the Plant Pathology farm, Stillwater, Oklahoma, to determine the efficacy of carbofuran (Furadan 4F, 2-3-dihydro-2,2 dimethyl-7-benzofuranyul methylcarbamate Lot No. m100077) using ultra low volume (ULV) in furrow, at plant application methods for controlling _D_. _dipsaci_ on alfalfa. The field has a history of _D_. _dipsaci_ infestation since its artificial inoculation in 1980. The previous seedling alfalfa stand had been decimated the spring of 1985 by the combined effect of _D_. _dipsaci_ infestation and herbicide phytotoxicity. The field had been summer and winter fallowed prior to planting.

A Ford 1600 tractor equipped with a toolbar mounted Planet Jr. planters set at 30 cm centers was used for planting. Six rows of "Buffalo" alfalfa seeds were planted per plot at a rate of 14.5 Kg/ha. Carbofuran was applied in a continuous stream in the furrow behind the seed drop using an ULV microtubule applicator developed at OSU (49). Treatments consisted of applications of carbofuran at: 0.28, 0.56, 0.86, and 1.12 Kg a.i./ha applied in 46 l/ha of water;

water alone was used as the control. Treatments were used as the main plot in a randomized complete block design with 6 replications. Plot size was 9.1 x 2.4 m.

Stand count was assessed on 2 randomly predetermined 30 cm sections of both rows 2 and 5 of each plot for a total length of 60 cm for each row. Survival counts of alfalfa seedlings was also studied. Fifty plants 2.4 cm apart or more were selected on row 3 to monitor long term stand survival. Intervening plants were removed and processed to determine nematode population levels 3 weeks after planting.

Data for stand persistance was taken twice, 22 and 43 days after planting from rows 2 and 5. Counts for determination of survival were started 28 days after planting; they were conducted weekly for a total of 6 weeks from row 3. Forage yield for treatments was determined by harvesting with a sickle bar mower and weighing the total forage for each 21.84 m^2 plot. Forage was farvested on July 10, August 4,1986, and May 5, 1987. One forage aliquant sample was taken and 50 g were soaked in aerated water for 24 hours. Emerged nematodes were collected on a 400 mesh soil screen and their populations determined.

Automated Experimental Extraction Procedures for *Ditylenchus dipsaci*

The apparatuses for the first three extraction systems have a majority of components in common. Described in the direction of water flow (from top to bottom) each apparatus has three parts: a) the extraction funnel, b) the trap, and c) the reservoir (FIGURE 1). The extraction funnels, 8 liter conical galvanized fuel funnels (FIGURE 1a), differed between systems only in their water intake and discharge. The funnels were suspended 10cm above the trap.

All three traps consisted of a 20cm diameter brass soil screen rim without a screen, nested in an intact 325 mesh (.043 mm opening) soil screen (FIGURE 2). This 325 mesh trap screen was modified by gluing a 5 cm diameter plastic disk (FIGURE 2a) to the screen below the funnel discharge and installing a 11 cm long, 0.5 cm OD vinyl tube air vent (FIGURE 2c) through the screen mesh (FIGURE 2b). The plastic disk was to disperse the funnel discharge flow preventing the nematodes from being forced through the screen. The vent tube prevented air from being trapped under the wet screen during operation. The trap screen was seated in a 5 liter plastic bucket (FIGURE 1b). The seams between each component were sealed with a 5 cm wide rubber band cut from an automobile inner tube. A plastic basket (FIGURE 3a) was fabricated of polypropylene mesh (0.5 cm openings) and a saran (chic lumite Corp) mesh disk (0.7 cm openings, 20 cm dia.) was glued to its bottom (FIGURE 3b). Holes were cut into the side and bottom of the basket to allow insertion of the trap bucket overflow tube (FIGURE 3c). The basket was then inverted in the trap bucket so that the saran mesh was parallel with and 1 cm below the bottom of the soil screen and the mouth of the overflow tube

Figure 1. Components of three automated bulk nematode extraction systems.

A. Galvanized fuel funnel
B. Trap bucket (TB)
C. Reservior bucket
D. Submersible centrifugal pump
E. Kitchen strainer
F. Top mist system (TMS)
G. Vinyl tube for gravity flow
H. Top Flooding System (Water intake)
I. PVC pipe syphon
J. Bottom flooding system

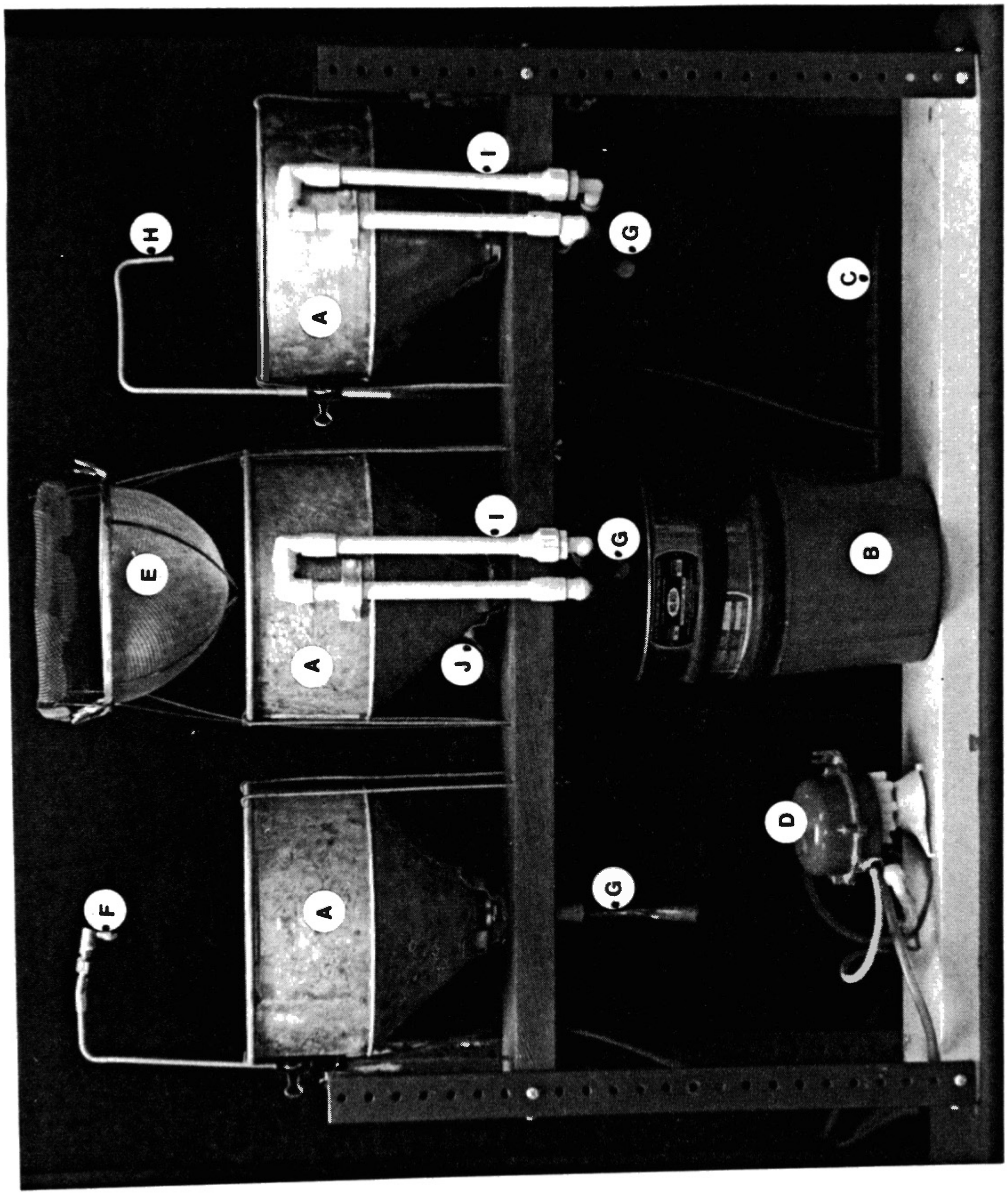
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Figure 2. Modified trap screen

A. Plastic disk for dispersing discharge flow
B. Air vent tube
C. 325 mesh (.043 mm opening) soil screen

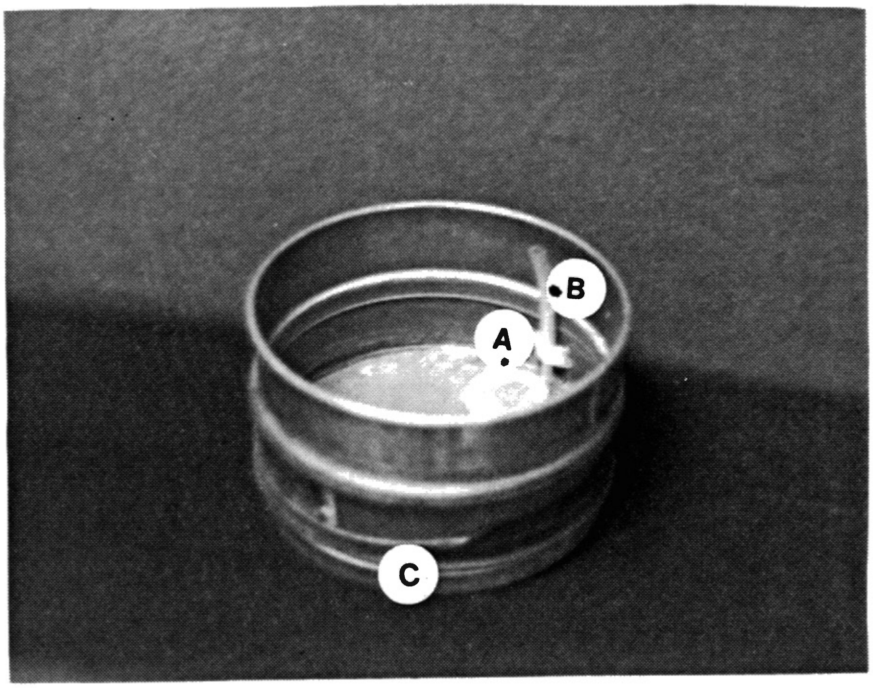
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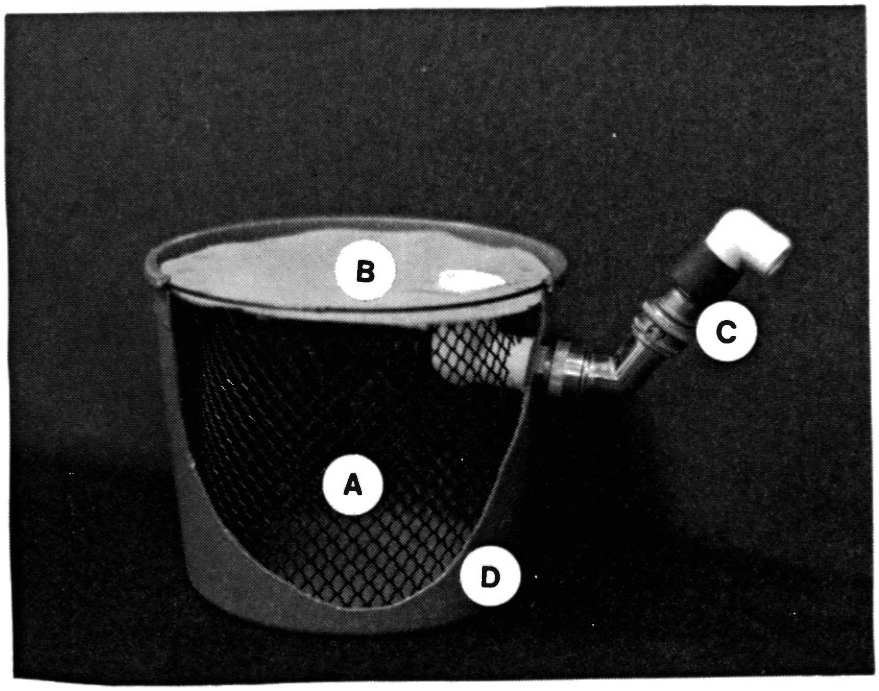
Figure 3. Cutaway view of the trap bucket (TB)

A. Polypropylene support basket
B. Turbulence deflector saran disk
C. "Gooseneck" overflow and water level control
D. Plastic bucket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D

was even with the saran mesh surface. This configuration allowed water discharged from the funnel to pass rapidly through the 325 mesh screen, trapping the nematodes on the screen. The saran disk functioned as a barrier reducing turbulence in the bottom of the trap bucket which would resuspend previously collected nematodes and causing them to be lost through the overflow. Therefore the water passed through the 325 mesh screen, flowed rapidly between it and the saran mesh and out the overflow tube during funnel discharge cycles. Nematodes on the 325 mesh screen crawled through the screen and settled into the trap bucket during the interval between discharge cycles.

The roughly U-shaped overflow tube was constructed of PVC (polyvinyl chloride) pipe fittings and metal garden hose connections. Rotation of a "gooseneck" (FIGURE 3c) on the discharge end of the overflow tube allowed the water level in the trap to be maintained approximately 1 mm above the soil screen surface.

The overflow tube emptied into a 15 liter reservoir bucket (FIGURE 1c). A 110V submersible centrifugal pump (Teel 1P808A) (FIGURE 1d) in each reservoir bucket was used to recycle the water through the system at desired time intervals. Pump cycles were controlled with electric timers.

One hundred twenty-five grams of the alfalfa forage to be extracted was contained in a 1 mm mesh 21 cm diameter kitchen strainer from which the handle had been removed

(FIGURE 1e). The strainer containing the forage was suspended in the funnels during extraction. One hundred twenty-five grams of forage was processed under all the extraction procedures.

Top Mist System (TMS)

Water was pumped from the reservoir, through a 6 mm ID vinyl tube, to a 0.3 mm ID aluminum pipe with a mist nozzle attached (FIGURE 1f). The mist nozzle was suspended 16 cm above the forage strainer basket and the spray was directed to wet the total surface of the forage. Nozzle delivery was 450 ml/min and the spray cycle was controlled at 2 min/15 min. Thus 900 ml of water was cycled through the forage every 15 minutes. Funnel drainage was by direct gravity flow through a 1.25 cm vinyl tube which directed the flow onto the plastic flow dispersion disk on the trap screen (FIGURE 1g).

Top Flooding System (TFS)

Water delivery was essentially identical to TMS except that the water was pumped onto the forage directly from the aluminum tube with no mist nozzle attached (FIGURE 1h). The water was purged from the funnel by a PVC pipe siphon which was activated immediately upon total submersion of the forage (FIGURE 1i). The funnel fill and purge cycle was timed to occur once per hour and last 4.5 minutes. Water delivery rate was controlled with an inline ball cock valve at the minimum flow required to prime the purge siphon upon filling of the funnel. Discharge water was directed from the siphon onto the plastic flow dispersion disk through a 1.25 cm vinyl tube (FIGURE 1g).

