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The pathogenicity of the nematodes, Pratylenchus penetrans and Tylenchorhynchus claytoni, on turfgrasses.

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The Pathogenicity of the Nematodes, *Pratylenchus penetrans* and *Tylenchorhynchus claytoni*, on Turfgrasses

by

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I. Introduction

There are more than one hundred and fifty golf courses in Massachusetts, with approximately a dozen new ones being constructed annually. It has been estimated that the annual cost of maintenance alone for Massachusetts courses is from ten to fifteen million dollars. Of this expenditure a large portion is spent for the control of various disease inciting organisms which attack turf.

Included in this group of organisms are different species of plant parasitic nematodes although the importance of nematodes on turfgrass has not been thoroughly investigated, and their effects on grass have been based mainly on field observations. Few pathogenicity studies have been conducted and only a limited number of nematode species have actually been proved pathogenic to turf. With the advent of new culturing techniques for nematodes, it is now possible to maintain aseptic conditions which eliminate effects of other organisms and permit a more detailed investigation of the role of nematodes as plant pests.

Nematocides for controlling nematodes parasitic on established turfgrass have been used successfully. The alleged improvement in turfgrass vigor and density from the use of these nematocides may not necessarily be due exclusively to the reduction of parasitic nematodes.

The present investigation was undertaken to determine 1) the pathogenicity of two parasitic nematodes Pratylenchus penetrans (Cobb, 1917) Filipjev and Schuumans Stekhoven,

1941, an endoparasite and Tylenchorhynchus claytoni Steiner, 1937, an ectoparasite on turfgrass and 2) the effect on grass growth of the reduction of nematodes by nematocides.

II. Review of Literature

A. Nematodes Reported Associated with Roots of Turfgrasses

Literature on plant-parasitic nematodes associated with turfgrass consists primarily of field survey reports. Many investigators merely list those genera found associated with turfgrass roots; others suggest that nematodes definitely injure turf. Very few experiments have been conducted to determine the nature of the relationship of nematodes with turf.

In the Tampa Bay area of Florida, Kelsheimer and Overman (23) reported that Belonolaimus gracilis, Trichodorus sp., Criconemoides spp., Hoplolaimus coronatus, Ditylenchus dipsaci and Radopholus similis were found infesting lawn grass roots. They also observed that both the stubby-root (Trichodorus) and sting (Belonolaimus) nematodes caused a stubby-root condition similar to that incited by these nematodes on other plants. Also in Florida, Christie, Good and Nutter (9) and Good, Christie and Nutter (15) examined many turf samples from various locations within the state. Their findings increased the list of nematodes associated with turf injury to include Dolichodorous, Pratylenchus, Xiphinema, Hemicycliophora and Rotylenchus. They further reported that the sting and lance (Hoplolaimus) nematodes appear to cause the most injury to turf and that the lance nematode usually feeds internally on St. Augustine grass.

Yellow tuft, a disease of bentgrass, was suggested by Tarjan and Ferguson (59) to be caused by Panagrolaimus and

Eucephalobus. The disease was characterized by numerous small shoots arising from stoloniferous nodes. Leaves of these shoots were mostly yellow or necrotic.

Parris (39) attributed the decline of lawn grasses in Mississippi to attacks by plant endo- and ectoparasites. Stylet nematodes (Tylenchorhynchus spp.) were most frequently encountered in the lawn samples he surveyed.

Good et al. (16) surveyed turf nurseries in Georgia and noted that Trichodorous christiei was the most common species found. However, dagger, ring and stylet nematodes were also found throughout the state. Somerville et al. (55) examined 232 turf samples from 20 states. From these they obtained the six most prevelant nematode genera in order of frequency of occurrence: stylet, meadow, spiral, lance, dagger and ring. Their data also showed that turf samples from the northern states consistently contained stylet nematodes. Surveys of turf areas conducted by other workers in the northern states also showed that the stylet nematode was most prevelant.

Troll and Tarjan (64) found both known parasites and suspected parasitic nematodes occurring with roots of grasses growing on golf course greens in Rhode Island. They reported that Tylenchorhynchus claytoni and Rotylenchus erythrinae occurred most frequently in turf samples displaying symptoms of chlorosis and/or die back. Both Pratylenchus penetrans and Paratylenchus sp. were also extracted from turf samples, but in fewer numbers.

In a more recent survey of golf greens in Illinois, Taylor et al. (60) indicated that Tylenchorhynchus was the most abundant parasitic genus present, followed by Helicotylenchus and Pratylenchus.

Many of the same nematode genera associated with turfgrasses have been reported found with roots of both cereal and pasture grasses (13, 14, 20, 33, 37).

Field observations and surveys have revealed that there are a substantial number of known parasitic nematode genera associated with turfgrass roots, but no attempts to determine if the nematodes were pathogenic were reported by these workers. Of all the genera identified in the field surveys Tylenchorhynchus, an ectoparasite, appeared to be most frequently associated with turf.

B. Nematode Pathogenicity Studies on Grasses

Nematode pathogenicity can be determined by approximating Koch's postulates (29,34). The application of these criteria, the constant association of the nematode with the disease, isolation of the organism in pure culture and the production of the disease by inoculation from pure culture into healthy plants, has been made easier with the recent development of new techniques for culturing nematodes aseptically. However, only a few nematode pathogenicity and host-parasite studies have been conducted on turfgrass and there are several host-parasite experiments on grasses other than those grown for turf.

Near Madison, Wisconsin, Perry et al. (42) found large

numbers of parasitic nematodes associated with unthrifty growth and necrotic root systems of Kentucky bluegrass. In greenhouse experiments they found that Kentucky bluegrass plants grown in pots and inoculated with a species frequently found in the field, Helicotylenchus digonicus, weighed less than control plants. Roots of the inoculated plants were discolored brown and small in diameter compared to normal roots. Histological studies showed that parasitized root cells became necrotic, host cells sloughed-off and the parasites fed on the phloem.

Rhoades (46) also demostrated the pathogenicity of nematodes on turfgrass. In greenhouse experiments he inoculated St. Augustine grass with either Belonolaimas longicaudatus or Trichodorous christiei. The root weights of St. Augustine grass infested with B. longicaudatus were greatly reduced compared to the controls. T. christiei reduced both top and root growth. The nematode also caused stubby tips on the rootlets but no discoloration or necrotic lesions.

Coursen and Jenkins (11) proved Paratylenchus projectus to be pathogenic to Kentucky 31 Tall Fescue. Plants grown in pots and inoculated with 5,000 or 10,000 nematodes were shorter than uninoculated checks and both inoculation levels increased tiller production. Although the tops of inoculated plants were shorter than those of checks, their roots weighed more than controls and no visible symptoms of feeding could be found on parasitized roots.

In a study of root galls caused by Ditylenchus radicicola on American beach grass, a non-turfgrass, Chiaravalle (8) showed that the nematode caused hypertrophy and hyperplasia of both cortical and stelar tissues. He also obtained stimulation of the aerial growth of Sudan grass, another non-turfgrass species, by inoculating D. radicicola into the soil. In Holland s'Jacob (52) demonstrated that the same nematode specie caused galls of annual bluegrass roots - a turf species. D. radicicola caused a severe stunting of annual bluegrass but did not injure either perennial ryegrass or timothy. Differences in host range indicate that D. radicicola from Rhode Island and Massachusetts may be a different species from that found in Europe.

Riggs et al. (47) inoculated both pasture and lawn Bermuda grass varieties with root-knot nematodes. The nematodes were more pathogenic on the pasture types because they caused more galls than lawn varieties. In a similar study, Sledge (53) reported that an unidentified root-knot nematode completed its life cycle and caused a slight swelling of the roots at the site of infection on a number of Bermuda grass varieties.

In experiments conducted by McGlohon et al. (30), lance, pin, stunt, spiral, stubby-root and root-knot nematodes caused various reductions in top and root weights of tall fescue, orchard grass, dallisgrass, Bermuda grass, Italian ryegrass and bahiagrass. In every case, nematodes were parasitic, completing their life cycle, as well as patho-

genic, causing injury to the host.

There are a number of host-parasite and pathogenicity studies on nematodes affecting corn, cereal grasses and pasture grasses (12, 18, 31) but in only a few cases has injury to the grasses been shown. Most studies demonstrate only parasitism.

C. Effects of P. penetrans and T. claytoni on Plants Other Than Turfgrass

Both P. penetrans and T. claytoni have consistently been reported to be associated with turfgrass roots, but few studies have been conducted to show that they are pathogenic to turf. Both species have been shown to be highly pathogenic to many plants other than grasses.

Mountain and Patrick (35), working on the peach replant problem, showed that in the absence of bacteria and fungi, P. penetrans caused discoloration and necrosis of dermal and cortical cells, forming distinct lesions on roots. On apple seedlings, Pitcher et al. (43) found that P. penetrans caused a similar necrosis of dermal and endodermal tissues. Mid-cortical cells of apple roots exhibited little visible reaction to the organism, even though nematodes fed and reproduced in this tissue. In peach roots, the cortical cells turned black when infested with nematodes. Histochemical tests indicated that sensitivity to P. penetrans is related to the presence and concentration of phenolic substances in various tissues of both the peach and apple roots. It was found that in peach the glycoside amygdalin

was broken down by means of a B-glucosidase produced by P. penetrans. Apple roots do not contain amygdalin but nematode enzymes act against other phenolic substances, the break-down products of which could also cause discoloration. P. penetrans is a primary parasite of both apple and peach roots because it can invade, feed on and reproduce in them in the absence of other organisms. The nematode is also pathogenic to both apple and peach for it regularly caused discoloration and necrosis of root tissues. It is of particular interest that in neither host did the nematode penetrate beyond the root endodermis.

Under aseptic conditions, P. penetrans was shown to be pathogenic to strawberry (63). The organism did not invade the root endodermis, but caused discoloration and hyperplasia of the tissue. Working with celery as the host, Townsend (62) showed that P. penetrans under aseptic conditions caused necrosis of cortical and epidermal cells but would not penetrate the endodermis.

Under greenhouse conditions the average weight of Zinnia inoculated with P. penetrans was about one-half that of uninoculated plants (17). Balsam infested with P. penetrans weighed, on the average, one-fourth as much as their controls (17).

P. penetrans fed on root hairs and the epidermis of a strawberry and Ladino clover grown aseptically in test tubes (7). The nematode caused a disappearance of root hairs, swelling of root tips, darkening of the roots and stunting

of the plants. Shafiee and Jenkins (49), studying the host-parasite relations of P. penetrans and pepper plants, observed that the nematode caused mechanical destruction of parenchyma cells in the root cortex. They also noted an increase in potassium in the leaves of infested plants.

Tarjan (57) investigated the mineral nutrition of boxwood in relation to infestation by Pratylenchus spp. His experiments were not conducted under aseptic conditions. There was a difference in mineral elements in the stems, leaves and roots of nematode-infested boxwood compared to shrubs free of nematodes.

Experiments on pathogenicity and host-parasite relations of Tylenchorhychus claytoni and Tylenchorchynchus spp. have not been conducted on as wide of variety of plants as with those involving P. penetrans.