Bottom Flooding System (BFS)

This system is identical to TFS except that the water intake is located at the bottom of the conical portion of the funnel (FIGURE 1j). The anti-backflow valve was a flap of rubber sheet glued above the orifice and extended to cover it.

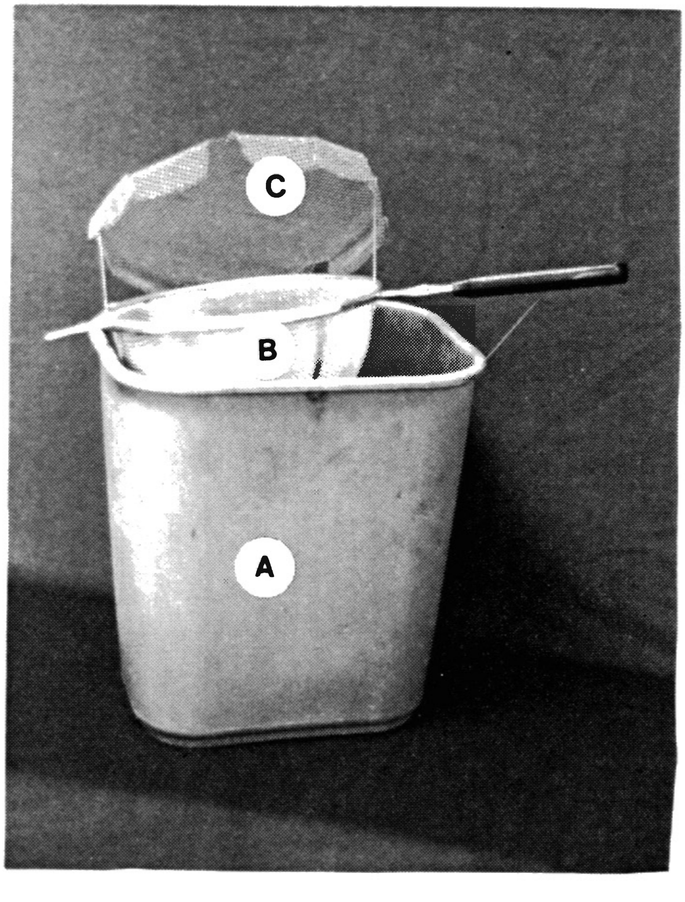
Manual Extraction Methods

Intermittent Soaking System (ISS)

A 15 liter plastic bucket (FIGURE 4a) was filled with 10 liters of water. The alfalfa forage to be extracted was contained in a kitchen strainer as described above, with the difference that this strainer had its handle (FIGURE 4b). The forage was soaked for 30 minutes once every 4 hour period and the strainer pulled out of the water, suspended on the rim of the basket for the forage to drain and aerate for 3 1/2 hours. The forage was maintained in the strainer by a 5 mm mesh screen wrapped over the rim of the strainer (FIGURE 4c).

Figure 4. Extraction apparatus used for the manual extraction systems.

A. Plastic bucket
B. Kitchen strainer
C. Mesh screen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B
A

Continuously Aerated Soaking
System 1 (CASS-1)

A plastic bucket identical to the one described under ISS was used. The alfalfa forage was loosely placed inside the bucket and covered with 10 liters of water. The system was aerated for 24 hours through 2 mm glass tube inserted into 4 mm ID plastic tube tapped into a manifold (1.25 cm OD PVC pipe). Air flow was regulated by a single aquarium air control valve on the manifold intake. Nematodes were recovered every 4 hours.

Continuously Aerated Soaking
System 2 (CASS-2)

This extraction procedure is similar to CASS-1; they differ only in the way nematode data was collected; nematodes under this system were collected at the end of the 24 hour period.

The six extraction procedures constituted the treatments. Forage samples were extracted for 24 hours to constitute one replication. The design was a randomized complete block and the experiment was replicated 5 times.

The nematodes extracted were recovered every 4 hours from the trap of TFS, TMS, BFS, and from CASS-1. Nematodes were also recovered at the end of the 24 hour extraction period from ISS and CASS-2 and also from the screen and the reservoir of TFS, TMS, and BFS. One 500 mesh screen was used to recover nematodes from each and their number estimated immediately by the following method. Nematodes were poured into a 150ml beaker and the volume of water brought to 100ml. Two 2ml samples were separately taken and nematodes counted under a stereoscopic microscope. The average number of nematodes per ml was calculated and used to estimate the suspension of each collection.

Fungicidal Control of _Catenaria_ Infection of _Ditylenchus_ Inoculum During Storage

Inoculum Collection and Quantification

Nematodes were extracted from alfalfa forage collected from Chickasha, Oklahoma. The thick alfalfa crowns were split and the other plant parts cut into sections about 15cm long. The excess soil was washed off with tap water and undetermined quantities of forage were soaked in about 10 liters of water in a 15 l capacity bucket. The water was aerated as described under CASS-1. Nematodes were collected every 24 hours for at least 3 days by pouring the water bucket through a 400 mesh screen. Recovered nematodes were separated from soil and plant debris by the OSU tub technique (1). Clean nematodes were stored in the refrigerator (4°C) during the extraction process to slow infection by zoosporic fungi. The initial inoculum level was determined by first concentrating the nematodes in 200 ml water by settling and decanting. The flask containing the nematodes was gently shaken to thoroughly mix the inoculum and 3 - 3 ml samples were withdrawn. Each sample was collected into a counting dish and the entire population of each subsample was counted with the aid of a stereoscopic microscope. The average number of nematodes/ml was then calculated and used to estimate the inoculum density in the 200 ml suspension. The final volume of the suspension was brought to 500 ml in a 1000 ml graduated cylinder and set aside.

Fungicide Efficacy

Metalaxyl [N-(2,6-Dimythelphenyl)-N-(methoxy-acetyl)-4 cyclohexene-1,2-dicarboximide)] and captan (Cis-N-(Trichloromethyl)-4 cyclohexene-1,2-dicarboximide), were evaluated for their efficacy in protecting nematode inoculum against *C. anguillulae* infection during storage. The study was set up on the laboratory bench at room temperature. A set of 500 ml flasks were used as containers for the nematodes and fungicide mixtures.

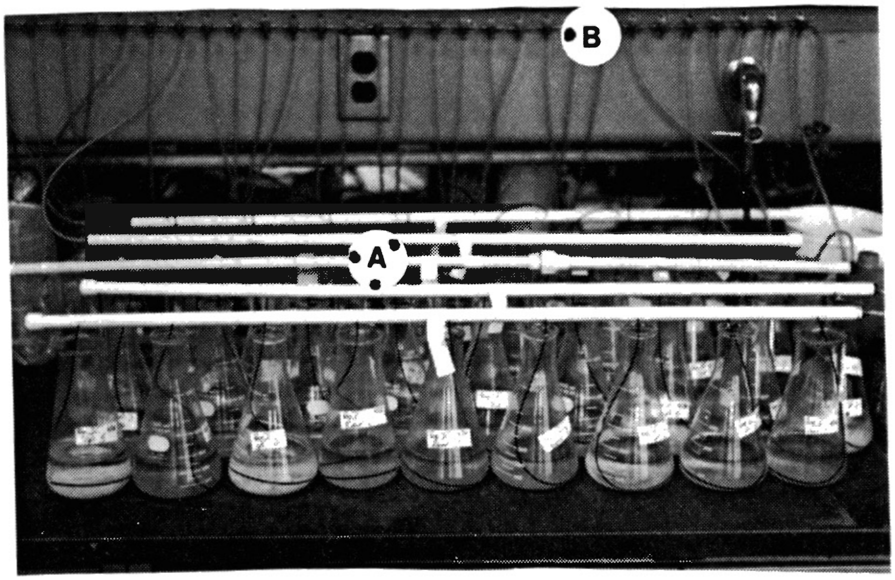
Four concentrations of 10, 200, 500, and 1000 ppm active ingredient (a.i.) were used for each fungicide and water alone as a control. The desired fungicide stock concentration constituting one treatment was prepared by adding the necessary amount of chemical to 800 ml of water. Two hundred ml of the above described suspension were then placed in a flask and 50 ml of nematode suspension added to constitute a final volume of 250 ml per flask and the desired concentration. The granules from the metalaxyl stock solution were settled at the bottom of the flask; the supernatant liquid was then poured slowly into another container and the granules discarded to clear the solution. The nine flasks from each replicate were randomly aligned on the bench (FIGURE 5) and aerated by a 1.2 mm ID PVC tube (FIGURE 5a) taped into a manifold (1.25 cm OD PVC pipe) suspended above the flasks. Air flow was regulated by a single aquarium air control valve (FIGURE 5b). Each replicate was set up on a different day to give time for data gathering. The study design was a randomized complete block with 5 replications.

The original nematode suspension from each treatment was determined at the beginning of each test.

One 10 ml sample was withdrawn from each flask and the number of visibly healthy and infected nematodes counted with the aid of a stereoscopic microscope. Data on healthy and infected nematodes was collected each week for a total of 42 days. The nematode suspension in each flask was gently shaken to achieve uniform nematode distribution and one 10 ml sample was withdrawn from each flask; counts were then made and the data recorded. After the withdrawal of each weekly 10 ml sample, the level of water in the flasks was marked so that daily addition of water would compensate for the evaporation and maintain the original concentration.

Figure 5. Experimental apparatus used for _Ditylenchus_ inoculum storage in fungicide suspensions.

A. PVC manifold
B. Air control valve


B
A

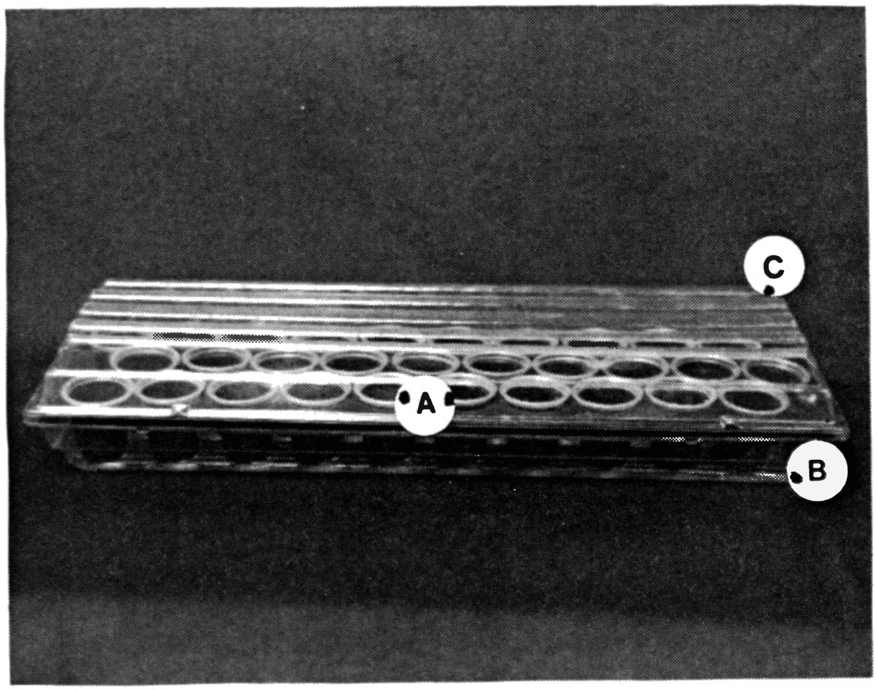
Effects of Fungicide Exposure
on *D. dipsaci*

An experiment was conducted in an attempt to determine the effect of fungicide exposure on *D. dipsaci* infectivity. Two testing procedures were used; one method for the first two replicates and a second method for the last three replicates of the fungicide protection study.

Method 1: 'Buffalo' alfalfa seeds were germinated in aerated water for 24 hours prior to planting. Thirty-five ml condiment cups with a 4 mm hole at the bottom were filled with about 3 g jiffy mix which was then covered with 9 g of fine sand. The cups were then bottom watered to saturation, one germinated seed planted in the middle of each cup and slightly covered with the same sand. Ten cups were used for each treatment. Ten active females and/or larvae from each weekly sample, or the total number of live nematodes when there was less than 10 nematodes in the sample, were used as inoculum. Each nematode was picked and washed off onto the planted seed with a few droplets of water. Cups were then placed in individual holes in a plexiglass receptacle (FIGURE 6a) fitted to a clear vinyl tray (FIGURE 6b) (A. H. Hummert Seed Co., PT-100C) which was then covered with a second tray (FIGURE 6c) to prevent evaporation. The entire unit was then maintained under a light bank. Individual plants were harvested and stained after a 7 day incubation to determine the number of nematodes inside the seedlings.

Figure 6. Moist chamber used for incubation of inoculated alfalfa seedlings.

A. Cups in plexiglass receptacle
B. Bottom vinyl tray
C. Top vinyl tray to reduce evaporation

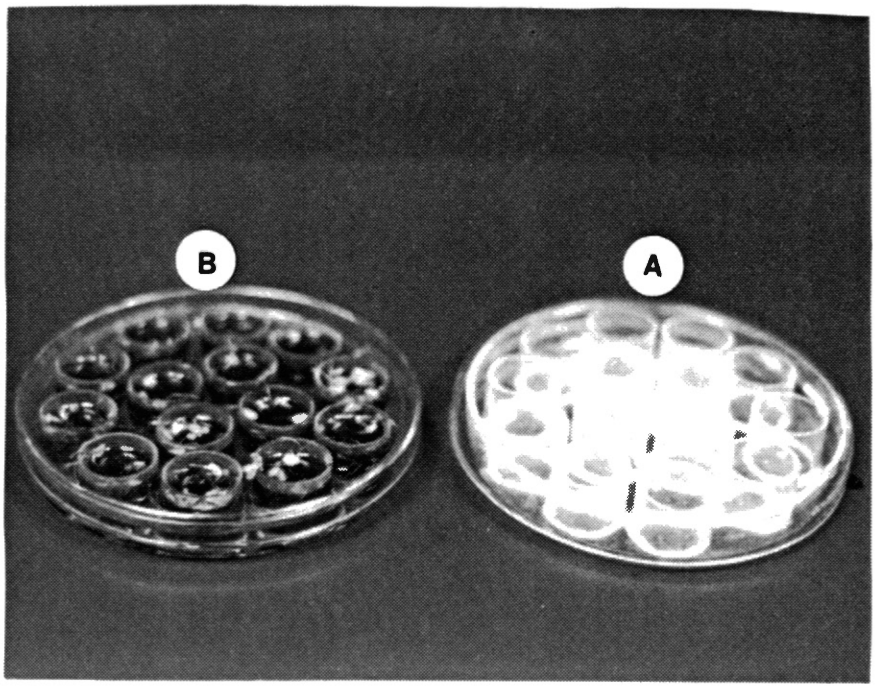

C
A
B

Method 2: This method differs from the first one only in that 3 cups were used and 6 germinated seeds were planted per cup. Nematodes from each sample were rinsed on a 500 mesh screen to wash off the fungicide and then collected in condiment cups. The nematodes were concentrated and transferred onto the seeds with a dropper. When the numbers of recovered nematodes was too low, the total count was used to inoculate only one cup with 6 seeds. Cups were then placed in the container described above (FIGURE 6) and incubated for 7 days before the seedlings were harvested and stained.

The staining procedure was the same for both methods. Glass tubes, 25 mm ID and 21 mm long were glued to the inside of a 137 mm wide 21 mm deep glass petri dish bottom. This produced a container with 14 small discrete cylindrical chambers (FIGURE 7a). About 6 ml of lactophenol cotton blue (31) was pipetted into each compartment and the space in between the compartments filled with water up to half the chamber's height (FIGURE 7b). The petri dish was then covered and heated on a hot plate until the water reached a rapid boil. Seedlings were transferred to the hot lactophenol cotton blue and stained for 3 to 5 minutes. At the end of the staining period, the plants were transferred to small vials with forceps and covered with phenol for overnight clearing of the plant tissue. Each vial was then shaken and the contents poured into a 86 mm glass petri dish and the nematodes in the plant tissue were counted with the

Figure 7. Petridish staining apperatus.

A. Empty apparatus showing small discrete chambers
B. Apparatus with lactophenol cotton blue and alfalfa seedlings


B
A

aid of a stereoscopic microscope. Dissection of some seedlings was sometimes necessary to separate the entangled nematodes inside the tissue. A few nematodes were observed outside plant tissue at the time of counting but were included in the counts.

Purification and Identification of Isolate "C"

Seven Oklahoma isolates of a zoosporic fungus, presumably *Catenaria anguillulae*, were collected from *D. dipsaci* and an unidentified dorylaim. Two of the isolates survived to pure culture: one isolate, termed isolate "C", was purified from infected *D. dipsaci* collected from Harrisson alfalfa field at Yale, Oklahoma; the second isolate, termed isolate "A" was purified from an unidentified Dorylaimoid nematode collected at K. B. Cornell wheat field, Custer, Oklahoma. Isolate A, however, was contaminated and subsequently lost. This study was therefore conducted only with the geographic isolate "C".

The fungus was isolated in the following manner. Twenty to thirty infected and non infected nematodes were washed for 3 minutes on a 500 mesh screen with 10% chlorox in a wash bottle. The nematodes were then rinsed with abundant sterilized tap water and transferred onto 3% agar, in a thin film of water. After 3 to 5 days, fungal structures could be observed developing from inside many of the nematodes. Infected nematodes were aseptically transferred under the hood to PDA plates to promote faster growth of the fungus. A second transfer was made from the edge of the growing fungus on the original PDA plates, to a set of new ones. The fungus was maintained by monthly transfer to new medium. Positive identification of _C. anguillulae_ was made after purification by comparison with the descriptions in the literature (16). During this study, _Catenaria_ cultures on PDA were blended, dried for 24 hours in an isolation chamber, and kept in petri dishes at room temperature. Ninety days after desiccation, the fungus could still grow on PDA plates and infect _Ditylenchus_ and _Panagrellus redivivus_. The dried fungus could still infect _Ditylenchus_ and _Panagrellus_ 8 months after desiccation, but did not grow on PDA.

Effect of Naturally Occurring Soil Antagonists on the Control of _D. dipsaci_ by _C. anguillulae_

A comparison of the effect of _C. anguillulae_ on _D. dipsaci_ infection of alfalfa seedlings was made using one lot of field soil with its natural biota intact and one disinfected by soil fumigation.

Field soil was collected from an adjacent area of the field in which the chemical control study was conducted. One lot of 114 liters was collected in plastic pails, mixed, and divided into two 57 liter lots. One lot was stored under shelter while the other was spread evenly on a polyethylene tarp. A 0.68 kg can of methyl bromide (MC_2, Dow Chemical Co.) was installed in a simplex applicator (Soil Fumigants Co.). The applicator was placed on the soil surface and the tarp was folded into an envelope and sealed prior to release of the chemical. The envelope was opened after 3 days and the soil was allowed to air dry for 1 day. Both soil lots were passed through a soil grinder, screened through a 30 mesh screen and removed to the laboratory in separate covered containers. The experimental units consisted of small pots fashioned by melting a single drainage hole in the bottom of 35 ml condiment cups (Fill-Rite Inc). Six grams of Jiffy Mix potting mixture (A. H. Hummert Seed Co.) was placed in the bottom of each cup to prevent soil loss and 25 g of natural or fumigated field soil was added. All cups were bottom watered to field capacity.

The study consisted of 5 replications of 6 treatments arranged in a randomized complete block design. The treatments compared were:

1. Fumigated field soil without nematodes and fungus (FS/O).
2. Fumigated field soil to which only Ditylenchus was added (FS/D).
3. Fumigated field soil to which both Ditylenchus and Catenaria were added (FS/D+C).
4. Non-fumigated field soil without nematodes and fungus (nFS/O).

5. Non-fumigated field soil to which Ditylenchus alone was added (nFS/D).

6. Non-fumigated field soil to which both Ditylenchus and Catenaria were added (nFS/D+C).

The fungus isolate described as isolate "C" in the previous section was used in this study. Two plates of 4 month old cultures were blended at low speed for 25 seconds in a Waring blender. The mixture was suspended in 50 ml of sterile water and poured into a clean beaker. To assess the density of zoosporangia, 1 ml was drawn from the suspension described above and added to 49 ml of water. Three samples, 3 ml each, were taken and separate counts were made with the aid of a stereoscopic microscope. Two to 3 drops of lactofuschin red were added to stain the fungal material. The 3 numbers obtained from counting all the sporangia in each dish were averaged for determination of the spore density in the suspension. One ml of fungal suspension was added to the desired cups.

Nematodes were extracted from alfalfa forage as previously described under the fungicide experiment section.

Twenty-five (25) females and/or juveniles were picked into 1 ml of water before being deposited on top of the fungus. PDA plates with no fungus but kept in the same environment as the fungus cultures, were processed under the same conditions and used as a control in all the cups where no fungus was added. The cups were placed in a moist chamber (FIGURE 4) and incubated for 24 hours at room temperature. Three 'Buffalo' alfalfa seeds, germinated for 24 hours in aerated water, were planted at 15 mm centers 10 mm from the edge of the cup. The seeds were covered with 1 mm of the appropriate soil and 1 ml of sterile water was added to all cups. The cups were placed in the vinyl tray moist chamber previously described and incubated for 7 days before the seedlings were harvested and stained. The study was repeated twice, and the design was a randomized block with 5 replications.

Catenaria anguillulae as a Biological Control of *D. dipsaci* in a Foliar Environment

The efficacy of using *C. anguillulae* as a biological control of *D. dipsaci* in a foliar environment was investigated in separate studies using zoosporangium and zoospore inoculum in protectant applications prior to nematode inoculation.

Zoosporangial Testing

Single germinated alfalfa seeds were planted in condiment pots as described in the section on fungicide exposure. An excess of plants were produced so that sufficient 4 to 7 day old seedlings could be selected for uniformity at the initiation of each replicate of each study. All studies were of a randomized complete block design with 7 replications in time. The zoosporangial suspensions were prepared from 3 month old fungus culture on nutrient broth. The culture was first washed with tap water on a 500 mesh screen and the fungal mat collected in 100 ml of water. The fungus was commutated at low speed for 25 seconds in a Waring blender. Two serial dilutions were prepared from the first concentration of sporangia. The 3 rates subsequently obtained and water as a control were used to constitute 4 treatments:

Rate 0 (Rt0) = water as a control
Rate 1 (Rt1) = 27 sporangia/droplets of H_2O
Rate 2 (Rt2) = 83 sporangia/droplets of H_2O
Rate 3 (Rt3) = 565 sporangia/droplets of H_2O

An excess of condiment cups were prepared and planted. After 4 to 7 days seedlings were selected for height uniformity.

Freshly extracted nematodes from dry alfalfa forage were concentrated in a counting dish and used for inoculum.

One droplet of the desired concentration of sporangia was placed between the cotyledons of an alfalfa seedling with a dropper. Five J-4 nematodes were individually picked into the droplet. The seedling was immediately covered with a non-vented condiment cup to retard the evaporation of the droplet. Inoculated seedlings were left in place until evaporation of all droplets. The cups were then removed and replaced by a clear vinyl tray. The unit was incubated for 4 days at 25C and 12/12 hours dark/light, the seedlings harvested, stained and nematodes counted.

Zoospore concentration was determined with the aid of a compound microscope at 200 x magnification. First the surface area of the microscope field was calculated by measuring its diameter with a micrometer. The surface area of the cover slip to be used was also calculated. Two drops from the rate to be determined or 0.1 ml, was deposited in the middle of a clean micro slide and carefully spread with the cover slip. The slide was put under the microscope and the number of zoospores counted within ten random microscopic fields. The number of zoospores were then averaged and used to estimate the inoculum level both under the cover slip (0.1 ml of suspension) and in the 5 ml suspension.

Zoospores as Inoculum

Zoospores were produced in the following way. One plate of 3 month old culture was blended at low speed for 25 seconds in a Waring blender. Fifty ml of a 1:1 ratio of sterilized water and sterilized pond water were added twice to the blender and poured into an autoclaved 500 ml flask. The flask was then sealed with a piece of aluminum foil and set on the laboratory bench. Millions of zoospores were produced after three days of incubation in the flask.

Three rates of zoospore concentrations were prepared in the following way: one ml from the original zoospore concentration was added to 4 ml of water and another 1 ml transferred from the first dilution to 4 ml of water. The three rates and water as a control were used as treatments.

1. Water as control or rate 0 (Rt0)
2. 2076 spores/droplet for rate 1 (Rt1)
3. 10380 spores/droplet for rate 2 (Rt2)
4. 51907 spores/droplet for rate 3 (Rt3)

The rest of the experiment was conducted as described under zoosporangial study.

CHAPTER IV

RESULTS AND DISCUSSION

Carbofuran Effects on Stand Establishment

The results of the stand counts 22 and 43 days after planting are summarized in TABLE I. All the carbofuran treatments significantly (P=.05) reduced alfalfa stand. This reduction in stand counts was likely a phytotoxic effect which resulted in 31 and 40 percent (TABLE I) seedling loss (P=0.01) under the highest carbofuran rate (1.12 Kg a.i./ha) at the time both counts were taken. This negative effect on stand was not observed by Sheaffer et al. (53) and Greenwood et al. (28), who applied respectively, 2.2 Kg a.i./ha "Furadan" 10G prior to planting and broadcast of 13 Kg a.i./ha "Furadan" 10G. Although regression analysis was not done, the data tends to suggest that as carbofuran rates increase, the stands at 22 and 43 days decrease (TABLE I).

Carbofuran Effects on Seedling Survival

Although initial stands were reduced by carbofuran, statistical analysis of the data on the seedling survival indicates that all carbofuran treatments significantly (P=0.01) improved alfalfa survival over a period of 6 weeks

TABLE I

EFFECT OF CARBOFURAN APPLICATION ON ALFALFA SEEDLING STAND COUNT (1)

Treatment	Observation Period			
	22 Days		43 Days	
	No. Plants (2)	C-T	No. Plants (2)	C-T
Control	29.8	-	23.4	-
0.28 Kg/ha	24.8	5.0	18.8	4.6
0.56 Kg/ha	22.1	7.7	16.2	7.2
0.84 Kg.ha	19.5	10.3	14.2	9.2
1.12 Kg/ha	20.5	9.3	14.0	9.4
LSD = 0.10		4.1		3.5
0.05		4.9		4.2
0.01		6.7		5.8
CV =		17.4		20.3

1) - Counts recorded from 2 randomly predetermined 30cm sections of rows 2 and 5.

2) - Numbers are averages of the 4 counts made (2 each) on rows 2 and 5 at each time period.

as compared to the untreated control (FIGURE 8). From an initial 50 plants per row on all treatments, the control declined to an average of 36 plants by the end of 6 week monitoring period. All carbofuran treatments resulted in significantly less decline in stand by the end of this period as shown in FIGURE 8. All carbofuran treatments through the monitoring period produced a slower rate of plant mortality than the control. The check lost significantly (P=0.01) higher numbers of seedlings the 2 weeks following the selection of the 50 seedlings. It is also notable that the differences between treatments became greater as the normal seasonal increase in abiotic stress occurred. Greenwood et al. (28), Belanger et al. (5), working with stem and soil nematodes respectively reported beneficial effects of post plant field application of carbofuran on alfalfa.