Krusberg (24) reported that corn, wheat, sudangrass and Irish potato were favored hosts for increasing population of T. claytoni. His tests were conducted on plants growing in previously sterilized soil to which he added a known number of nematodes. In another more extensive study on feeding habits and host range of T. claytoni, Krusberg (25) found that the nematode could reproduce on a wide range of plants. He noted that on alfalfa roots, the nematode fed only on epidermal cells, not root hairs. Cell staining reactions and lack of punctures indicated no injury from feeding. Krusberg did not find any histological evidence of nematode injury to tobacco roots, but he did demonstrate a considerable reduc-

tion in weight of T. claytoni infested tobacco roots grown in pots.

T. claytoni reduced the root systems of hybrid corn in greenhouse tests (36). An evaluation of resistance was based on a comparison of root weights and nematode populations. Inbreds susceptible to T. claytoni had reduced root systems and increased nematode population, but around resistant inbreds, the nematode number decreased,

In a greenhouse test, Sher (50) observed that T. claytoni stunted Azaleas. He found that the dry weights of the tops of inoculated plants were less than those of the uninfested plants. No distinctive symptoms were noted on the root systems.

Host-parasite relations have been studied with other species of Tylenchorhynchus. For example, Reynolds and Evans (45) investigated the effect of T. dubuis on the growth of cotton. They found that the nematode reproduced, fed on the root epidermis and caused stunting of the host. Larvae were observed inside small secondary roots.

Still another species, T. martini, was determined by Birchfield and Martin (2) to be parasitic and pathogenic on sugar cane. In controlled studies conducted in the greenhouse, the nematode reproduced on and reduced the root systems of this grass.

The above experiments show that P. penetrans and T. claytoni are both parasitic and pathogenic to a number of different plant species. Pathogenicity of P. penetrans, an

endoparasite, is exhibited by both root lesions and cell necrosis. T. claytoni, causes a stunting of either the top or root growth of infested plants but root cell injury is not noted.

D. Chemical Control of Nematodes

Numerous reports appear in the literature concerning effective chemical control of parasitic nematodes infesting agronomic and ornamental crops but many do not relate how control affects the vegetation (22, 32, 58). Where investigators have shown these effects they are usually manifested by the plant as increased yields, larger top and root growth, better plant color, increased vigor, or a combination of these. The majority of nematocides have considerable toxicity to a wide range of organisms so that increased crop response coupled with nematode removal is only an indication that nematodes were responsible for poor growth.

Wilcox et al. (65) reported that corn, cotton, and sorgo growing on soil in which parasitic nematodes were controlled by fumigants, Dowfume MC-2, D-D, Dowfume W-85, Nemagon, Fumazone and allyl alcohol, produced higher yields and developed more vigorous and healthy root systems but oats and clover yields were not affected by fumigation.

Slootweg (54) reported that a nematocidal fumigant controlled parasitic nematodes and appeared to stimulate the growth of narcissi. Apt and Gould (1), working on the control of P. penetrans, also found that fumigation controlled the nematode and increased yields.

Increased yields and quality are not always credited to nematode control. Peachey and Winslow (40) attributed improved yield and quality of carrots grown on land treated with nematocides to the partial sterilization effect of the soil treatment and not to the control of nematodes.

Poor stands and low yields of alfalfa in Rhode island were frequently associated with Pratylenchus pratensis and a high incidence of crown rot (56). Fumigants controlled nematodes and increased yields, but crown rot incidence continued high, indicating that crown rot was independent of the nematocide treatment. Thus, either the suppression of nematodes or unknown factors were related to increased yields.

Hollis et al. (19) determined the relationships between nematodes, fumigation and fertilization in rice culture. They found that soil fumigation and fertilizers exerted independent additive effects on rice yields and both were necessary for maximum yields.

Experiments on chemical control of nematodes on turf-grass do not always report the gross effects on vegetation but often merely relate the chemical effect on the parasite.

Tarjan and Cheo (58) obtained a significant reduction of parasitic nematodes after an application of nematocide drenches to established bentgrass.

The symptoms of nematode damage to turf and chemical control measures were discussed by Bloom and Wuest (3). They noted that turf in experimental plots treated with a

nematocide showed less damage to the foliage due to fungi than did untreated plots. The foliage resistance to fungi was claimed to be due to the elimination of nematodes which allowed the grass to develop a good root system and in turn healthier leaves. No definite data on top or root weights were given, although it is stated that grass grew better because nematodes were killed.

In another study, applications of chemicals to established turf were shown to control parasitic nematodes for as long as four months, but again gross effects to the grass were not given (27).

Powell (44) suggested that nematodes were a major factor in the decline of a lawn in Athens, Georgia. The turf did not respond to an application of fertilizer, but improved after a nematocide was applied. In still another report, Christie and Perry (10) found a marked improvement in plant growth and an increase in the spread of grass by rhizomes after an application of a nematocide to Bermuda grass.

The most striking improvement of turf vegetation from applications of nematocides to nematode-infested Kentucky bluegrass was reported by Perry et al. (42). The growth of bluegrass plants and their root systems was greatly improved through the use of all the nematocides in the test. The color of the grass in treated plots was reported to be deeper green and the leaves longer and broader. The roots of treated plants penetrated deeper into the soil than the checks.

Based on the above literature, it appears that both

P. penetrans and *T. claytoni* can severly injure turfgrass roots. With this in mind, the present investigation was undertaken to find both the exact nature of the damage and the gross effects these organisms might have on turf.

III. Selection of Turfgrasses for Tests

Both Pratylenchus penetrans and Tylenchorhynchus claytoni are found associated with the roots of a number of different grass species (9, 15, 16, 60, 64). Because of this wide host range, a series of preliminary histochemical tests, using the methods of Thomas and Orellana (61) and Pitcher et al. (43), were conducted in an attempt to find turfgrasses supplying a range of visible changes which would be possible indicators of nematode effects.

Applications of pectinol to varieties of castor bean have been used to rapidly measure castor bean resistance to Botrytis. Thomas and Orellana (61) found that resistant bean capsules remained firm and green when sprayed with a pectinol solution and moderately susceptible varieties turned brown. The browning is characteristic of oxidation and polymerization of phenolic compounds.

Pitcher et al. (43) showed that in the presence of P. penetrans both the epidermal and endodermal tissues of apple root quickly discolored but the cortical cells became only slightly discolored. In histochemical tests, sections of apple roots immersed in diazotized sulphanilic acid(DSA) reacted in a similar manner. The endodermis and epidermis rapidly turned a dark brown whereas the cortical cells became a light yellow. These reactions were interpreted to indicate the presence of large concentrations of phenolic substances in the darker colored tissues with lesser amounts

in the cortex. Similar tests conducted on peach roots showed that in the presence of P. penetrans and/or DSA all three root tissues discolored immediately. Pitcher concluded that in both apple and peach roots the tissues that showed a rapid discoloration in the presence of P. penetrans also contained large amounts of phenolic substances.

The final selection of grasses for histological and greenhouse studies was based on their response to applications of DSA and/or pectinol. The species chosen provided a wide range of coloration from the pectinol and DSA used. The following grass seed were tested.

Bluegrass

Common Kentucky Poa pratensis L.

Delta Kentucky Poa pratensis L. var. Delta

Merion Kentucky Poa pratensis L. var. Merion

Bentgrass

Astoria Colonial Agrostis tenuis Sibth. var. Astoria

Highland Colonial Agrostis tenuis Sibth. var. Highland

PennCross Agrostis palustris Huds. var. PennCross

Red Top Agrostis alba L.

Velvet Agrostis canina L.

Fescue

PennLawn Festuca rubra L. var. PennLawn

Creeping Red Festuca rubra L.

Kentucky 31 Festuca arundinacea Schreb.

Ryegrass

Annual Lolium multiflorum Lam.

Perennial Lolium perenne L.

Bermudagrass

Cynodon dactylon (L.) Pers.

Seeds of the 14 grass species and varieties were surface sterilized by placing them for 25 minutes in a five per cent sodium hypochlorite solution (Clorox) containing a non-ionic wetting agent. The seeds were then rinsed several times in sterile distilled water and placed for germination on two per cent water agar in Petri dishes.

Germination times varied with the grass species, but all seedlings were allowed to grow until small root systems developed. Entire plants from each species were removed from Petri plates and placed in Syracuse watch glasses. Each plant was then sprayed with a 25 per cent solution of pectinol (Pectinol 59L, Rohm and Haas, a commercial preparation of pectinases) in distilled water containing a wetting agent. The sprayed plants were incubated at a relative humidity of 100 per cent for 16 hours at a temperature of 33° C. Roots of all turfgrass species so tested turned various shades of light brown to yellow. The roots of three of the pectinol-treated grass species - annual ryegrass, creeping red fescue and Kentucky bluegrass - exhibited colors of light brown, very light brown and yellow respectively.

Untreated plants of the 14 grass species were further

tested for the possible production of phenols by dipping their root system in DSA. The root epidermal cells of all species tested exhibited shades of brown to yellow similar to those treated with pectinol. Ryegrass, creeping red fescue and Kentucky bluegrass showed the same varying shades of color as in the first test.

Both preliminary histochemical tests indicated that ryegrass was the most susceptible to an organism-incited disease, creeping red fescue less susceptible and Kentucky bluegrass the least. Thus, rye, fescue and Kentucky bluegrass were selected as test plants on the basis that they might react differently to injury caused by P. penetrans and T. claytoni.

IV. Histological Studies

A. Procedures

1. Nematode Cultures

P. penetrans and T. claytoni were aseptically cultured separately on Dupuits alfalfa callus tissue growing in nutrient agar using a modification of the technique described by Krusberg (26).

Alfalfa seeds were surface sterilized and scarified in concentrated sulfuric acid for 15 minutes, rinsed four times in sterile distilled water and transferred to sterile two per cent water agar in Petri plates. After germination the seedlings were placed on nutrient agar slants in 25 x 150 mm test tubes. Surface-disinfected P. penetrans and T. claytoni were then added to their respective cultures for propagation.

The nutrient agar (see appendix A) consisted of a modified Knop's solution less coconut milk as described by Krusberg. A growth hormone, 2, 4-dichlorophenoxyacetic acid, was added to stimulate callus tissue formation.

2. Growing the Host

Rye, fescue and Kentucky bluegrass to be inoculated with P. penetrans were grown aseptically in test tubes containing nutrient agar. Grass plants inoculated with T. claytoni were grown in sterilized soil in test tubes.

Seeds of ryegrass, creeping red fescue and Kentucky bluegrass were surface sterilized, rinsed and placed on two per cent water agar for germination. Annual ryegrass germi-

nated in two to four days, fescue in five to seven days and Kentucky bluegrass after seven days. Fifty-six germinated seedlings of each species were then transferred to 168 nutrient agar slants. Agar was prepared according to a formula described by Bredakis (4) (appendix B).

Because of the difficulty encountered in maintaining live T. claytoni on the three test plants in nutrient agar, soil was used as a growth media in later experiments.

Twenty-five grams of sandy loam soil and 12 ml of the nutrient solution (Table 1) were added to each of 90 test tubes which were then steam sterilized. Upon germination 30 seedlings of each grass species were transferred to the soil media in the tubes which were then placed under a bank of three Grow-lux lamps giving continuous illumination in a growth chamber held at 70° C.

3. Nematode Extraction

Nematodes for inoculation were extracted from cultures aseptically. Conical centrifuge tubes, 15 ml capacity, were filled three-quarter full with distilled water. A plug of non-absorbent cotton was inserted into each vial so that it just touched the water. Vials were capped with aluminum foil and then autoclaved. Callus tissue containing the nematodes was transferred to the top of the cotton plugs. Nematodes settled to the bottom of the vial in four to eight hours and were extracted in a small amount of water.

4. Host Inoculation

Ryegrass was inoculated with P. penetrans five days after being transferred to nutrient agar while fescue and Kentucky bluegrass was allowed to grow 14 and 20 days respectively before inoculation.

Ryegrass and red fescue were inoculated with T. claytoni eight days after the seedlings were transferred to the soil media. Kentucky bluegrass was inoculated 14 days after transfer.

To assist in inoculation and to aid in maintaining aseptic conditions, an inoculating chamber was fitted with a dissecting microscope. The cover of the chamber was polyethylene with a hole which allowed the eyepiece to protrude beyond the material but yet fit snugly under the removable eyepiece. The chamber and microscope within were sterilized with ultraviolet light for 24 hours prior to inoculation. A small amount of water and nematodes was poured from the nematode-containing centrifuge vials into a sterilized watch glass sitting on the microscope stage. Autoclaved micropipettes plugged with cotton were used to transfer nematodes from watch glasses to tubes containing seedlings. Seventy-five to 100 P. penetrans were added to each of the 42 agar slant replications for each grass species. Fourteen plants for each grass species were maintained as uninoculated controls. Only 26 test tubes per grass species were inoculated with T. claytoni. Each ryegrass tube and each red fescue tube received 75 to 100 nematodes, but only 40 T. claytoni

were pipetted into the soil media anchoring Kentucky bluegrass. Four controls were grown for each of the three grass species grown for T. claytoni studies.

Nematodes tend to become trapped in surface water. To insure nematode movement through the agar slants a very small amount of sterilized lukewarm two per cent water agar was pipetted into the test tubes to absorb water added with nematodes.

5. Histological Preparation

In the first 14 days after inoculation of turfgrass species with P. penetrans, two plants of each species were removed from agar slants every three days and examined microscopically. Thereafter, two plants per species were removed twice a week.

For the possible detection of phenols and lesions, the roots of all the infested plants, when removed from the agar, were placed in a drop of diazotized sulphanilic acid (DSA) on a glass slide. Roots from one of the two plants were examined carefully and sections containing nematodes and/or eggs were cut out and placed in Craf III, a chromo-acetic solution fixative (21). For further examination the entire root of the second plant was boiled for 60 seconds in acid fuschin-lactophenol and then placed in clear lactophenol to clear. Roots of control plants were also subjected to DSA, fixative and/or acid fuschin and lactophenol.

Two T. claytoni infested plants from each grass species were taken out of their growth media one week after inocu-

lation and every week thereafter for eight weeks. The ninth week, the remaining inoculated plants plus the controls, were extracted. The roots were thoroughly washed in water to remove adhering soil particles before placing them in DSA. They too were examined microscopically and then subjected to chromo-acetic fixation and/or acid fuschin and lactophenol.

To check for microbiological sterility, each test tube containing a grass species infested with T. claytoni was opened under aseptic conditions and small pieces of root placed on sterile potato dextrose agar.

T. claytoni were extracted from each of the infested soils by the sugar flotation method as described by Caveness and Jensen (5).

All chromo-acetic fixed root sections were dehydrated, cleared and imbedded in Tissue Mat. Sections were cut on a rotary microtome, mounted on slides and stained with safranine and fast green, as described by Jensen (21).

6. Laboratory Observations

Using techniques described by Lavallee and Rohde (28), several observation trials were made of the feeding habits of T. claytoni and P. penetrans. Transparent boxes 46 x 22 x 5 mm were filled with nutrient agar to an approximate depth of four millimeters. Prior to gelation an aseptically-germinated grass seedling was inserted into agar to a depth that assured complete root coverage. Three replications were made for each grass specie and for each nematode specie. Twenty-four hours after the transplant, an eight millimeter

diameter agar cylinder was removed from the center of each chamber and 10 to 15 nematodes of the desired species were pipetted into the cavity. The hole was then filled with warm ungelled agar establishing a continuous medium through which nematodes could easily move to grass roots. Three replications of each turfgrass species were left uninoculated and instead, each root was punctured with an insect mounting pin. No attempt was made to maintain aseptic conditions during a three day observation period. At the end of each trial both wounded and nematode infested plant roots were dipped in DSA and the inoculated roots were also stained with acid fuschin and cleared with lactophenol.

Laboratory observation trials were repeated just to further note nematode movement and their effects on the grass roots.

B. Results

1. Observations

In all of the inoculated chambers, at least a few nematodes reached the grass roots. A number of nematodes of both species reached the surface of the agar and apparently did not return beneath the surface to feed, while some moved away from the root area.

Both ryegrass and fescue roots appeared more attractive to P. penetrans than Kentucky bluegrass roots. On the third day of observation, several P. penetrans were still wandering through the agar in which Kentucky bluegrass was growing, but none could be seen moving through the agar containing

fescue or rye. On Kentucky bluegrass the first nematode reached a root in four and one-half hours, but then immediately moved away.

In a second trial, P. penetrans feeding could be observed on both ryegrass and fescue roots two hours after inoculation. Several nematodes, upon reaching the rye and fescue roots, appeared to stay in the same area of contact. They also probed at various distances from the root cap, but never at the cap.

In the first trial, P. penetrans was observed penetrat- a fescue root in the region of elongation 24 hours after inoculation, but the method of penetration could not be determined. In the second trial, one nematode was seen penetrating a ryegrass root while another nematode fed in the same area. Penetration appeared to be intercellular. One of two nematodes had also laid an egg outside the root. Lesions could not be detected either after penetration or when the roots were tested with DSA to determine if phenols were present. After the roots of the first test species were stained and cleared, four P. penetrans were found in one small rye root. One nematode was located between two lateral root nodes; the other three found just behind the root cap.

The stained fescue plant had three small roots and each had two or more P. penetrans in it; one contained an egg. The nematodes were located at various distances from the root cap. No nematodes were found in the Kentucky bluegrass roots.

Examination of the stained roots of the second test revealed P. penetrans in relatively the same areas of the roots in ryegrass and fescue as in the same grass species of the first trial. However, only one root of Kentucky bluegrass had a nematode and it was located considerably behind the root cap. In a third trial, in which only Kentucky bluegrass was used, no P. penetrans were found in any of the roots.

The needle wounds made in the root cells in each of the three grass species did not turn brown. When the injured cells were placed in DSA, they did not show any indication of strong phenol accumulation.

T. claytoni specimens appeared to feed or at least probe the root cap for a short time and then move to other areas of the root. Feeding occurred behind the root cap on all three species of grass. Stylet puncture marks were not discernible on any root cells that were being fed upon.

T. claytoni moved very slowly through the agar in comparison to P. penetrans, and died very shortly after the agar became contaminated with bacteria. T. claytoni was never seen penetrating any of the roots and was never found inside a root.

2. Histological

Top growth of the three grass species grown in test tubes for histological studies and infested with either P. penetrans or T. claytoni was not affected by the nematodes under the experimental conditions described.

Figure 1. Annual ryegrass roots invaded 24 hours after inoculation with P. penetrans.

A. P. penetrans partially within the root. Note the second nematode close by. x430

B. Enlargement of the above photograph. Note the nematode's head within the cell and what appears to be the entry hole (arrow). x430

C. Egg laid by one of the above two nematodes outside the root. x430

Figure 2. Creeping red fescue root invaded by P. penetrans.

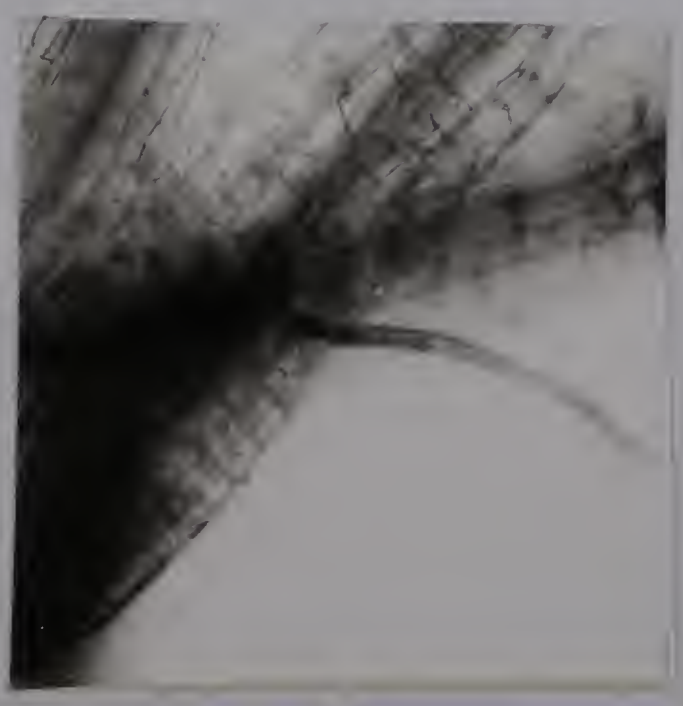
D. P. penetrans appear to have entered the fescue root intercellularly in the region of elongation. x100

E. Enlargement of the above exposure. The nematode has penetrated the root parenchyma. Note the bulging of the cell wall. x100

A

B

C



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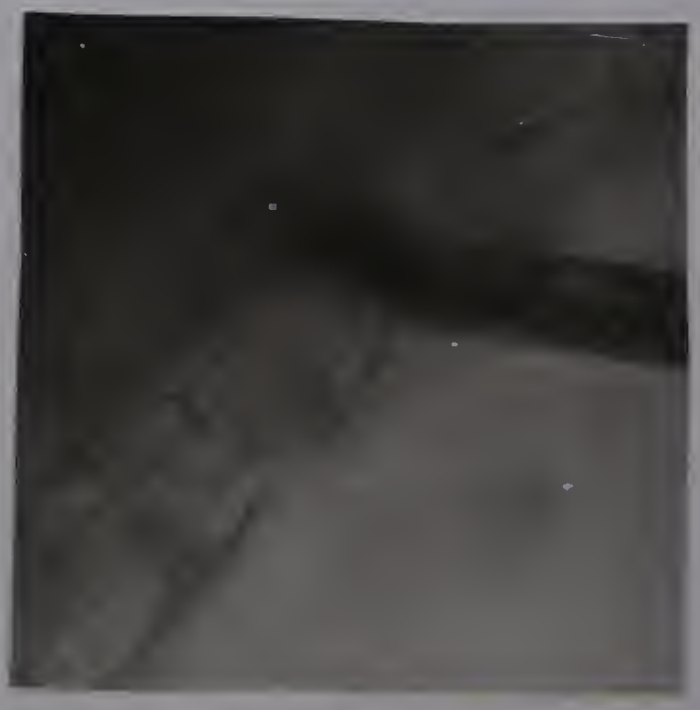


E

P. penetrans and their eggs were located only in the cortex of both primary and lateral roots of each grass species and almost always aligned parallel to the endodermis.

Several lesion nematodes were found within roots of annual ryegrass two days after inoculation and three days after inoculation in creeping red fescue. More than 120 Kentucky bluegrass plants were inoculated with P. penetrans, but nematodes were found in the roots of only two plants.

Eleven P. penetrans and eggs were detected in necrotic leaf cells of ryegrass. Fifty-two eggs of P. penetrans were counted in a 15 mm section of annual ryegrass, and nine nematodes were found in a half-inch section of a lateral creeping red fescue root.