Nematicidal Efficacy of Carbofuran

Although very low numbers of stem nematodes were detected from harvested alfalfa seedlings at the beginning of the study, the data indicate that nematodes could not be recovered from forage at the time of the 2nd harvest, that is 15 weeks after the application of carbofuran. Nematicidal effect and subsequent increase in forage of alfalfa fields infected with Ditylenchus, have been attributed to carbofuran, although at relatively high rates (5,28). Because initial levels of soil stem nematodes were

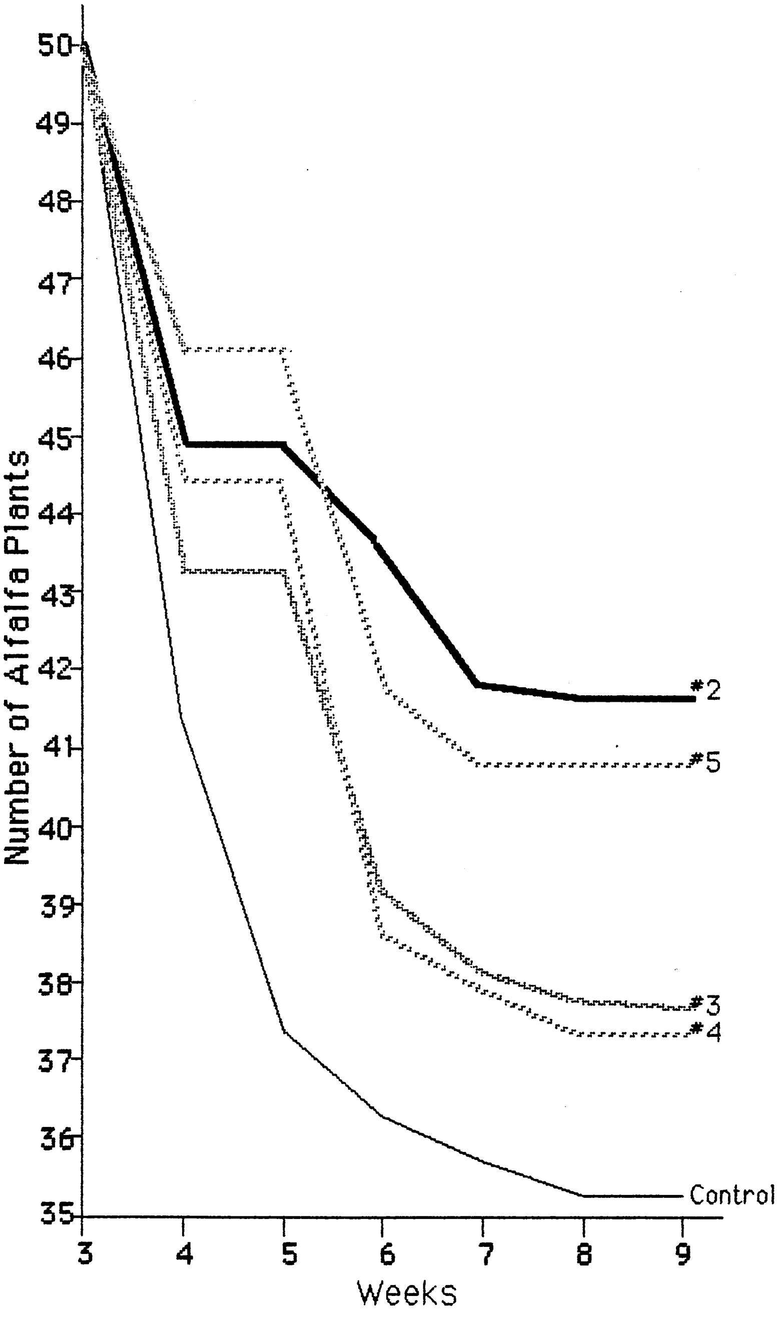
Figure 8. Effect of carbofuran application on seedling survival.

1. Control
2. 0.28 Kg a.i./ha
3. 0.56 Kg a.i./ha
4. 0.84 Kg a.i./ha
5. 1.12 Kg a.i./ha

a.i. = active ingredient

Survival counts were made on a single row in each plot in which the seedlings had been thinned to 50 plants a minimum of 2.5 cm apart 3 weeks after planting. The number of plants presented are means of 6 replications.

LSD (Week 9) (0.10) = 0.9
(0.05) = 1.1
(0.01) = 1.6


Number of Alfalfa Plants
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
3
4
5
6
7
8
9
Weeks
#2
#5
#3
#4
Control

not evaluated, the absence of nematodes from forage in the early part of the study cannot be attributed solely to nematicidal activity of carbofuran against _D. dipsaci_. However, the data on recovered nematodes 365 and 430 days after planting (TABLE II) points toward an initial nematicidal effect of the chemical. An analysis of variance of the data at harvest 1, indicated that _Ditylenchus_ numbers were significantly lower (P=0.05) under treatment 5 (TABLE II). This relationship is not uniform throughout the treatments nor is it confirmed by the nematode populations recovered after 430 days (TABLE II). Very low numbers of nematodes were recovered under all treatments and no significant differences were found between any two treatments.

Forage Yield Data

Analysis of the total yields taken at three harvest dates revealed the lowest rate of carbofuran, produced a significantly higher yield (P=0.10) than the control (FIGURE 9). The better results of survival counts are only slightly reflected on the forage yield since only one significantly higher yield was observed. The apparent initial phytotoxicity is compensated by the late beneficial effects of carbofuran, but in general, slight forage yield increases resulted. Lin et al. (41) reported that application of carbofuran at 500 ppm resulted in weights of alfalfa seedlings well below that of the non-inoculated controls. A

TABLE II

INFLUENCE OF CARBOFURAN APPLICATION ON FOLIAR POPULATION LEVELS OF *DITYLENCHUS DIPSACI*

	Time of Harvest - Days After Planting[1]	
Treatments	365 Days	430 Days
1. Control	1171.0	2.20
2. 0.28 Kg/ha	351.7	0.33
3. 0.56 Kg/ha	211.5	17.33
4. 0.84 Kg/ha	511.5	10.83
5. 1.12 Kg/ha	104.2	0.00
LSD = 0.10	887.4	19.60
0.05	1073.1	23.70
0.01	1463.5	32.30
CV =	198.6	320.50

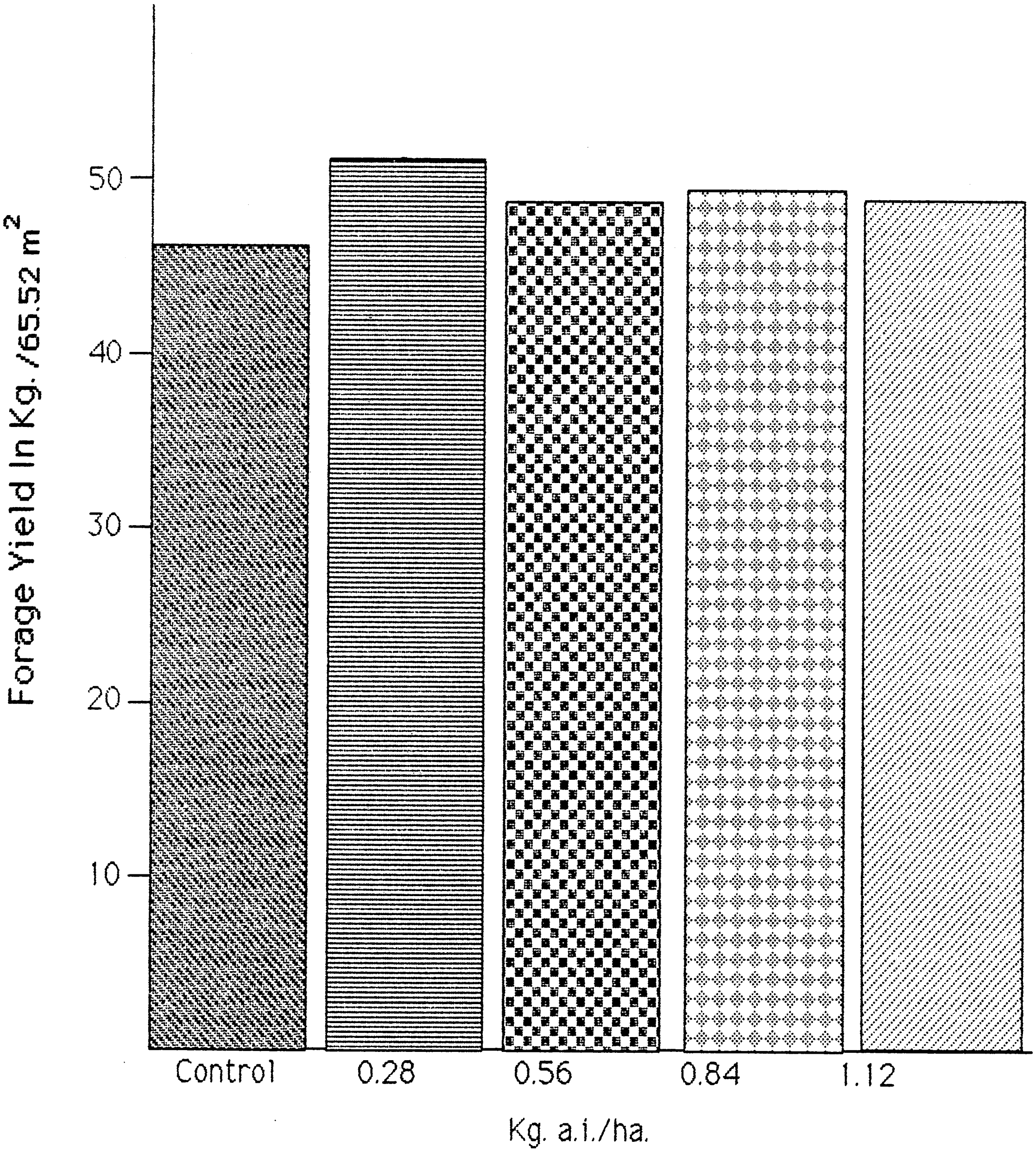
1) - Data are means of *D. dipsaci* recovered from 50g subsamples collected at harvest.

2) - Forage was harvested 4 times but no nematodes were recovered at harvest 1 and 2, 110 and 135 days respectively, after planting.

Figure 9. Effect of carbofuran on total alfalfa forage yield.

1) Field was planted april 23, 1986 and forage was harvested July 10, August 4, 1986 and May 15, 1987.

LSD (0.10) = 4.2
(0.05) = 5.0


Forage Yield In Kg. /65.52 m^2
50
40
30
20
10
Control
0.28
0.56
0.84
1.12
Kg. a.i./ha.

consistent inverse relationship is not observed either between nematode populations and forage yield except for the control which has the lowest forage yield and the highest _Ditylenchus_ populations. Treatment 5 with the lowest nematode populations had the second lowest forage yield behind the control.

A normal continuous decline in forage production was also observed probably due to hotter and dryer environmental conditions (FIGURE 10). The less severe decline in forage yield observed under treatment 2 might be the result of relatively low number of nematodes and moderate phytotoxicity at the onset of the study. The more severe decline in forage production observed under the control, explained its overall lowest yield.

The overall results of this study indicated an initial phytotoxicity of carbofuran which was followed by a beneficial effect on surviving plants. Nematicidal activity, in combination with other factors is suggested by the nematode and forage data. The insecticidal rates of carbofuran, especially the 1.12 Kg a. i. /ha, may be an economical control measure against _D. dipsaci_ on alfalfa. More studies are needed to clearly assess if its phytotoxic effect is outweighed by nematicidal and positive physiological activities.

Figure 10. Comparison of the effect of carbofuran application in forage yields at three harvest dates.

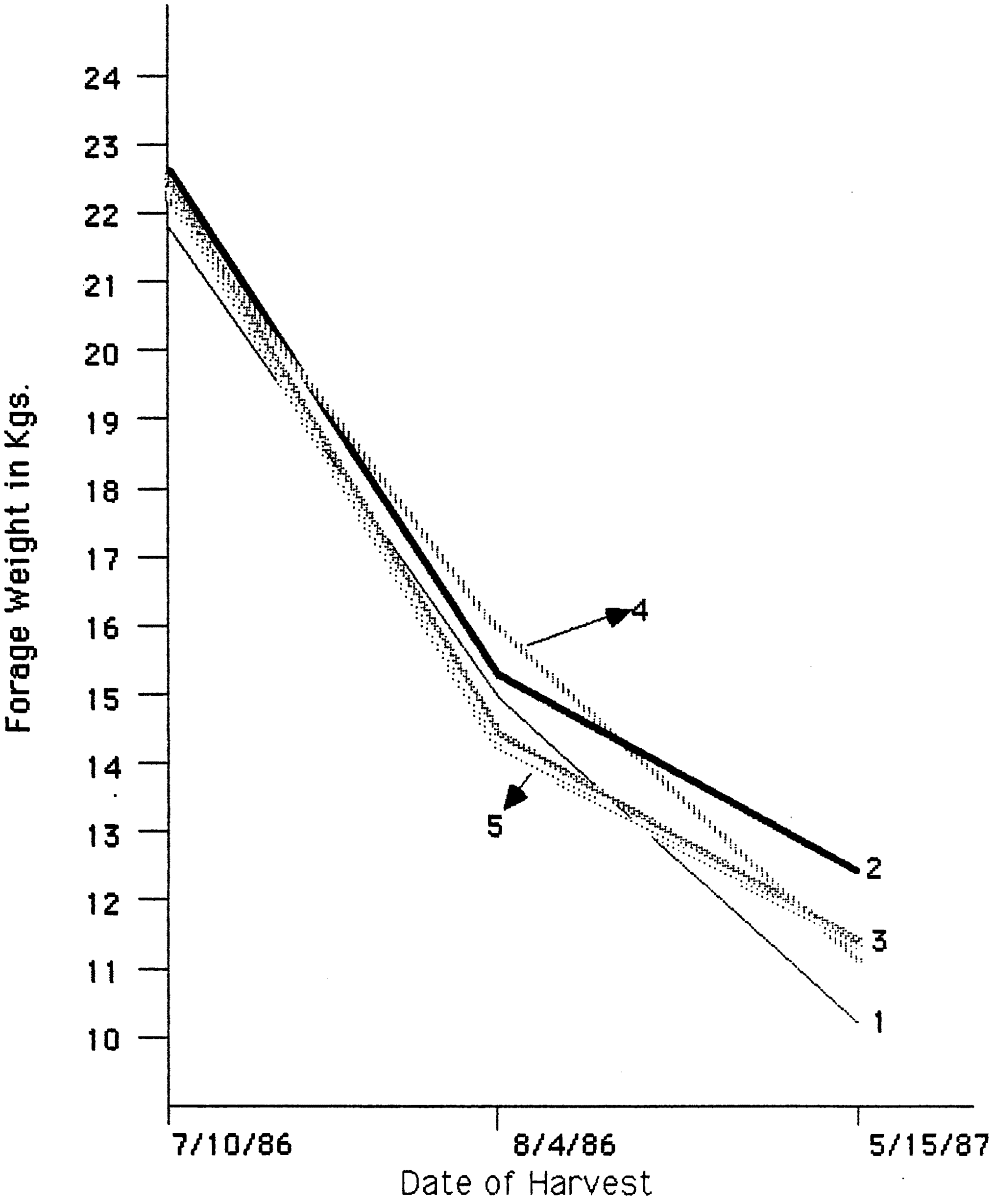
1. Control
2. 0.28 Kg a.i./ha
3. 0.56 Kg a.i./ha
4. 0.84 Kg a.i./ha
5. 1.12 Kg a.i./ha

a.i. = active ingredient

1) Forage for each treatment was harvested over 65.52 m^2.