Nematode lesions were never apparent in roots of any of the inoculated grass species either before or after placing them in DSA. The parasites moved through parenchyma cells of the cortex, tearing cross walls, but injured cells did not become discolored. In a number of roots two or more nematodes were observed in the same root area, but again they did not appear to cause cell necrosis.

Cortical cells of stained sections of ryegrass and fescue roots infested with P. penetrans did not appear to have thickened cell walls. Cells containing or adjacent to nematodes never showed discoloration and the stelar tissue appeared unaffected.

When placed in DSA, sections of roots from some of the three grass species turned slightly dark in color which

Figure 3. T. claytoni shown probing an annual ryegrass root 24 hours after inoculation. x430.

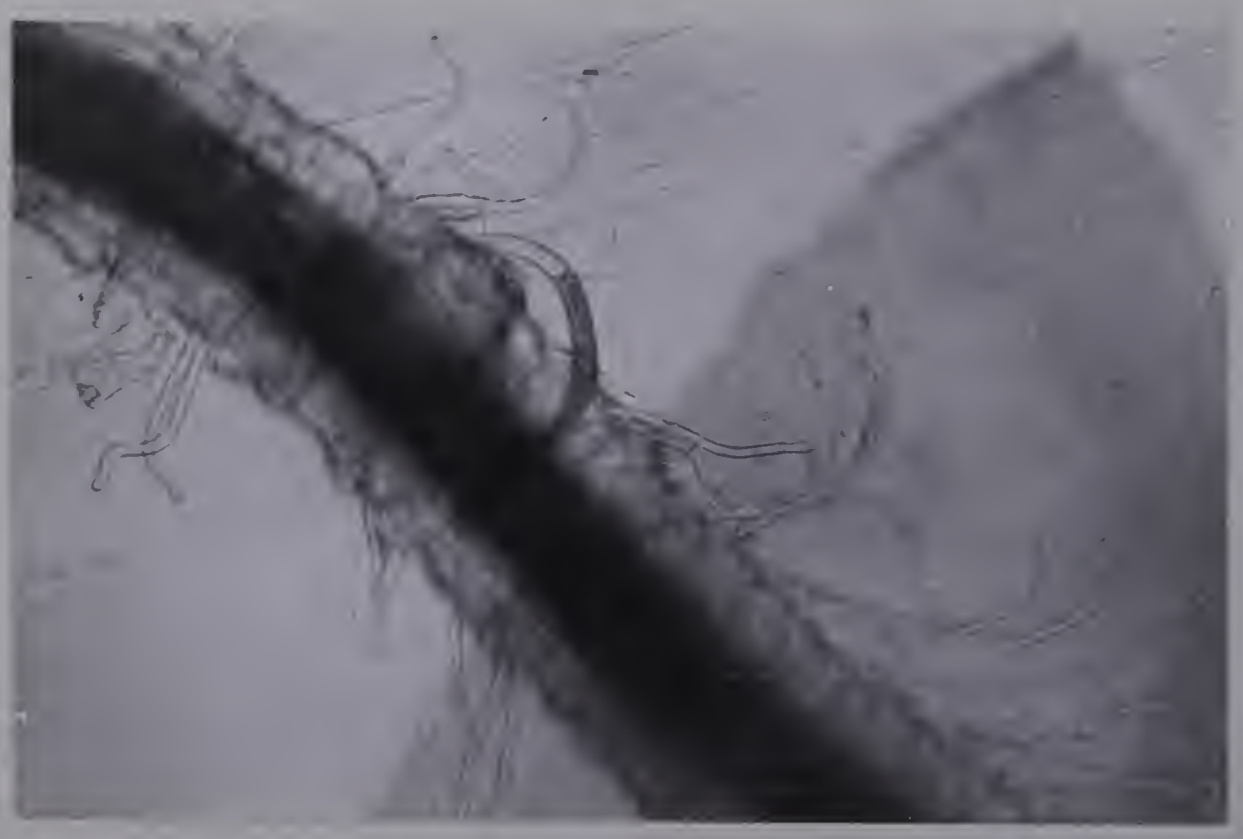


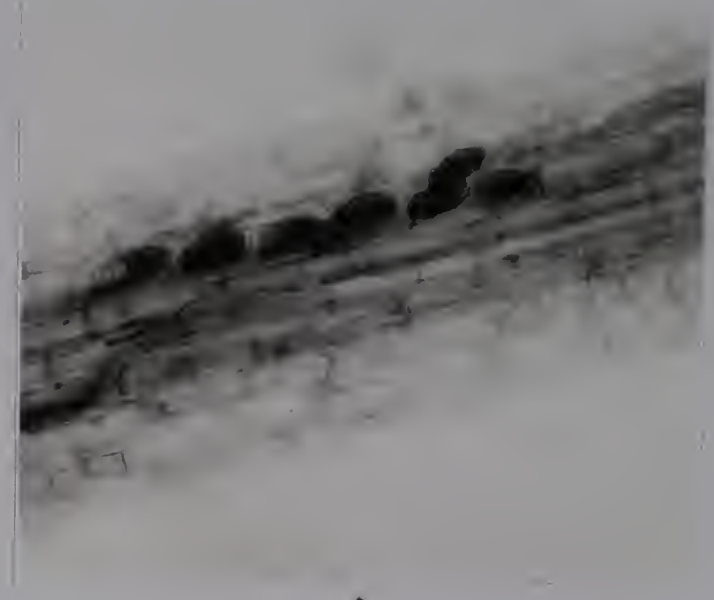
Figure 4. T. claytoni shown probing a creeping red fescue root. Note that the nematode appears to be feeding next to a lateral node. x430.

Figure 5. Annual ryegrass roots infested with P. penetrans and their eggs. All roots were stained with diazotized sulphanilic acid.

A. Seven nematode eggs within the cortical cells. One nematode is seen above the eggs. x100

B. Several eggs and nematodes within the cortex of ryegrass. x100

C. A single nematode within the cortex. x100



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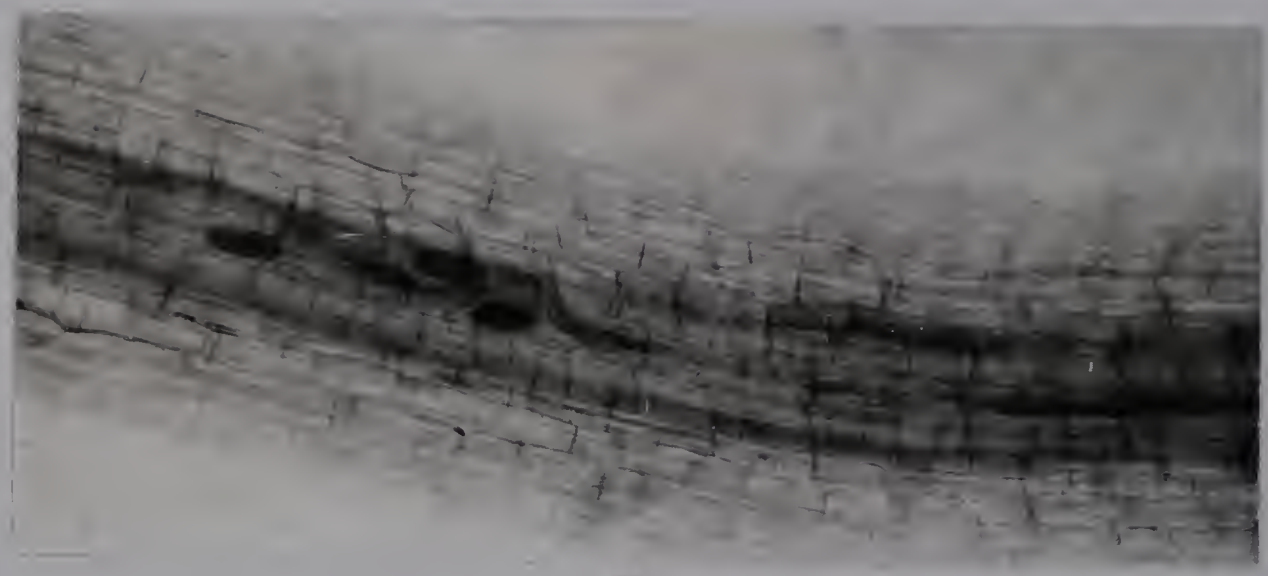

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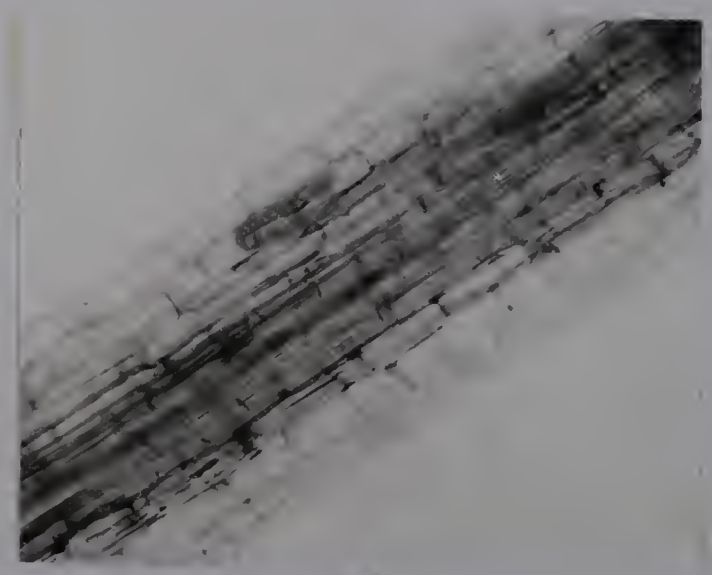


C

Figure 6. Kentucky bluegrass roots invaded by P. penetrans. Roots were stained with acid fuschin and cleared with lacto-phenol.