LSD (1st Harvest) (0.10) = 1.8
(0.05) = 2.1

LSD (3rd Harvest) (0.10) = 1.8
(0.05) = 2.5


Forage Weight in Kgs.
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
7/10/86
8/4/86
5/15/87
Date of Harvest
4
5
2
3
1

Efficacy of the Extraction Procedures

Top Mist System (TMS)

Significantly ($P=0.01$) lower nematode populations were obtained under TMS in comparison to TFS, BFS and ISS (FIGURE 11). The rate of nematode emergence was also lower, since 80% recovery was achieved after 16 hours while the same rate was achieved after 12 hours for TFS, BFS, and CASS-1 (FIGURE 12). The very low efficacy of TMS is a result of an inadequate wetting process. The mist of water created uneven flow of water through the forage, leaving pockets of plant material from which emerged nematodes were not washed down adequately. This was confirmed by the higher numbers of nematodes recovered by soaking the forage for about 10 minutes after the test was terminated. The mist might also be too weak to provide any efficient downward washing effect of extracted Ditylenchus. Two (2) percent of the nematodes were collected from the screen and 21% were passed over into the RB.

Top Flooding System (TFS)

The most efficient method of nematode extraction in terms of total numbers extracted was TFS (FIGURE 11). Over 80 percent of the nematodes recovered emerged after 12 hours of extraction which indicates a high rate of emergence (FIGURE 12).

Figure 11. Means of Total _Ditylenchus_ extracted from dry alfalfa forage by six extraction techniques.

TFS: Top Flooding System
TMS: Top Mist System
BFS: Bottom Flooding System
CASS-1: Continously Aerated Soaking System 1
CASS-2: Continously Aerated Soaking System 2
ISS: Intermittent Soaking System

1) Data are the means of the total number of _D. dipsaci_ recovered from 5 replications.
2) 125 g of forage were processed for 24 hours and the experiment was replicated 5 times

LSD (0.10) = 3378.7
(0.05) = 4085.8
(0.01) = 5572.4

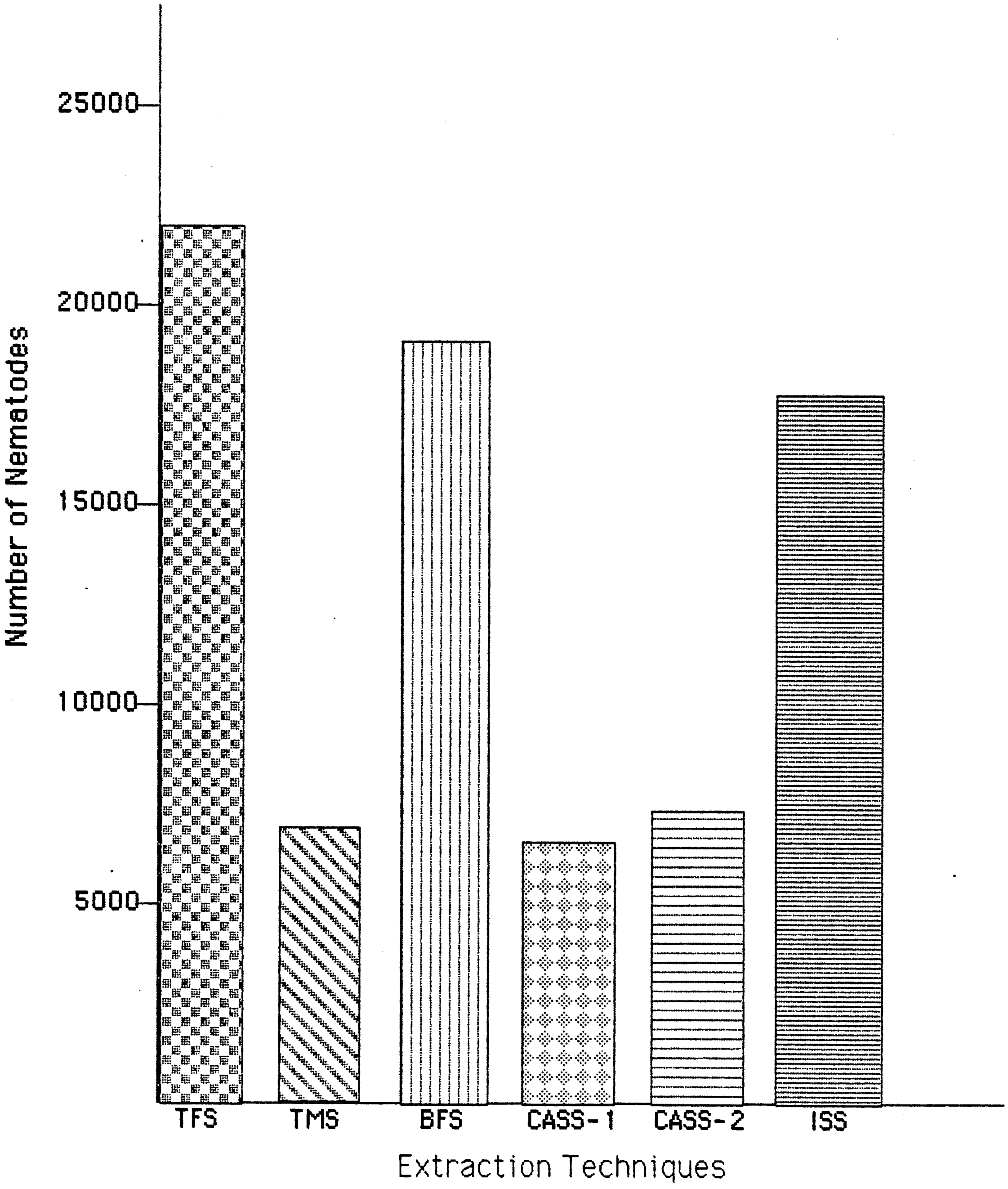
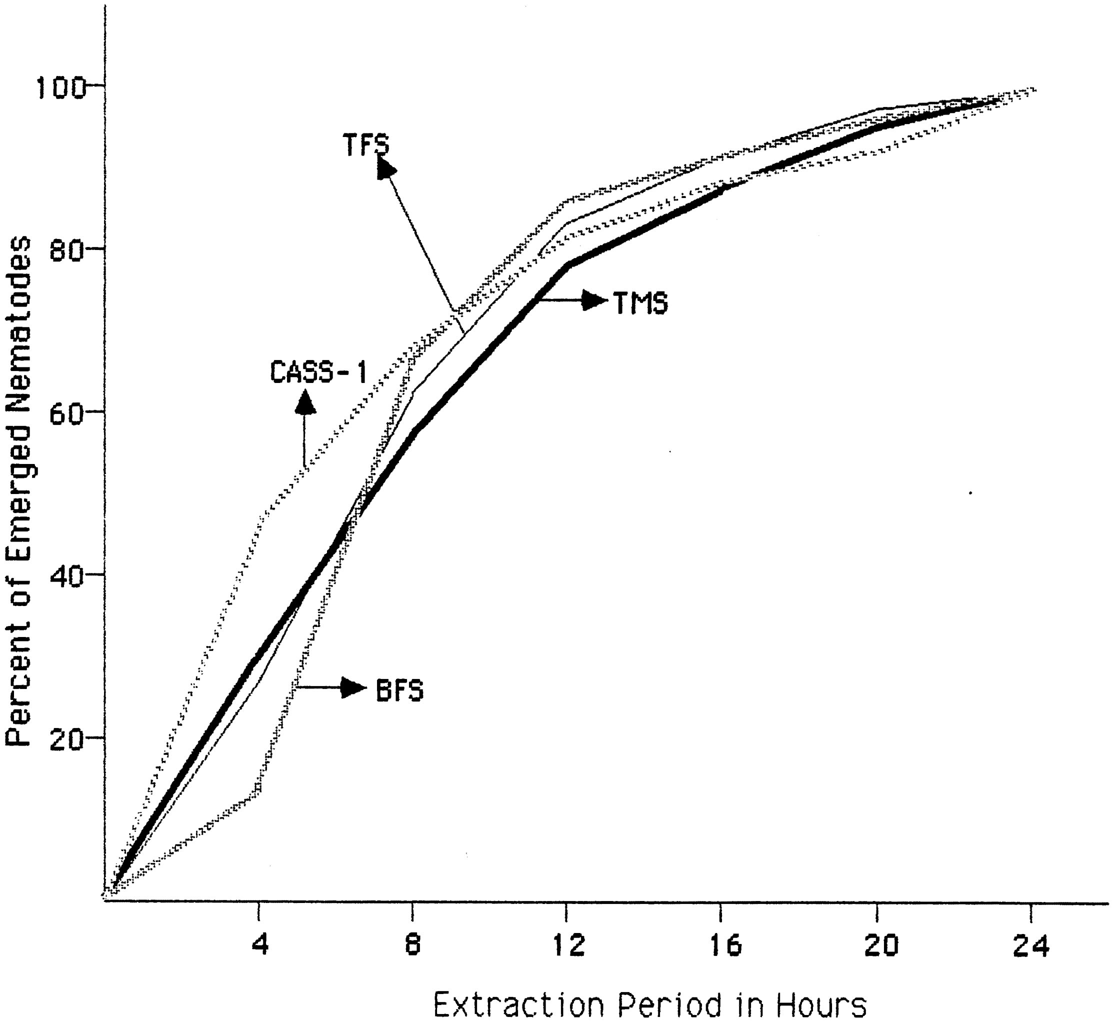

25000
20000
15000
10000
5000
Number of Nematodes
TFS
TMS
BFS
CASS-1
CASS-2
ISS
Extraction Techniques

Figure 12. Rate of nematode recovery per 4 hour period from Ditylenchus infected alfalfa forage.

TFS: Top Flooding System
TMS: Top Mist System
BFS: Bottom Flooding System
CASS-1: Continously Aerated Soaking System 1

1) Emerged nematodes were recovered every 4 hours over a 24 hour period
2) Each sample represented 125 g dry alfalfa forage


TFS
TMS
CASS-1
BFS
100
80
60
40
20
4
8
12
16
20
24
Percent of Emerged Nematodes
Extraction Period in Hours

Oostenbrink reported extracting over 80% of *D. dipsaci* after 21 hours from plant material using a funnel spray method (43). The better efficiency of TFS procedures is essentially due to the additional oxygenation of the water stream pouring onto the forage and its washing effect on emerged nematodes.

Only 1.4% of the total nematodes extracted was retained on the screen of the trap bucket. No significant differences were observed between screens of the different systems.

The trap bucket (TB) collected 87% of the nematodes recovered while 11% of the nematodes were retained in the reservoir of the recycling water.

Bottom Flooding System (BFS)

The bottom flooding system (BFS) was the second most efficient extraction technique on the basis of quantitative extraction and the most efficient in terms of nematode emergence from the forage (FIGURE 11, 12). Eighty-five percent nematode recovery was observed after 12 hours. The lower total of nematodes recovered compared to TFS is probably due to the additional aeration of the latter system during the wetting process of the forage. A very low percentage (1.6) of extracted nematodes were retained on the screen. In contrast to TFS, 33.2% of the *Ditylenchus* were in the water of the reservoir bucket (RB).

Intermittent Soaking System (ISS)

The important role played by aeration and a complete uniform soaking and drainage is better illustrated by ISS which produced significantly (P=0.01) higher nematodes than the other manual techniques (FIGURE 13). The only difference between the 3 manual extraction methods is the aeration period the forage is subjected to. In the ISS the substrata was aerated for 3 and 1/2 hours between soakings and the result is clearly indicated by the much higher nematode populations collected. Although lower numbers of Ditylenchus are obtained in ISS than the most efficient TFS procedure, no significant difference was observed (FIGURE 11).

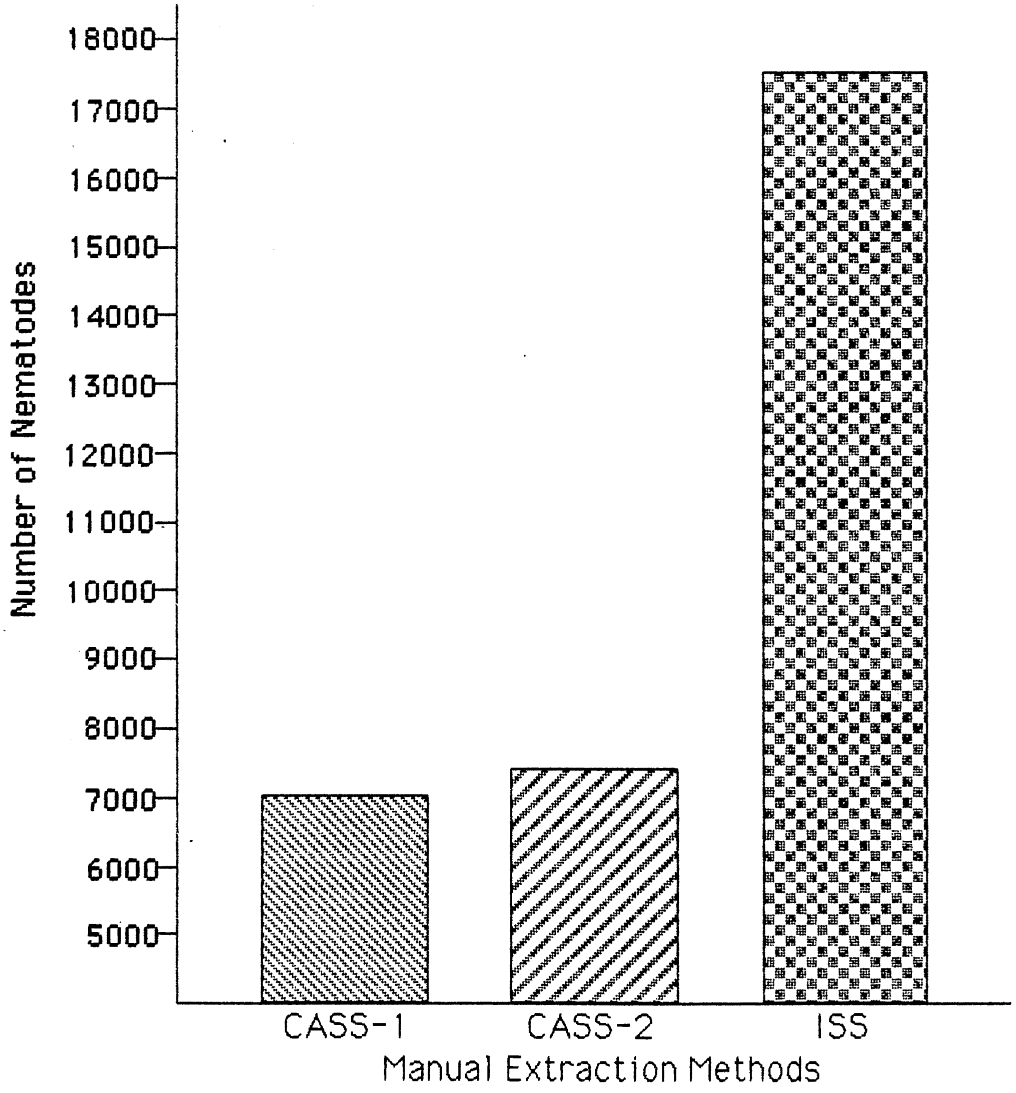
Continuously Aerated Soaking System 1 (CASS-1)

The least efficient extraction procedure was CASS-1 with significantly (P=0.01) lower number of nematodes recovered compared to TFS, BFS, and ISS (FIGURE 11). A comparison between CASS-1 and the 2 other manual extraction indicates a significant (P=0.01) reduction in nematodes extracted between CASS-1 and ISS (FIGURE 13). This is another demonstration of the important role of aeration in nematode extraction. Chapman (14) reported that his aeration techniques increased the total number of nematodes extracted, but not the pattern of emergence. The aeration provided to CASS-1 was apparently not enough to prevent the

Figure 13. Total numbers of recovered nematodes from alfalfa forage by use of 3 manual extraction methods.