A. P. penetrans eggs. Part of a nematode can be seen (arrow). x430

B. A nematode within the root. Note part of an egg can be seen (arrow). x430

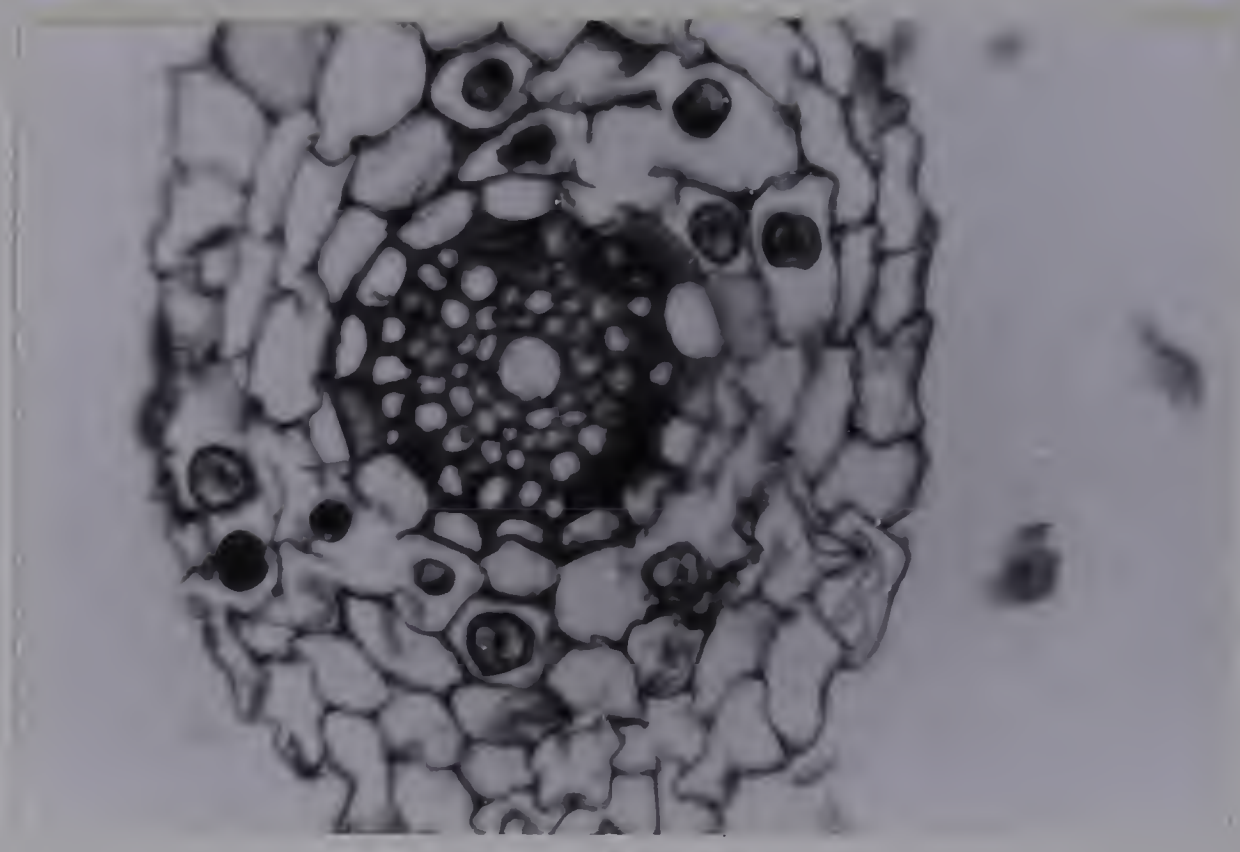
A

B

Figure 7. Cross sections of annual ryegrass and creeping red fescue roots showing P. penetrans nematodes lying in the cortical parenchyma.

A. Creeping red fescue roots. Note approximately 12 nematodes within the cortex. x430

B. Annual ryegrass root only two nematodes can be seen within the cortex neither of which appeared to cause necrosis of the cells. x430



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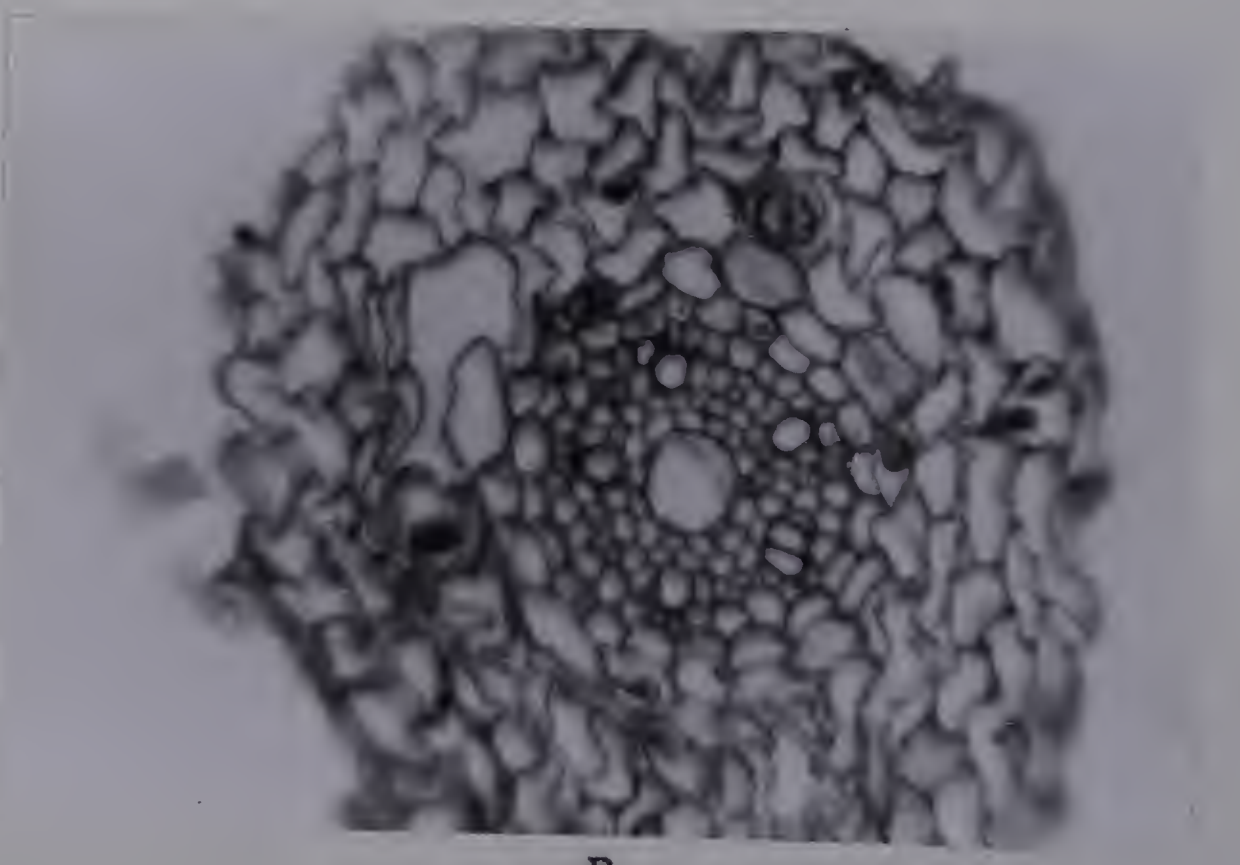


B

indicated a possible accumulation of phenols. Also, thin sections cut from these dark areas and stained with fast green further showed possible injury of the epidermal cells. however, the injury did not appear to be caused by nematode feeding.

Root sections, wounded with a sterile needle to simulate a stylet wound, readily took up stain; however, a similar reaction was not seen in any of the roots that had been infested with T. claytoni.

T. claytoni was never observed within roots of any of the grass species.

V. Effects of Nematodes on Turfgrass

A series of experiments were conducted in the greenhouse to determine the gross effects of P. penetrans and T. claytoni on ryegrass and creeping red fescue. Tiller counts, herbage and root weights of the inoculated plants were compared with the controls.

A. Procedures

1. Experiment One

Fifteen one cubic foot wooden boxes coated, inside and out with a non-coal tar based paint were filled with steam sterilized sandy-loam soil. The soil pH had been adjusted to 6.3 by the addition of ground limestone. Each box of soil received a 10-10-10 fertilizer and an extra amount of superphosphate prior to planting of the grass. The amount of fertilizer placed in each box was at the rate of two pounds of actual nitrogen per 1000 square feet of soil. The superphosphate was added at the rate of 25 pounds of material per 1000 square feet.

Surface sterilized ryegrass and creeping red fescue seedlings, germinated on aseptic two per cent water agar in Petri plates, were transplanted to the boxes. Each of 10 boxes was planted with 64 ryegrass seedlings; the remaining five were each planted to 64 seedlings of creeping red fescue.

Approximately 10,000 P. penetrans were introduced into the soil 8 to 10 days after transplanting, in three boxes of

ryegrass and three of red fescue. Four boxes, two for each species of grass, acted as controls. Three of the remaining five boxes of ryegrass were each inoculated to T. claytoni and two were kept as controls.

All inoculum was aseptically propagated on alfalfa callus tissue growing on nutrient agar. Nematodes were obtained by removing the agar and the tissue from the test tube, placing on a Baerman funnel, and collecting in a Syracuse watch glass for counting.

The nematodes, in water, were pipetted into a small hole made in the soil at the base of each grass plant and then the hole was covered with soil.

The boxes of turf were watered daily but were not limed or fertilized during the test. Soil temperature was recorded daily for approximately a month but when it did not go over 85° F., readings were discontinued.

Tiller counts were made only twice on all plants, the second count about 20 days after the first.

Two and one-half months after transplanting, the grass in each box was cut to a height of three inches every 20 days. The clipping height was maintained using an electric shear, resting on and moving across a three inch high template.

The top growth was clipped four times and green weights recorded (Table 1). After the fourth cutting, the grass plants were subjected to additional stress.

The boxes of grass were placed under a black plastic tent and grown without light for two weeks. During that

period, observations on their top growth were noted and the top herbage was cut twice, weighed and recorded. The first cutting was made seven days after placing them in the dark and again at the termination of the test. Root weights and nematode counts were also noted at the end of the experiment.

To aid in getting soil and root nematode counts, a cup-cutter, one used for cutting the hole in a golf putting green, was employed to remove two plugs from each turf box. The plugs were four inches in diameter, six inches deep, each containing about four plants.

Nematodes were extracted from 500 grams of soil in each plug, using a modification of the Seinhorst elutriator technique (48). Washed roots were placed in water on Baerman funnels to obtain P. penetrans. Roots were kept on the funnels four days, after which they were air dried and weighed.

2. Experiment Two

Ten thousand nematodes inoculated to plants growing in boxes in experiment one were found to be insufficent to influence plant growth in such a large volume of soil. Therefore, it was decided to conduct the next two tests in small pots. The small amount of soil placed in pots provided conditions favorable to the nematode.

Each of 30 four-inch plastic flower pots received 452 grams of a sterilized sandy-loam soil that had been autoclaved for 40 minutes at 15 pounds pressure.

Prior to transplanting the plants, the soil in each pot was fertilized with a 10-10-10 at a rate equivalent to two pounds of nitrogen per 1000 square feet and limed with dolomite lime equivalent to 50 pounds per 1000 square feet.

Each pot, containing 10 aseptically germinated seedlings, was replicated 10 times for each of the three grass species - ryegrass, fescue and Kentucky bluegrass. Fourteen days after transplanting from agar, three pots of each turf grass species were each inoculated with 5000 P. penetrans and three pots were inoculated with 5000 T. claytoni. Two uninoculated controls were maintained for each grass species.

Plants were watered daily and fertilized every two weeks with 24-12-12 at the rate of one eighth of a pound of nitrogen per 1000 square feet. Tiller counts were made twice on all plants, and the experiment was terminated three and one-half months after plants were inoculated. Nematodes were extracted from the soil of each pot and from the roots infested with P. penetrans. To determine the yield of P. penetrans from the infested ryegrass roots, roots were kept on Baerman funnels for 32 days. Each day nematodes were counted and fresh aerated water was added to the funnels to replace that drawn off.

The top growth in each pot was cut, oven dried, weighed and recorded. The root growth of only those plants inoculated with T. claytoni and their respective controls, were oven dried, weighed and noted (Table 4).

All data were statistically analysed.

Figure 8. Containers used in determining the effects of nematodes on turfgrass.

A. One cubic foot boxes of soil in which red fescue was grown. Note very little difference in top growth between nematode inoculated plants and the controls.