CASS-1: Continously Aerated Soaking System 1
CASS-2: Continously Aerated Soaking System 2
ISS: Intermittent Soaking System

LSD (0.01) = 7416.1


18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
Number of Nematodes
CASS-1
CASS-2
ISS
Manual Extraction Methods

development of anaerobic conditions. Those anaerobic conditions could have been compounded by a buildup of leachates from the plant tissue. The leachates may have become toxic to the stem nematodes, worsening the anaerobic conditions. Water in that system was changed every 4 hours, but this did not improve the recovery of nematodes. Despite the low number of quantitative nematode recovery, a high rate of emergence was observed under CASS-1. Over 40 percent of the nematodes emerged 4 hours after the beginning of the extraction process (FIGURE 12). The first samples were very dirty, but samples became cleaner in time as the amount of leachates decreased. This decrease in leachates might explain the slight increase in nematodes recovered under CASS-1 at the end of the 24 hour extraction period (FIGURE 12).

Continuously Aerated Soaking System 2 (CASS-2)

The adverse conditions detrimental to nematode extraction described under CASS-1 also occurred in CASS-2 as indicated by the low total recovery (FIGURE 11). As shown on that figure, there were significantly ($P=0.01$) lower numbers of nematodes between CASS-2 and TFS, BFS and ISS. The single sample recovered at the end of the 24 hour period was very dirty, probably due to leachate accumulation.

In general, TFS and BFS were the two most efficient procedures in terms of quantitative extraction. The rate of nematode emergence was comparable after 12 hours. Relatively clean samples were recovered from the trap and reservoir buckets. The screens of the TB's can be backwashed between sample to reduce the dirt and plant material retained during syphoning process. The forage could also be quickly washed before the extraction process in order to reduce trashy materials.

The automated intermittent systems, especially TFS and BFS can be efficiently used for field extraction and inoculation. Constant attendance during extraction is not required and the recycling process allows the use of very little water. Bulk samples of plant materials can be efficiently processed for a long period of time, and an addition of antibiotics in the trap buckets could prevent or slow down the development of contaminating organisms. Like the other two automated intermittent systems, TFS could be improved by combining the trap bucket and the reservoir bucket into one unit.

The 3 manual extraction methods can be used to process bulk samples, but unattended and/or field extraction would be more difficult.

Results of Nematode Exposure To Fungicide

Live Nematode Data

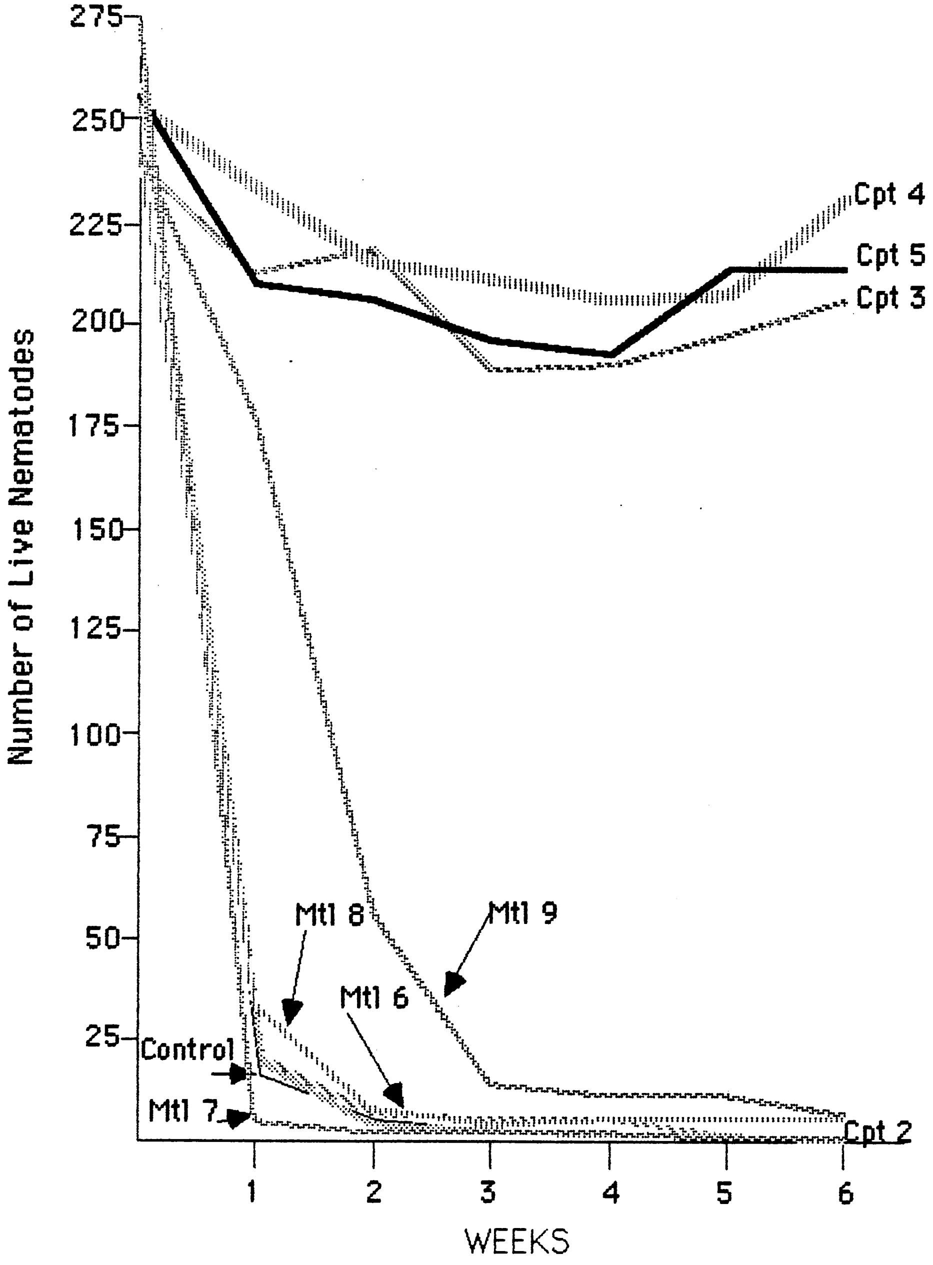
Weekly averages of live nematodes recovered over a 6 week period is presented in FIGURE 14. An analysis of the

Figure 14. Influence of fungicide concentrations on *Catenaria* infection of *Ditylenchus* dipsaci.

Control 1
Cpt 2 = 10 ppm
Cpt 3 = 200 ppm
Cpt 4 = 500 ppm
Cpt 5 = 1000 ppm
Mtl 6 = 10 ppm
Mtl 7 = 200 ppm
Mtl 8 = 500 ppm
Mtl 9 = 1000 ppm

1) 10 ml samples were collected each week and the number of live nematodes counted
2) Numbers represent averages of 5 replicates

LSD (Week 6) (0.10) = 71.1
(0.05) = 85.5
(0.01) = 115.0


Number of Live Nematodes
275
250
225
200
175
150
125
100
75
50
25
1
2
3
4
5
6
WEEKS
Cpt 4
Cpt 5
Cpt 3
Cpt 2
Mtl 9
Mtl 8
Mtl 6
Control
Mtl 7

initial numbers of live nematodes for all the treatments, showed that no significant difference was found (P=0.01). This indicates that uniformity was achieved in the preparation and repartition of the inoculum. Significantly greater numbers of live *D. dipsaci* (P=0.01) were recovered after 3 weeks in the 3 higher rates of captan compared to all other treatments.

The very big difference between the initial populations and the numbers of nematodes at the end of the first 7 days, indicates that water alone, as well as the fungicide concentrations in Cpt 2, Mtl 7, Mtl 8 are totally inefficient in protecting *D. dipsaci* from *Catenaria* infection in liquid medium. The three higher concentrations of Captan and to a lesser extent the highest concentration of metalaxyl appeared to have sustained greater nematode survival at week 1. By week 2, the treatments were clearly segregated into two groups; the Cpt 3, 4 and 5 group, and a second group constituted by the control, Cpt 2, Mtl 6, 7, 8 and 9. The nematode populations in the 2nd set of treatments continued to drastically decline and less than 10 live nematodes per weekly sample were recovered from week 2 to the end of the study (FIGURE 14).

The three highest rates of captan provided a high level of protection of the nematodes against *C. anguillulae*. Although no significant differences were detected between the 3 higher captan rates, a noticeable decrease in live nematodes was observed under Cpt 3, indicating that the 200 ppm concentration was not high enough to prevent noticable infection of the nematodes (FIGURE 14).

Metalaxyl 9 was intermediate in efficiency, for the first 2 weeks of the experiment, but very little protection was observed after 14 days.

Infected Nematode Data

Data on numbers of nematodes infected could not be accurately collected because of their decomposition and or clumping. This phenomenon was also observed by Sayre and Keely (50). The severity of *Catenaria* infection contradicts the findings of Boosalis and Mankau (8) who observed that a very small percentage of live and active nematodes were attacked by the fungus; the results are similar however, to those obtained by Esser and Ridings (20), Stirling and Platzer (55), and Gray (27). The apparent contradictions may be explained by differences in virulence of *Catenaria* isolates as suggested by Sayre and Keeley (50).

One storage procedure of *Ditylenchus* inoculum that needs to be investigated is to clean freshly extracted nematodes in captan concentrations from a few hours to one or two days, and wash off the fungicide before storing the nematodes in sterilized water.

In summary, metalaxyl, like benomyl, PCNB, captan, amphotericin B, cycloheximide, and griseofulvin used in previous studies (17,45), is not efficient in protecting *D. dipsaci* against *C. anguillulae* infection under the storage conditions studied here. Higher concentrations of Mtl might prove to be effective as suggested by Mtl 9 treatment, but such high concentrations might be detrimental to the inoculum; this needs to be investigated. High rates of captan (300 ppm or more) can significantly reduce nematode infection by C. anguillulae up to 6 weeks as measured in this study.

The rate of nematode infection suggests that the Catenaria isolate used in this study is virulent.

Testing for Infectivity of Inoculum Stored in Fungicide Suspension

Method 1

The results of the infectivity tests obtained under method 1 were unsatisfactory because of the very low numbers of nematodes stained in the seedlings.

Placing the inoculum on top of germinating seeds was found to be the best method of inoculation by Elgin et al. (19). The same study indicated however that 25 nematodes per seedling were necessary to recover 10 nematodes or more in about half of the plants. The one nematode per seedling was designed in our study in order to determine a precise percentage of infective nematodes. The negative results obtained after two replicates can be attributed to lack of nematode infectivity but also primarily to the very low inoculum level and perhaps to other undetermined factors. Beginning with the third replicate a second method for testing the infectivity was used. The second testing method was devised after nematodes failed to infect the alfalfa seedlings; this lack of infectivity was thought to result from the very low level of inoculum used under method 1.

Method 2

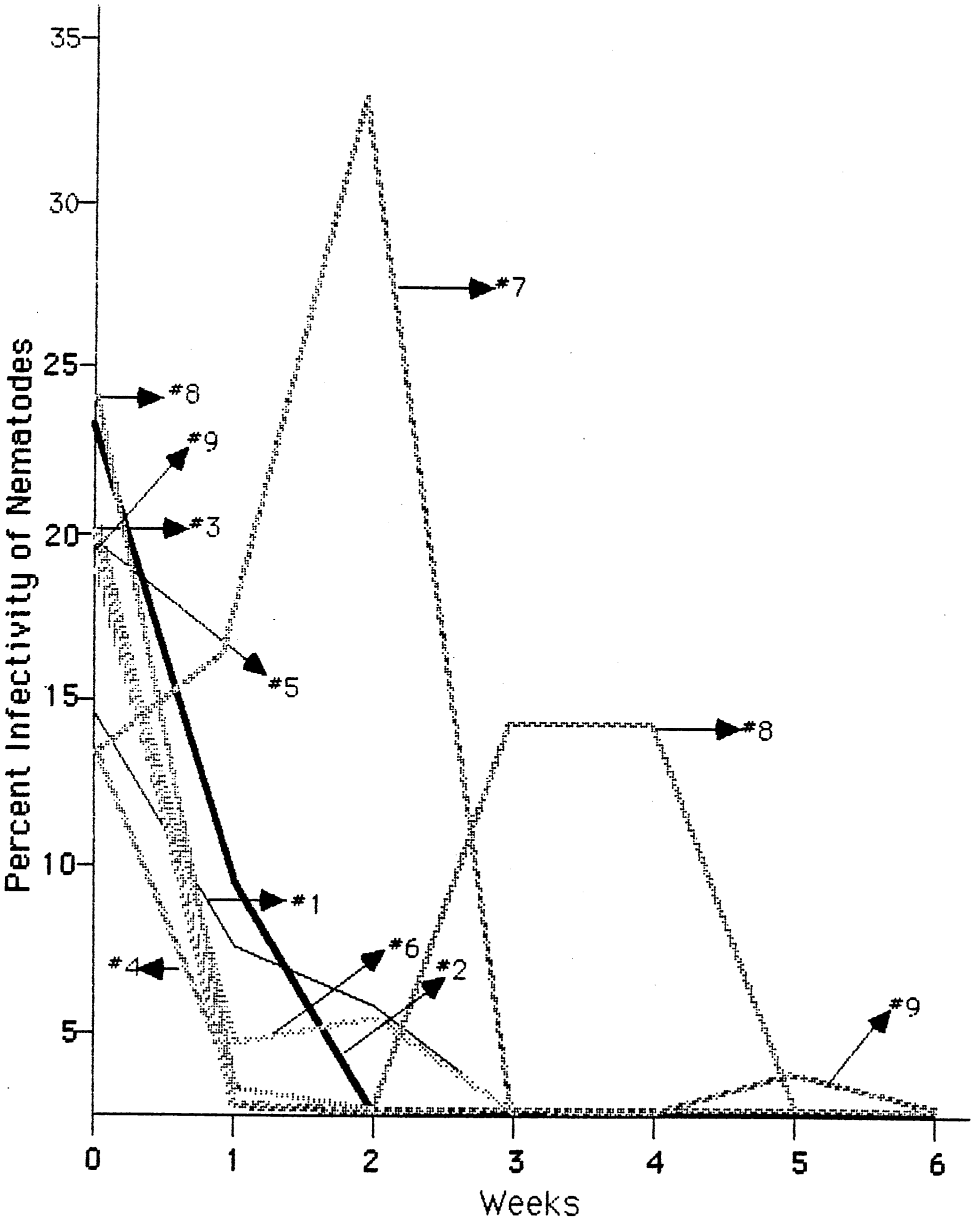
The results obtained under that second testing procedure are summarized in FIGURE 15. An examination of the number of nematodes stained in time indicated a drastic reduction in infectivity only 7 days after storage began in the different milieu. The decrease in nematodes recovered from inside seedlings might be explained by an identical decrease in the number of live nematodes sampled and inoculated for most treatments except for Cpt 3, 4, and 5 which maintain a high number of live nematodes, or at least not visibly infected by Catenaria. The low recovery of nematodes from seedling tissue for those 3 treatments beginning with week 1, cannot be entirely attributed to low inoculum level, inadequate moisture, nature of the soil or some other physical factors. This is clearly illustrated by the results of week 0, when the nematodes used for inoculum were added and immediately withdrawn from the different concentrations. What the data suggest is that most of the nematodes being recovered very quickly lose their ability to infect alfalfa, possibly due to some physiological changes caused by the fungicides.