B. Four-inch pots containing 10 grass plants. Note inoculated plants appear as healthy as the controls.

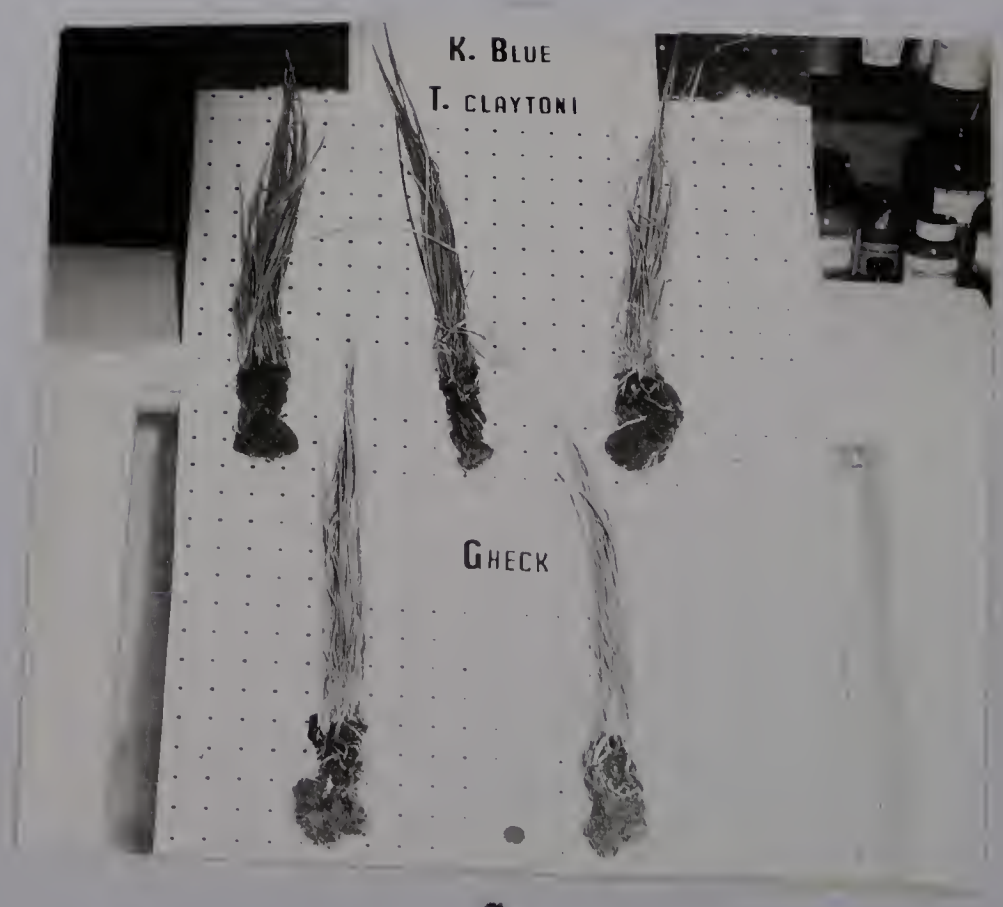
C. Kentucky bluegrass plants inoculated with T. claytoni compared with checks.


R. FESCUE
P. PENETRANS
CHECK

A


R. FESCUE
P. PENETRANS
CHECK

B


K. BLUE
T. CLAYTONI
CHECK

C

3. Experiment Three

The second experiment was repeated; thirty pots were each filled with 452 grams of steam sterilized sandy-loam soil. Lime and fertilizer were added to the soil to adjust the pH and fertilizer level. Fertilizer was not added during the course of the experiment.

Two months after inoculation, nematodes were removed from the soil of each pot and their numbers recorded. Roots of plants were washed in water and placed on Baerman funnels for four days. Nematodes, which came out of the roots during that time, were collected and counted. Both tops and roots from each pot were oven dried and weighed.

B. Results

1. Experiment One

After one month, the average number of tillers produced by annual ryegrass plants inoculated with 10,000 P. penetrans was 50 compared to the 47 average for the controls. Infested creeping red fescue averaged 53 tillers to 43 for the non-inoculated plants (Table 1).

The green herbage weights of ryegrass plants, infested with P. penetrans, varied considerably when compared to the weights of the controls. The first two cuttings of the top growth of the controls, were heavier than those from infested plants, but the results were just the opposite for the next two cuttings (Table 1). The top weights and root weights of infested plants, subjected to two weeks of darkness, also

outweighed the controls but not significantly.

There was a slight increase in top weight and root weight of creeping red fescue plants infested with P. penetrans and grown in the dark, compared to the weight of the check plants also grown in the dark (Table 2). However, the green weights of the controls, before darkness, were greater for the first three cuttings than the inoculated plants. The fourth cutting showed a slight increase in clipping weight for the infested plants over the controls.

Tiller counts and clipping weights produced by the boxes of annual ryegrass inoculated with T. claytoni were recorded.

The total number of P. penetrans, recovered from the soil and roots of annual ryegrass and creeping red fescue, were 1698 and 463 respectively (Table 2).

Prior to being placed in the dark the top growth of all grass species appeared normal; no stunting or chlorsis was observed. After two weeks of growing in the dark, there were no foliage differences between controls and nematode infested plants; all were severely chlorotic.

2. Experiment Two

The top growth of the nematode infested rye and fescue appeared as healthy as did the controls. No stunting or chlorosis occurred and both had the same number of tillers.

Very little difference in the oven dried top weights of two inoculated grass species was noted when compared to the

controls (Table 3).

The average number of P. penetrans recovered from the replications of infested rye grass soil was 212. The roots yielded an average of 3,808 nematodes over a 32 day extraction period (Fig. 9). An average of 3,481 P. penetrans was recovered from the infested soil in which creeping red fescue was grown. Red fescue roots in 12 days yielded an average of 579 nematodes. A statistical analysis of co-variance showed no significant differences between the dry matter of either P. penetrans inoculated rye or fescue and the dry matter of comparable non-infested plants.

No P. penetrans were recovered from either the inoculated soil or roots of Kentucky bluegrass. Also, plants of one inoculated replication and plants of one control replication died before the test was completed; therefore, top and root weights were not recorded.

In test two, there were no differences in tiller production by creeping red fescue and annual ryegrass inoculated with T. claytoni compared to their respective uninoculated controls. Kentucky bluegrass tillers were not counted.

When compared to check plants, the top growth of all three species of grass inoculated with T. claytoni did not appear to be affected by the nematodes. There were no significant differences in the oven dried top weights of each of the nematode infested plants when compared to the dry weight of their respective controls (Table 4). However, there appeared to be a considerable increase in the top

weight of infested bluegrass when compared to the controls.

An analysis of co-variance showed a significant reduction in root weight of infested creeping red fescue compared to its check (Table 4). Nematode-infested Kentucky bluegrass soil yielded the largest amount of T. claytoni, 15,571; soil in which red fescue was grown produced 9,799 nematodes, only 6,471 were extracted from the rye-infested soil.

The composite sample of soil taken from each pot was pH 6.3; calcium, potassium, phosphorpus and magnesium were high; nitrate nitrogen and NH_4 nitrogen were low.

3. Experiment Three

As in test one and two, when compared to non-infested grass species, the foliage of the three turfgrass species infested with either P. penetrans or T. claytoni were not influenced by inoculation with nematodes. The average root weight of infested annual ryegrass was significantly less than the average root weight of the control. No significant differences were noted between the average root weights of creeping red fescue and Kentucky bluegrass inoculated with P. penetrans and the average root weights of their controls (Table 5).

The combined average number of P. penetrans recovered from the soil and roots of infested annual ryegrass was 410. Inoculated creeping red fescue soil and roots yielded an average of 98 and Kentucky bluegrass yielded an average of 542.

The average number of T. claytoni recovered from soil, in which ryegrass, fescue and Kentucky bluegrass was grown, is listed in (Table 6).

An analysis of co-variance showed a significant increase in average top growth weight of annual ryegrass plants infested with T. claytoni as compared to average top weight of the check plants (Table 6). No significant differences were found between the average top growth weights of creeping red fescue and Kentucky bluegrass infested with T. claytoni and the weights of non-infested controls.

The average dry root weight of Kentucky bluegrass not infested with T. claytoni, was almost twice the weight of the infested plants but the difference was not statistically significant. There were no significant differences in the root weights of fescue and annual ryegrass, infested with T. claytoni compared to their controls.

At the end of this test the soils had a composite pH of 5.7. A soil fertility test inicated that calcium, phosphorous and nitrate nitrogen levels were low and potassium and ammonium nitrogen were medium high.

Figure 9. P. penetrans recovered from ryegrass roots.

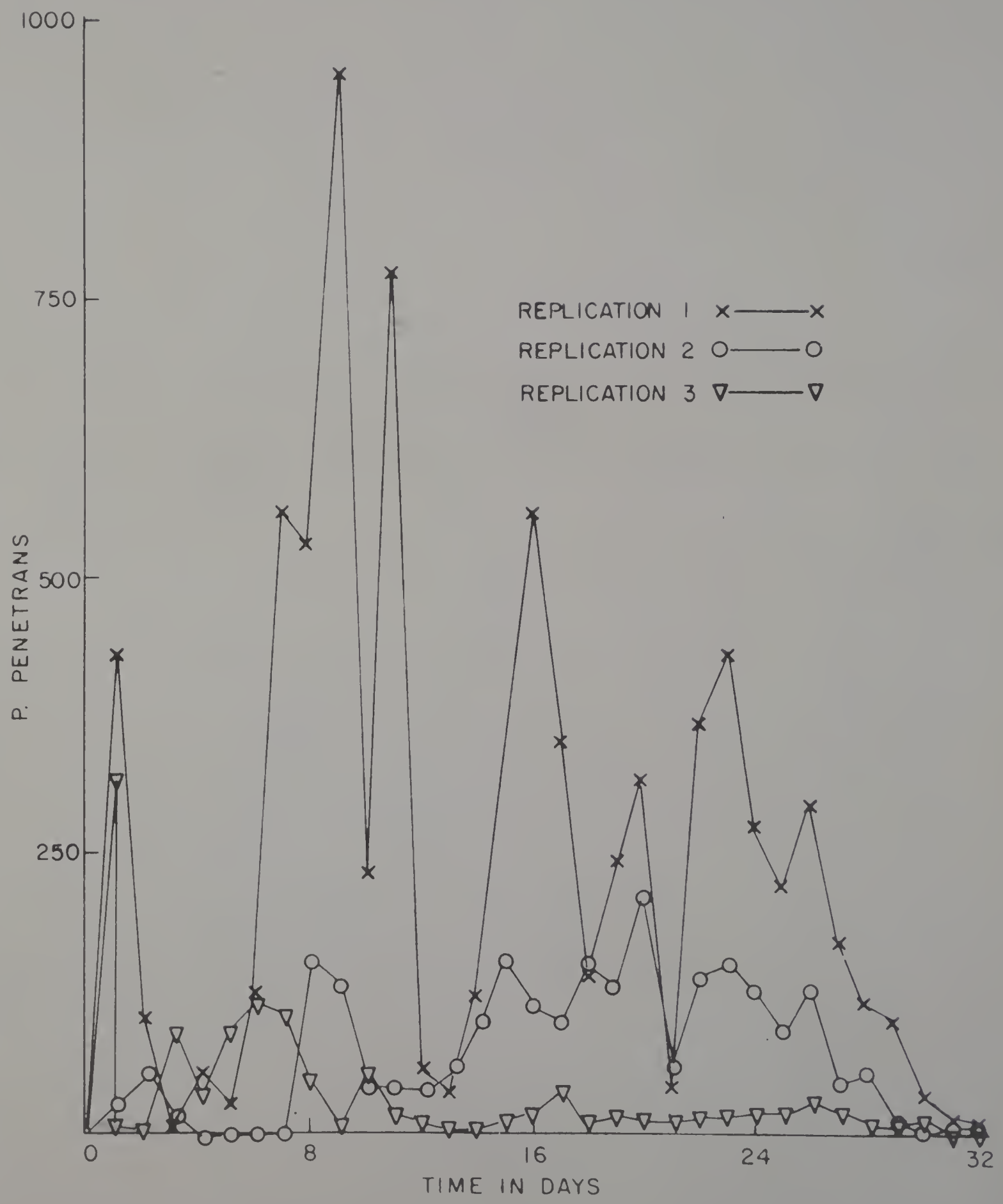


Table 1. Average number of tillers and four cuttings of plant tops from two species of grass infested with P. penetrans (Experiment One).