Two hypotheses are likely:

Figure 15. Percent infectivity of Ditylenchus dipsaci exposed to fungicide suspensions.

1. Control
2. 10 ppm a.i. captan (Cpt)
3. 200 ppm a.i. Cpt
4. 500 ppm a.i. Cpt
5. 1000 ppm a.i. Cpt
6. 10 ppm a.i. metalaxyl (Mtl)
7. 200 ppm a.i. Mtl
8. 500 ppm a.i. Mtl
9. 1000 ppm a.i. Mtl

1) Curves represent percentages of nematodes recovered from inoculated seedlings


Percent Infectivity of Nematodes
35
30
25
20
15
10
5
0
1
2
3
4
5
6
Weeks
#8
#9
#3
#5
#7
#8
#1
#4
#6
#2
#9

- The alfalfa seedlings escaped infection because the nematodes have lost their sense of orientation and therefore cannot find them;
- Or the nematodes can still find the seedlings, but have lost their infectivity.

A third possibility, alone or in combination with the two mentioned above, could be aging, although residual nematodes from the suspensions distributed to the treatments were capable of infecting alfalfa seedlings after a storage peroid of more than 4 weeks in the refrigerator (temperature about 4°C).

Catenaria _anguillulae_ as a Biological Control Agent

In Two Soil Environments

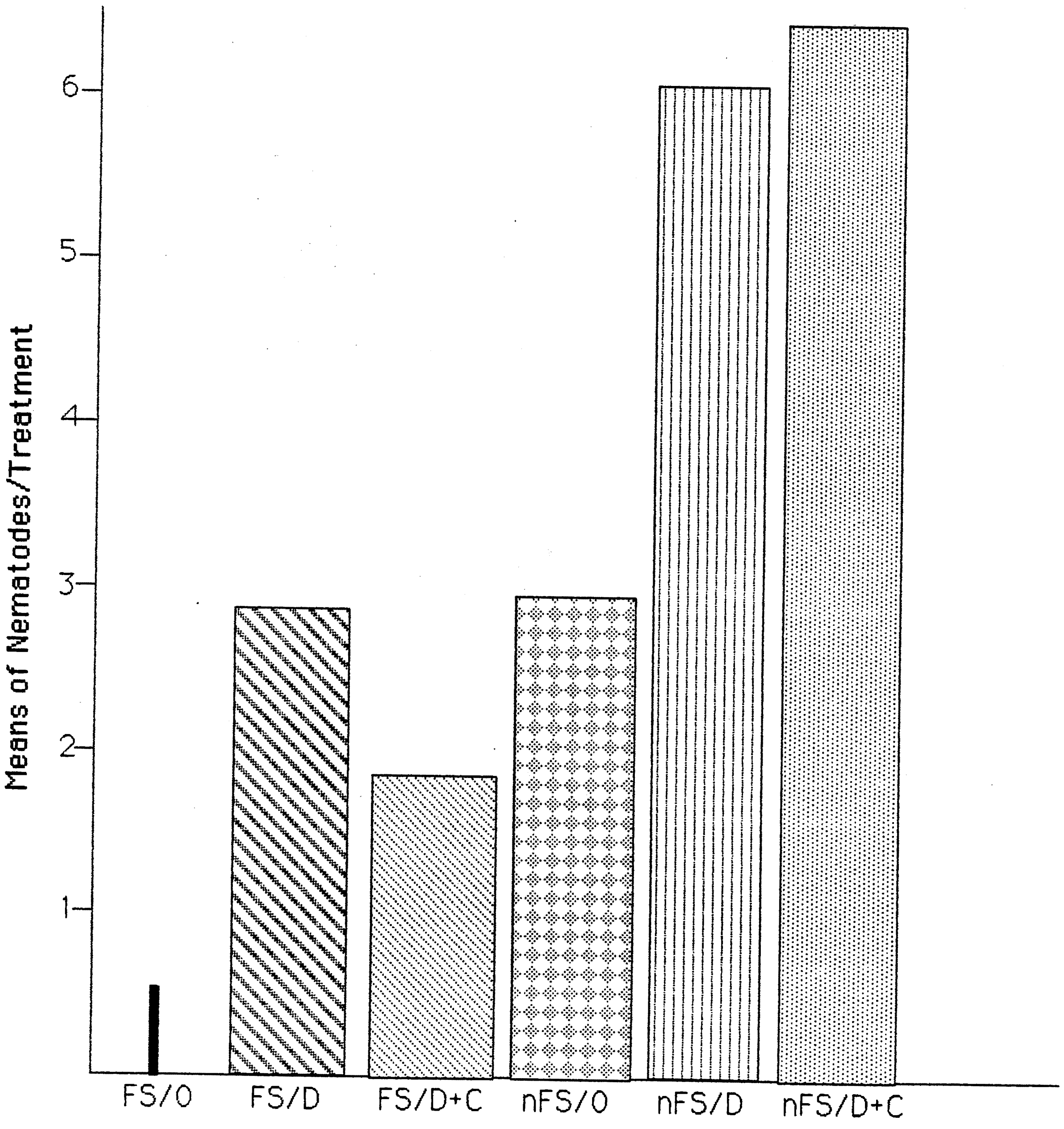
Significantly (P=0.05) higher numbers of _Ditylenchus_ were recovered from seedlings grown on non-fumigated field soil with _Ditylenchus_ (nFS/D) and non-fumigated filed soil with _Catenaria_ and _Ditylenchus_ (nFS/D+C) than on seedlings harvested from fumigated soil without fungus and nematodes (FS/O) and fumigated soil with both organisms added (FS/D+C). This is an indication of the adverse effect natural competitors are exerting on _Catenaria_ in the non-fumigated soil (FIGURE 16). The data indicates that significant difference (P=0.10) existed between nFS/D, nFS/D+C and nFS/O. The nematodes recovered under nFS/O was the result of natural soil background infestation by

Figure 16. Effects of naturally occuring soil antagonists on the control of Ditylenchus dipsaci by Catenaria anguillulae.

FS/0: Fumigated field soil without nematodes or fungus
FS/D: Fumigated field soil to which Ditylenchus was added
FS/D&C: Fumigated field soil with Catenaria and Ditylenchus was added
nFS/D: Non-fumigated field soil with Ditylenchus
nFS/D&C: Non-fumigated field soil with both organisms added

1) Nematodes recovered under NFS/0 represent background infestation by Ditylenchus
2) Numbers are means of recovered nematodes per 3 seedlings

LSD (0.10) = 4.3
(0.05) = 5.2


Means of Nematodes/Treatment
1
2
3
4
5
6
FS/0
FS/D
FS/D+C
nFS/0
nFS/D
nFS/D+C

Ditylenchus. The biological control effect obtained under non-fumigated field soil is illustrated by removing the background infestation from nFS/D and nFS/D+C. Significant differences would no longer exist between any of the treatments, including FS/D from which background infestation has been removed by fumigation. The very low number of nematodes stained in alfalfa seedlings under FS/D+C conditions [0.6 nematodes per plant, i.e. total number of nematodes (9) divided by the number of seedlings (15)] surely demonstrated an effective biological control. The low number of nematodes recovered under FS/D was not the result of any biological control effect since the soil was fumigated and no fungus was added with the nematodes. One explantion might be the low inoculum effect described by Elgin et al. (19). They reported that a minimum of 25 Ditylenchus was required per seedlings for half of the inoculated plants to be infected by 10 nematodes.

Boosalis and Mankau (8) could not detect any effect on soil nematode populations by the addition of Catenaria. The use of sporangia in this study might be in part responsible for the weak biological control effect observed. Sayre and Keely (50) discovered that apparently not enough spores were released from sporangia to reach the level capable of significantly protecting onion seedlings against Ditylenchus attack.

The reduction in onion bloat observed over the control (17% under high zoospore concentrations) was unsatisfactory for onion crop, but such an improvement in alfalfa crop forage might be economical.

Some other adverse effects encountered by zoospores in soil environment are encystment, competition and antibiotic processes, mechanical obstruction to their movement presented by soil particles, nematode movements away from them, sand particles brushing them off the nematode cuticle, and dilution of the chemical gradient needed by the zoospores to locate their hosts (50).

The overall results of this study strongly suggest that C. anguillulae under certain field conditions does exert a significant natural control pressure on soil nematodes.

The biological potential of Catenaria could be greatly enhanced by selecting already virulent isolates for resistance to soil antagonists. This is demonstrated by the isolate used in this study; non uniform virulence was observed under liquid and soil media.

In Foliar Environment

Zoosporangial suspension was first used as inoculum in the evaluation of C. anguillulae as a biological control agent of D. dipsaci in foliar environment. The results of the experiment were not significant enough to allow valid interpretation. The data indicated that more nematodes were recovered under the highest concentration of zoosporangia than under the control.

A second study was conducted using zoospores as inoculum to see whether the inefficiency of the fungus was due to non zoospore release or to some other factors. The results from that study on the use of Catenaria as a biocontrol agent to prevent the infection of alfalfa seedling stage by the stem nematode, D. dipsaci are illustrated in FIGURE 17. Statistical analysis of the data indicates a significant difference at the 10% level between the control and the Rt-3 treatment. This agrees with previous studies relating Catenaria infection of nematode to its zoospores concentration (33,34,50). A good illustration of the high zoospores concentration required for biological control effect is shown by Rt-2. The 10,000 spores/droplet used under that treatment did not result in any significant control of nematodes.

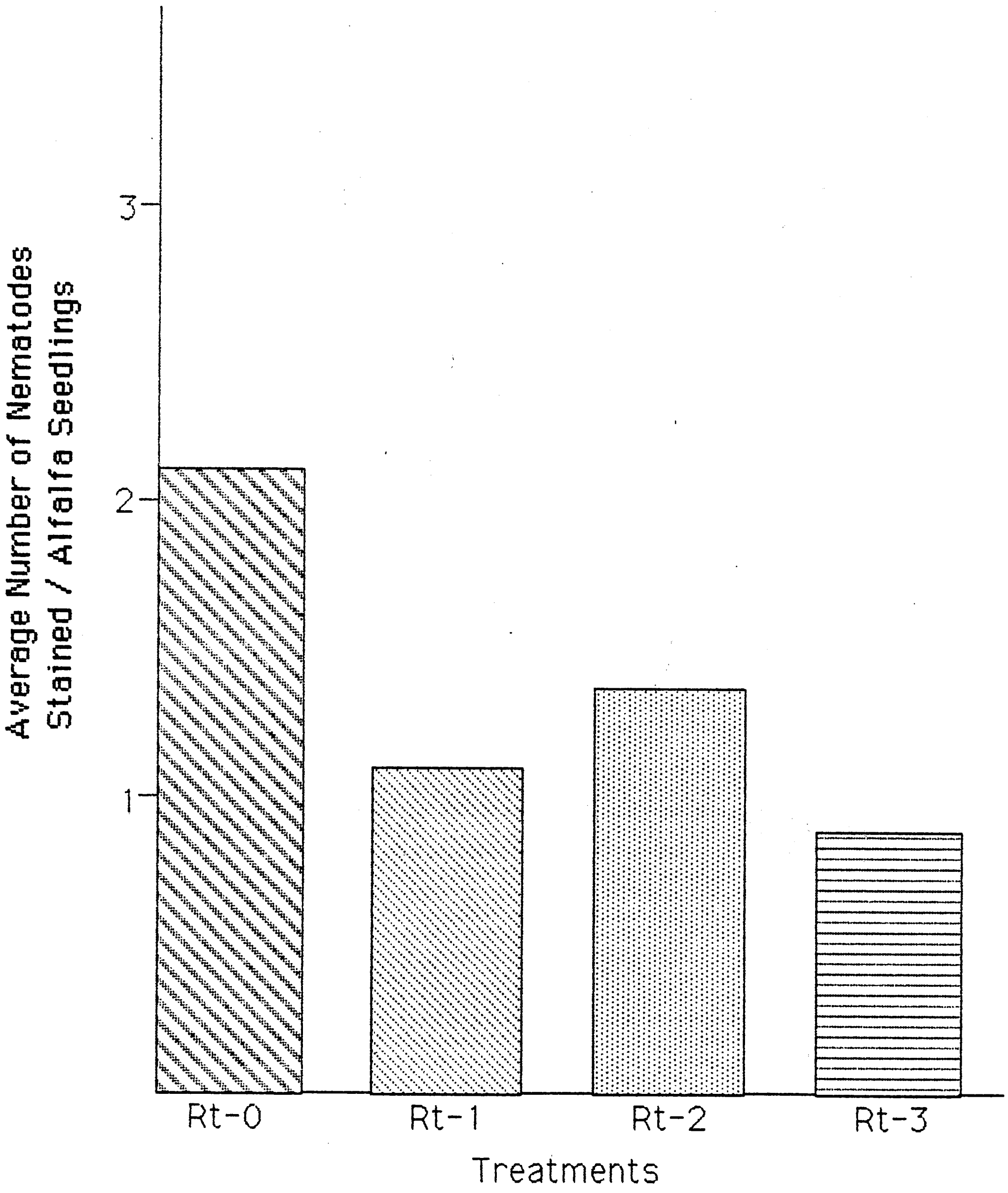
The better result of Rt-3 over Rt-2 agian indicated that high inoculum can produce an efficient biological control measure. Self-inhibitory process was not observed under Rt-3. This phenomenon, which is observed in many closely related species, occurs when high concentrations of zoospores induce the production of inhibitory substances such as lactic acid (11). Based on the result of this experiment it would be premature to draw conclusions regarding the future of foliar applications of Catenaria to control Ditylenchus on alfalfa. It is reasonable however, to assume that under the less hostile foliar environment, Catenaria could be more efficient as a biocontrol agent of

Figure 17. Catenaria anguillulae as a biological control agent of Ditylecnchus dipsaci in alfalfa seedling foliar environment.