Grass species	Tillers		Top green weights (in grams)							
			Infested				Control			
	Infested	Control	1	2	3	4	1	2	3	4
Annual ryegrass	50	47	145.5	110.9	160.8	209.8	193.6	128.1	134.2	146.0
Creeping red fescue	53	43	34.1	15.7	46.4	65.5	39.2	21.3	60.7	63.3

Data are means of three replications of inoculated plants and two controls.

Table 2. Average number of nematodes recovered and root and top weights of two grass species infested with P. penetrans and grown in the dark for two weeks (Experiment One).

Grass species	No. nematodes		Top weights (in grams)		Root weights (in grams)	
	Soil	Root	Infested	Controls	Infested	Controls
Annual ryegrass	1444	254	16.68	11.40	1.86	1.52
Creeping red fescue	168	295	15.54	14.91	3.18	3.70

Data are means of three replications of infested plants from four inch plugs and two replications of controls.

Table 3. Average number of nematodes recovered and top weights of two turfgrass species inoculated with P. penetrans (Experiment Two).

Grass species	No. nematodes Soil	No. nematodes Roots	Top weights (in grams) Inoculated	Top weights (in grams) Controls
Annual ryegrass	212	3808	3.79	3.70
Creeping red fescue	3,481	579	1.81	1.74

Data are the means of three replications of inoculated plants and two for controls.

Table 4. Average number of nematodes recovered and top and root weights of three turfgrass species inoculated with T. claytoni (Experiment Two).

Grass species	No. nematodes Soil	Top weights (in grams) Inoculated	Top weights (in grams) Controls	Root weights (in grams) Inoculated	Root weights (in grams) Controls
Annual ryegrass	6,471	3.25	2.97	2.51	2.81
Creeping red fescue	9,799	1.40	1.58	*2.12	5.56
Kentucky bluegrass	15,571	3.40	1.80	2.20	2.23

Data are the means of three replications of inoculated plants and two for controls.
*Analysis of co-variance indicates a significant difference between the inoculated root dry matter and the controls for creeping red fescue.

Table 5. Average number of nematodes recovered and top and root weights of three turfgrass species infested with P. penetrans (Experiment Three).

Grass species	No. nematodes Soil	Roots	Top weights (in grams) Infested	Controls	Root weights (in grams) Infested	Controls
Annual ryegrass	211.0	199.0	4.16	3.67	*2.75	8.92
Creeping red fescue	91.0	7.0	3.89	3.25	6.43	5.42
Kentucky bluegrass	467.0	75.0	3.53	3.75	3.51	3.10

Data are the means of three replications of infested plants and two for controls.
*Analysis of co-variance indicate significant difference between nematode infested dry matter and the controls in ryegrass roots.

Table 6. Average number of nematodes recovered and top and root weights of three turfgrass species infested with T. claytoni (Experiment Three).

Grass species	No. nematodes Soil	Top weights (in grams) Infested	Controls	Root weights (in grams) Infested	Controls
Annual ryegrass	2,137	*7.12	3.60	2.56	2.48
Creeping red fescue	4,661	4.52	3.23	5.23	4.89
Kentucky bluegrass	4,797	3.42	3.24	3.60	6.37

Data are the means of three replications of infested plants and two for controls.
*Analysis of co-variance shows a significant increase in nematode infested ryegrass top growth vs. controls.

VI. Nutrient Studies

A. Prodcedures

In the greenhouse experiments, there was no effect on the foliage of any of the turfgrass species inoculated with either P. penetrans or T. claytoni. The control plants did not show any signs of mineral deficiency. The top growth of ryegrass, fescue and Kentucky bluegrass and from those inoculated with T. claytoni in test two were analysed for potassium, calcium, magnesium and phosphorous and were compared with analysis of control plants. The roots of the fescue plants, infested with T. claytoni in both test two and three, were also analysed for potassium.

Samples were prepared for analysis by grinding each to a fine pulp in a Wiley intermediate mill. The pulp was then dried in an oven at 180° F. The dried samples were then ashed by the perchloric, nitric acid, liquid fire method as described by the AOAC (38).

Per cent potassium was determined by the flame photometer method (38). The per cent of magnesium and calcium was obtained using the spectophotometer as described in Analytical Methods for Atomic Absorption by Perkins-Elmer Instruction Manual (41). Phosphorous percentages were determined using Sherman's molybdenum blue method for total phosphorous (51). All results were statistically analysed.

B. Results

The percentages of potassium, calcium, magnesium and

phosphorous found in the top growth of all turfgrass species inoculated with either T. claytoni or P. penetrans were not significantly different from the tops of the controls (7, 8, 9, 10).

No significant differences were found in the per cent potassium uptake by fescue roots inoculated with T. claytoni in either test two or three compared to their respective controls.

Table 7. Average number of nematodes recovered and the average per cent nutrients in the top growth of three turfgrass species infested with P. penetrans (Experiment Three).

Grass species	No. nematodes		Per cent nutrients							
	Soil	Roots	Inoculated				Controls			
			K	Ca	Mg	P	K	Ca	Mg	P
Annual ryegrass	211.3	199.0	1.27	.73	.26	.265	1.31	.92	.29	.278
Creeping red fescue	91.0	7.0	1.34	.49	.25	.253	1.68	.47	.21	.285
Kentucky bluegrass	467.0	75.0	1.40	.46	.23	.213	1.65	.37	.21	.213

Data are the means of three replications of inoculated plants and two for controls.

Table 8. Average number of nematodes recovered and the average per cent nutrients in the top growth of three turfgrass species infested with T. Claytoni (Experiment Three).

Grass species	No. nematodes	Per cent nutrients							
	Soil	Inoculated				Controls			
		K	Ca	Mg	P	K	Ca	Mg	P
Annual ryegrass	2,137	.88	.59	.27	.207	1.11	.77	.24	.188
Creeping red fescue	4,611	1.21	.45	.19	.248	1.58	.45	.19	.233
Kentucky bluegrass	4,797	1.18	.36	.21	.205	1.48	.34	.21	.239

Data are the means of three replications of inoculated plants and two for controls.

Table 9. Average number of nematodes recovered and the average per cent nutrients in the top growth of three turfgrass species infested with T. claytoni (Experiment Two).

Grass species	No. nematodes Soil	Per cent nutrients Inoculated				Controls			
		K	Ca	Mg	P	K	Ca	Mg	P
Annual ryegrass	6,471	3.80	.41	.34	.454	3.49	.36	.16	.460
Creeping red fescue	9,799	2.77	.34	.16	.211	2.41	.33	.16	.206
Kentucky bluegrass	15,571	1.34	.35	.17	.199	1.53	.28	.23	.252

Data are the means of three replications of inoculated plants and two for controls.

Table 10. The average per cent of potassium in the top growth and roots of creeping red fescue inoculated with T. claytoni.

Test	Per cent potassium Tops		Roots	
	Inoculated	Controls	Inoculated	Controls
2	2.77	2.41	.37	.36
3	1.21	1.58	.28	.26

Data are the means of three replications of inoculated plants and two for controls.

VII. Experimentation on Field Control Measures

A. Experimental Plot Plan

Nematode experiments were conducted on a 50 x 60 foot area containing a mixture of Merion bluegrass and Pennlawn fescue which had become interspersed with a small amount of clover and other weeds.

The stand, established for at least four years, was growing in Walpole fine sandy loam located on the University's Brook Farm. Fertilizer and lime were not applied to the turf before or during this experiment, but the year before, the area had received approximately 50 pounds of ground limestone per 1000 square feet and at least 60 pounds of 10-10-10 fertilizer.

The second week of June 1964, the turf area was mowed to one and one-half inches and then aerified with a Ryan's greenaire aerifier. Grass clippings and turf plugs were raked off.

A plot was devised to determine any relationship between the rate of nematocide application, nematode reduction and density of the stand. The 50 x 60 foot area was divided into three 50 x 20 foot blocks. Each block was then divided into 5 x 5 foot plots, making 40 plots within each block, for a total of 120 treatment sites.

Eight nematocides and one fertilizer were selected for ease of application and lack of phytotoxicity. Each chemical was replicated four times and applied at the manufacturers'

recommended rates to plots within one block, one-half the recommended rates were applied to plots within the second block and double the recommended rates were applied to plots within the third block. Four of the plots within each block were used as controls.

Nematocides were thoroughly mixed in two gallons of water in plastic buckets and then applied by hand with watering cans. The chemical name, active ingredients and the manufacturers' rate of application of active material per acre are as follows:

Niagara 9227	organo phosphate	20 lbs.
Bayer 25141	organo phosphate	20 lbs.
Meta Systox R	organo phosphate	1½ pts.
Shell SD 7727	dichlorophenyl methanesulfonic	32 lbs.
Nemagon	dibromo chloropropane	5 gals.
Diazinon A1619	organo phosphate	40 lbs.
Diazinon 500	organo phosphate	40 lbs.
Smith, Kline and French 21701	coded	20 lbs.
Fertilizer 10-10-10	nitrogen, P_2O_5 and K_2O	860 lbs.

After the chemicals were applied, the plot was watered with a sprinkler for 24 hours.

The turfgrass area was mowed weekly with a 20 inch reel-type mower set at one and one-half inches.

One month after chemical application, a grass catcher was attached to the mower, and clippings from two cutting passes in each treatment site were collected weekly for five

weeks. A sixth harvest was made 26 days after the fifth; grass samples were oven dried, weighed and recorded. An analysis of co-variance was conducted on the top yields from all plots in relation to treatments.

Chemical phytotoxicity, color of the turf and increase in clover content were observed and recorded.

Two months after application of the chemicals each plot was examined for nematodes. Four soil plugs, each three quarter inch in diameter and six inches long, were taken at random from each plot with a soil probe and mixed. Nematodes were extracted from a 50 grams aliquot of soil from each plot by the sugar flotation method and counted in a sectioned-off Syracuse watch glass. All parasitic nematodes were identified to genus (Table 11).

Three days after the last harvest of clippings, two plugs, four inches in diameter and six inches deep, were obtained from each plot with a golf course cup cutter. The roots in each plug were washed free of soil; the top growth cut-off and the roots oven dried and weighed.

B. Results

All fertilizer treatments significantly increased the mean grass clipping weight (Tables 12, 13, 14). The top weights from all plots treated with the nematocide, Bayer 25141, at the recommended and double the manufacturers' suggested rates also showed a significant increase. However, there were only slight differences in the mean top weights from all three concentrations of Shell SD 7727, Diazinon 500,

Table 11. Nematodes and their occurrence from Merion-fescue turfgrass plots treated respectively with recommended, one-half and double rates of nematocides.