RT-0	Control (tap water)
RT-1	2076 spores/droplet of water
RT-2	10380 spores/droplet of water
RT-3	51907 spores/droplet of water

1) Five J-4 were picked into one droplet of spore suspension between cotyledon

LSD (0.10) = 1.0
(0.05) = 1.3


Average Number of Nematodes Stained / Alfalfa Seedlings
3
2
1
Rt-0
Rt-1
Rt-2
Rt-3
Treatments

nematodes. Conditions for zoospore encystment are likely to be different from the ones encountered in soil environments. A moist foliar environment would keep the zoospores in a film of water that could delay their encystment, increasing their chance of infecting outside nematodes.

If the mechanism of spore release from zoosporangia were better understood and could be controlled, the fungus could be applied in its sporangial form which is known to survive desiccation (10,16). The gradual release of spores under moist conditions is requisite to cause enough infection of nematodes coming out of plant tissue to prevent a population explosion. Another problem encountered in biological control research is mass production of inoculum. In the case of Catenaria, enough inoculum can easily be produced for laboratory and greenhouse studies. If the results obtained in future studies are satisfactory, efforts will probably be made to produce enough inoculum for field studies. The fact that infected nematodes were recovered on the first day relatively clean alfalfa forage was processed, suggests that Catenaria may be surviving in some form in the foliar environment. It is possible, however, that early nematode infection may be the result of apparatus contamination.

Although the zoosporangial suspension as inoculum did not provide satisfactory data, any future application of C. anguillulae as a biological control agent in foliar environment will probably be achieved through the application of the zoosporangia which are the surviving structures of the fungus. Higher numbers of Ditylenchus may have allowed for a greater percent of nematode infection, allowing the difference between treatments to be ellucidated.

CHAPTER V

SUMMARY AND CONCLUSIONS

1. The results of the stand counts suggested a positive correlation between phytotoxicity and carbofuran rates.

2. A positive physiological effect of carbofuran seemed to develop, following the initial phytotoxic effect on alfalfa seedlings.

3. Twelve months after planting, a significantly higher number of nematodes were recovered from the control forage indicating the consequences of early nematicidal activity of carbofuran.

4. Six extraction procedures of *Ditylenchus dipsaci* from alfalfa forage were evaluated on the basis of rate of nematode emergence and quantitative extraction efficiency.

5. The top flooding system, the technique which was most aerated, was most efficient on both parameters under consideration.

6. Despite aeration, anaerobic conditions appeared to quickly develop when the forage was continuously immersed.

7. The evaluation of 2 fungicide concentrations revealed that high rates of captan suppressed nematode infection by *Catenaria anguillulae*, while metalaxyl

concentrations were ineffectual in preventing infection of *Ditylenchus* by the fungus.

8. The alfalfa seedlings infectivity test data suggested that the nematodes lost their infectivity after 2 weeks in the fungicide suspensions.

9. *Catenaria anguillulae* was found to be a weak biological control agent of *D. dipsaci* in soil environment.

10. High zoospore concentrations significantly reduced seedling infection by the stem nematode in foliar environment.

11. As a general conclusion, it can be said that the suggestion of a positive biological control effect makes it worthwhile to investigate the use of *Catenaria* in foliar environments to control *D. dipsaci* on alfalfa.

LITERATURE CITED

1. Alby, J. 1975. "The incidence and host parasite relationships of selected nematodes and forage grasses". M.S. Thesis, Okla. State Univ. Stillwater. 42 p.

2. Annon. 1982. "Alfalfa production and pest management in Oklahoma". Circular E. 826.

3. Barron, G. L. 1977. The Nematode-Destroying Fungi. Topics in Mycology No. 1. Can. Biol. Publ. Ltd. Guelph, Ontario, Canada.

4. Baerman, G. 1917. Eine einfache methode zur auffindung von Anchylostomum -(New ato den)- Larven in Erdproben. Geneesk. Tijdschr. Ned.-Ind. 57:131-137.

5. Belanger, G., J. E. Winch, and J. L. Townsend. 1985. "Carbofuran effects on establishment of legumes in relation to plant growth, nitrogen fixation and soil nematodes". Can. J. Plant Sci. 65:423-433.

6. Birchfield, W. 1960. "A new species of Catenaria parasite on nematodes of sugarcane". Mycopath. et Mycol. Appl. 13:184-185.

7. Boelter, R.H., F.A. Gray, and R.H. Delaney. 1985. "Effect of Ditylenchus dipsaci on alfalfa mortality, winterkill, and yield". J. Nematology 17:140-144.

8. Boolsalis, M.G. and R. Mankau. 1965. "Parasitism and predation of soil microorganisms". p. 374-391. In: K.F. Baker and W.C. Snyder (ed.) Ecology of soil-borne plant pathogens. Univ. of Calif. Press.

9. Brown, E.B. and J.B. Goodey. 1965. "Observations on race of stem eelworm attacking lucerne". Plant Path. 5:28-29.

10. Butler, J.B. and A. Humphries. 1932. "On the cultivation in artificial media of Catenaria anguillulae, a chytrideacean parasite on the ova of the liver fluke, Fasciola hopudicola". Sci. Proc. R. Dublin Soc. (N.S.) 25:301-324.

11. Cantino, E.C. 1955. "Physiology and phylogeny in the water molds - a reevaluation". Quarterly Rev. Bio. 30:138-149.

12. Caveness, F.E. and H.J. Jensen. 1955. Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue". Proc. Helm. Soc. Wash. 22:87-89.

13. Chang, D.C.N., W.F. Campbell, and G.D. Griffin. 1973. "Ultrastructure changes induced by stem nematodes in hypocotyl tissue of alfalfa". J. Nematology 5:165-562.

14. Chapman, R.A. 1957. "The effects of aeration and temperature on the emergence of species of Pratylenchus from roots". Plant Dis. Rep. 41:836-841.

15. Cobb, N.A. 1918. "Estimating the nema population of soil". USDA Agric. Tech. Cir. 1.

16. Couch, J.N. 1945. "Observations on the Genus Catenaria". Mycologica 37:163-193.

17. Dhillon, S.M. and E.G. Platzer. 1981. "Laboratory evaluation of fungicides for the control of Catenaria anguillulae, a fungus parasite of Romanomermis culicivorax (Nematoda: Mermithidae)". Proc. Calif. Mosq. and Vector Control Assoc. 50:92-93.

18. Dropkin, V.H., W.L.Smith, and R.F. Myers. 1960. "Recovery of nematodes form infected roots by maceration". Nematologica 5:285-288.

19. Elgin, J.H., D.W. Evans, and L.R. Faulkner. 1975. "Factors affecting the infection of alfalfa seedlings by Ditylenchus dipsaci". J. Nematology 7:380-383.

20. Esser, R.P. and W.H. Ridings. 1974. "Pathogenicity of selected nematodes by Catenaria anguillulae". Soil Crop Sci. 33:60-64.

21. Fielding, M.J. 1965. "Observations on the length of dormancy in certain plant infesting nematodes". Proc. Helminthol. Soc. Wash. 18:110-112.

22. George, D.C. 1936. "Stem nematode causing damage to alfalfa in Arizona". Plant Dis. Rep. 20:144-145.

23. Gibbons, L.N. and G.S. Grandison. 1966. "An improved centrifugal-flotation technique for the isolation of Ditylenchus dipsaci". Nematologica 12:642-643.

24. Goodey, T. 1938. "Observations on the destruction of the stem eelworm *Anguillulina dipsaci*, by the fungus *Arthrobotrys olioospora*". Pres. J. Helminthol. 16:159-164.

25. Graham, J.H., F.I. Frosheiser, D.L. Stuteville, and D.C. Erwin. 1979. *A compendium of alfalfa diseases*. St. Paul, Minn.: American Phytopathological Society.

26. Graham, J.H., K.H. Kreitlow, and L.R. Faulkner. 1972. "Diseases in C.J. Hanson, alfalfa, Science and technology." Agronomy 15:355-370. Am. Soc. Agron., Madison, Wisconsin.

27. Gray N.F. 1984. "The effect of fungal parasitism and predation on the population dynamics of nematodes in the activated sludge process". Ann. Applied Biology 104:143-149.

28. Greenwood, P.B., G.W. Yeates, and G.W. Sheath. 1984. "Effect of nematicide on stem nematodes (*Ditylenchus dipsaci*) populations and productivity and survival of 3 lucerne cultivars in North Otago". New Zealand Journal of Agricultural Research. 27:557-562.

29. Ho, H.H. and C.J. Hickman. 1967. "Asexual reproduction and behavior of zoospores of *Paytophthora megasperma* var sojae". Can. J. Bot. 45:1963-1981.

30. Hogg, D.B. and J.L. Wedberg. 1983. "Studies in the use of carbofuran to enhance alfalfa stand establishment". Proc. Forage and Grassland Conf. Eau Claire, WI. 97-98.

31. Hooper, D.J. 1970. "Handling, fixing, staining and mounting nematodes". In: *Laboratory Methods for Work with Plant and Soil Nematodes*. Minist. Agric., Fish and Food. London, England. 44.

32. Hussey, R.S. 1971. "A technique for obtaining quantities of living *Meloidogyne* females". J. Nematology 3:99-100.

33. Jaffee, B.A. 1986. "Parasitism of *Xiphinema rivesi* and *X. americanum* by zoosporic fungi. Journal of Nematology. 18:87-93.

34. Jaffee, B.A. 1986. "Infection of *Xiphinema* spp. by zoosporic fungi during Baerman funnel extraction". J. Nematology 18:614.

35. Jatala, P. 1986. "Biological control of plant-parasitic nematodes". Ann. Rev. Phytopathol. 24:453-489.

36. Jenkins, W.R. and D.P. Taylor. 1967. *Plant Nematology*. Reinhold Publ. Co., New York, 270 pp.

37. Karling, J.S. 1938. "A further study of *Catenaria*". Am. J. Bot. 25:328-335.

38. Krusberg, L.R. 1963. "Effect of galling by *Ditylenchus dipsaci* on pectins in alfalfa". Nematologica 9:341-3??.

39. Krusberg, L.R. 1967. "Pectinase in *Ditylenchus dipsaci*". Nematologica 13:443-451.

40. Kuhn, J. 1857. Uber das vorkommen van *Anguillulae* an erkrankten Bluhtenkophen von *Dipsacus jullonum* L. Zeitschr. Wiss. Zool. 9:129-137.

41. Lin, S.C., B.R. Funke, and J.T. Schulz. 1972. "Effects of some organophosphate and carbamate insecticides on nitrification and legume growth". Plant Soil. 37:489-496.

42. McBurney, T. 1981. "Field assessment of stem nematode (*Ditylenchus dipsaci*) from visual symptoms in peas". Nematologica 27:115-117.

43. Oostenbrink, M. 1960. "Estimating nematode populations by some selected methods". In: *Nematology* (Ed. by J.N. Sasser and W.R. Jenkins). Univ. of North Carolina Press, Chapel Hill, N.C. 85-102.

44. Orton, W.A. and A. Ames. 1908. "Plant diseases in 1907". In: U. S. Dept. of Agric. Yearbook 1907:582.

45. Platzer, E.G. and B.J. Brown. 1976. "Physiological ecology of *Reesimermis mielseni*". In: "Proceedings of the 1st International Colloquium on Invertebrate Pathology and IXth Annual Meeting of the Society for Invertebrate Pathology". Queen's University at Kingston, Canada. pp 263-267.

46. Reed, B.M. 1977. "A structural of host-parasite relationships of *Ditylenchus dipsaci* (Kuhn) Filipiev and *Medicago sativa* L.". (Unpub. Ph.D. dissertation, Okla. State Univ. 71pp.).

47. Russell, C. C. 1971. "*Ditylenchus dipsaci* (Kuhn) Filipjev alfalfa nematicide trials". Proc. Okla. Ag. Chem. Conf. (1971), pp. 74-75.

48. Russell, C.C. 1982. "The stem nematode in the Southeastern United States". In: Riggs (ed) *Nematology in the Southeastern Region of the United States*. 157-173. Southern Coop. Bull. 276.

49. Russell, C.C. 1986. "An accurate, inexpensive, low pressure, ULV in-furrow nematicide applicator". J. Nematology 18:641.

50. Sayre, R. M. and L. S. Keeley. 1969. "Factors influencing Catenaria anguillulae infection in a free-living and a plant-parasitic nematode". Nematologica 15:492-505.

51. Sayre, R.M. 1986. "Pathogens for biological control of nematodes". Crop Protection 5:268-276.

52. Seinhorst, J.W. 1950. "De betekenis van de toestand van grond voor het optreden van aantasting door het stengelaaltje (Ditylenchus dipsaci (Kuhn) Filipjev)". Tijdschr. Plziekt 56:291-349.

53. Sheaffer, C. C., D. L. Rabas, F. I. Frosheiser, and D. L. Nelson. 1982. "Nematicides and fungicides improve legume establishment". Agron. J. 74:536-538.

54. Sorokine, N. 1876. "Note sur les begetaux parasites des anguillulae". Ann. Sci. Nat. 4:62-71.

55. Stirling, A.M. and E.G. Platzer. 1978. "Catenaria anguillulae in the meynithid nematode Romanomermis culieivorux". J. Invert. Path. 32:348-354.

56. Taylor, A.L. and W.Q. Loegering. 1953. "Nematodes associated with root lesions in Abaca". Turrialba 3, NS (1-2):8-13.

57. Thorne, G. 1961. Principles of Nematology, McGraw-Hill, New York, 553 p.

58. Villot, A. 1874. "Monographie des Dragonneaux". Arch. Zool. exper. gen. 3:184-185.

59. Yuksel, H.S. 1960. "Observations of the life cycle of Ditylenchus dipsaci on onion seedlings". Nematologica 5:289-296.

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