Nematode spp.	Occurrence Recommended rate			Occurrence One-half rate			Occurrence Double rate		
	Treated	Fertilizer	Water	Treated	Fertilizer	Water	Treated	Fertilizer	Water
Saprophytic	6365	1700	1065	6095	1485	1585	8715	1090	1340
Pratylenchus	950	150	525	1091	230	194	1435	450	200
Tylenchorhynchus	35	0	10	120	70	115	110	130	20
Paratylenchus	475	65	100	485	145	50	735	95	135
Criconemoides	1495	70	100	2170	995	170	830	0	555
Tylenchus	130	35	40	305	10	0	90	100	20
Spiral	5	170	20	0	0	0	30	0	20

Nematodes were extracted from 50 grams of soil from each plot, identified, counted and combined.

Figure 10. A view of the Merion bluegrass area which received nematocide treatments and the instruments used for gathering data.

A. Overall view of the turf area divided into 5 by 5 foot plots. Note the mower with a grass clipping catcher.

B. Turf plot showing the golf cup cutter used to take plugs for root samples. Turf plug is seen in the foreground.

A

B

Diazinon A1619, Niagara 9227, Nemagon and the check. The three chemical rates of SKF 21701 and those of Meta Systox R yielded the least average top growth weight.

The mean top weight of all treatments including the fertilizer in relation to each of the three treatment rates indicated that the recommended and double dosage significantly increased top growth in comparison to mean clipping weights taken from plots treated with one-half the chemical rates. There was little difference between the yields of the former two rates (Table 15).

Compared to the controls, there appeared to be some differences between parasitic nematode control by the tested nematocides at different rates (Tables 12, 13, 14). The least number of parasitic nematodes was extracted from Bayer 25141 plots treated at recommended rates. Diazinon 500 plots, followed by Niagara 9227 and Meta Systox R also yielded a low number of nematodes. Only SKF 21701 plots contained a higher number of parasites than the checks (Table 12).

At the one-half dosage, the lowest number of nematodes was extracted from Diazinon 500 and Bayer 25141 plots. The control areas also contained a relatively low number of nematodes while fertilizer treated plots contained a high amount (Table 13).

Plots to which Diazinon A1619 and Diazinon 500 were applied at twice the suggested amount, yielded an average of 28 and 48 parasites respectively. Nemagon, Niagara 9227 and

Shell SD 7727 treated plots all averaged fewer than 100 parasites, but Bayer 25141 plots averaged 181.2 nematodes compared to 252.5 found in the controls (Table 14).

Shell SD 7727 and SKF 21701 applied at double the manufacturers' suggested rates caused a speckled chlorosis of the grass leaves. Plants outgrew the injury within a few weeks.

Fertilizer was the only chemical that improved the color of the turf which appeared about 10 days after application and was still apparent at the time of the last cutting on August 31.

At the termination of the experiment, an increase in clover was noted throughout the entire turf stand, but the increase did not appear to be caused by any of the chemicals.

Root weights taken from the control plots were heavier than any of those obtained from plots treated with recommended rates of nematocides. The control plots had the highest number of parasitic nematodes. Plots receiving applications of Bayer 25141, at the manufacturers' suggested rates, had the lowest average number of nematodes and the fourth lowest root weight. The fertilizer plots yielded the lowest root weight (Table 12).

Shell SD 7727 applied on turf plots at one-half the suggested rate yielded a high mean root weight of 33.40 grams but contained an average of 223 nematodes. A mean of only 32 parasites were extracted from Diazinon 500 treated plots but the average root weight was only 19.11 grams. Root

taken from the fertilizer and water areas weighed 17.64 and 13.34 grams respectively. Nematodes from these respective plots average 362 and 131. Nematodes from the Meta Systox R plots compared to the average number taken from the controls, were 130 and 131. The root weights were 24.51 grams for the former and 13.34 grams for the latter (Table 13).

Bayer 25141 applied to turf plots at twice the suggested rate yielded an average root weight of 25.0 grams and a mean of 181 nematodes. Plots, treated with a double dosage of Diazinon A1619, had an average root weight of 24.77 grams and an average of 28 parasitic nematodes. The fertilizer treated areas and the controls had the highest average number of nematodes but yielded the lowest mean root weight (Table 14).

There was a statistically significant difference in the mean root weights in relation to the chemical rates (Table 15). Double the suggested rates yielded the heaviest root weight and the recommended treatment rates produced the least mean weight.

Results of soil pH and fertility tests taken at the conclusion of the experiment are shown in table 16.

Table 12. Average number of parasitic nematodes recovered from the soil, average clipping weights and the average root weights of a Merion-fescue grass mixture treated with recommended rates of nematocides.

Plot treatment	Dosage in 8 gal. of water	Number nematodes	Average clipping weights six cuttings (in grams)	Average root weights (in grams)
Niagara 9227	90.0 cc	1/ 72	2/ 15.25	3/ 14.29
Bayer 25141	30.0 cc	46	16.91	13.48
Meta Systox R	1.2 cc	76	12.68	19.63
Shell SD 7727	141.0 cc	143	14.18	15.91
Nemagon	43.0 cc	95	14.81	16.94
Diazinon A1619	90.0 cc	103	15.02	17.62
Diazinon 500	90.0 cc	68	14.68	13.11
SKF 21701	45.0 cc	185	13.17	11.52
Fertilizer	2.0 lb.	125	27.56	9.36
Water		173	13.24	19.97

Data are the means of four replications.
1/ LSD (.05)=113
2/ LSD (.05)=2.17
3/ LSD (.05)=12.66

Table 13. Average number of parasitic nematodes recovered from the soil, average clipping weights and the average root weights of a Merion-fescue grass mixture treated with one-half recommended rates of nematocides.

Plot treatment	Dosage in 8 gal. of water	Number nematodes	Average clipping weights six cuttings (in grams)	Average root weights (in grams)
Niagara 9227	45.0 cc	1/ 82	2/ 15.02	3/ 20.68
Bayer 25141	15.0 cc	47	13.20	27.87
Meta Systox R	0.6 cc	130	12.73	24.51
Shell SD 7727	70.0 cc	223	14.46	33.40
Nemagon	21.0 cc	135	13.66	15.50
Diazinon A1619	45.0 cc	186	13.27	16.91
Diazinon 500	45.0 cc	32	14.44	19.11
SKF 21701	22.0 cc	211	13.15	16.75
Fertilizer	1.0 lb.	362	24.66	17.64
Water		131	14.39	13.34

Data are the means of four replications.
1/ LSD (.05)=113
2/ LSD (.05)=2.17
3/ LSD (.05)=12.66

Table 14. Average number of parasitic nematodes recovered from the soil, average clipping weights and the average root weights of a Merion-fescue grass mixture treated with double the recommended rates of nematocides.

Plot treatment	Dosage in 8 gal. of water	Number nematodes	Average clipping weights six cuttings (in grams)	Average root weights (in grams)
Niagara 9227	180.0 cc	1/ 76	2/ 12.88	3/ 25.74
Bayer 25141	60.0 cc	181	17.93	25.01
Meta Systox R	2.4 cc	157	13.05	20.13
Shell SD 7727	282.0 cc	90	14.15	24.47
Nemagon	86.0 cc	65	13.57	20.50
Diazinon A1619	180.0 cc	28	13.90	24.77
Diazinon 500	180.0 cc	42	13.77	24.66
SKF 21701	90.0 cc	153	13.30	20.29
Fertilizer	2.0 lb.	207	26.67	18.05
Water		252	13.43	17.41

Data are the means of four replications.
1/ LSD (.05)=113
2/ LSD (.05)=2.17
3/ LSD (.05)=12.66

Table 15. Adjusted means for chemical rates of application on the top growth and the root growth of a Merion-fescue turf treated with nematocides.

Chemical rates	*Adjusted means top growth	**Adjusted means root growth
Double	15.19	22.09
One-half	14.19	20.07
Recommended	15.76	15.24

*LSD (.05)= .34
**LSD (.05)=3.34

Table 16. Results of chemical soil tests from Merion-fescue turfgrass plots treated with nematocides.

Test	Recommended rate	One-half rate	Double rate
pH	6.8	6.6	6.8
Calcium	high	medium	medium high
Potassium	medium high	medium high	medium high
Phosphorous	medium high	high	high
Magnesium	high	high	high

Tests conducted on combined random samples of soil from each area to which different chemical rates were applied.

VIII. Discussion and Conclusions

P. penetrans-infested roots of the three turfgrass species when placed in diazotized sulphanilic acid showed no signs of phenol production. No necrotic lesions of either dermal cells or cortical cells were ever detected during or after nematode invasion.

In the absence of bacteria and fungi just a few P. penetrans were located in the roots of only two Kentucky bluegrass plants out of 120 that were inoculated for histological studies. Large numbers of the nematode were found in the roots of both ryegrass and fescue roots, indicating a difference in susceptibility or tolerance to P. penetrans by the three turfgrass species. There is a possibility that successful penetration and establishment of the nematode in Kentucky bluegrass roots might depend on the presence of other organisms.

The endoparasite P. penetrans was often located next to the endodermis in the roots of both annual ryegrass and creeping red fescue. Neither endodermal nor vascular tissue appeared to be affected by the nematodes. Staining of sections of these tissues also failed to reveal any unusual reactions which might be called nematode injury.

The apparent lack of phenol production and absence of discolored lesions in root tissues of the three turfgrass species in the presence of P. penetrans shows that grasses do not follow the symptom pattern of other plants. Symptom difference might be due to the failure of phenol production

by the grass root tissues that are attacked by the nematodes. It would appear from the histochemical tests that there is a difference in phenol content between the three grasses.

Healthy Kentucky bluegrass roots immersed in diazotized sulphanlic acid, showed little or no color change, indicating the absence of phenolics or possibly they were tied up. Pectinol tests also indicated that Kentucky bluegrass might be less susceptible to disease. Root tissues of the three host grasses did not discolor in the presence of P. penetrans. Either the three grasses did not produce phenolics in the presence of the nematode or the histochemical tests were not indicative of phenol production. The apparent lack of phenol production by the nematode infested grass roots appeared to indicate that the three grasses are not very sensitive to the organism. The damage P. penetrans causes to annual ryegrass, creeping red fescue and particularly Kentucky bluegrass was not visibly evident.

Despite the failure to produce root discoloration and/or lesions, P. penetrans caused a severe necrosis of ryegrass leaf cells. The necrotic area was not tested for phenols but the very dark brown to black discolored tissues suggest their presence. The invasion of the grass leaf cells by the root endoparasite was unusual but it does afford a possibility to better study phenolics and their relation to nematode resistance.

Although the nematodes did not cause necrosis of root tissues, they appeared to tear the cortical parencyhma cells

of all three grass species as they moved through them. Injury to these cells may affect the uptake of nutrients and food storage, particularly under field conditions where these factors are likely to be limiting.

Under aseptic conditions, histological studies failed to show any evidence of feeding injury by T. claytoni on the roots of the three turfgrasses. It is possible that failure to detect root damage was due to self-healing by the cell along with replacement of cellular material as was found for a species of Trichodorous by Chen (6). T. claytoni was observed feeding on the roots of annual ryegrass, creeping red fescue and Kentucky bluegrass but pathogenicity was not established by histological studies.

The results of the first greenhouse experiments revealed no detrimental effects by the nematodes on growth of either annual ryegrass or creeping red fescue. P. penetrans did not appear to either increase the tillering of annual ryegrass or significantly affect green top weight. The average green clipping weights obtained from the nematode-infested plants increased progressively compared to the average clipping weights of the controls (Table 1).

Creeping red fescue grown in boxes and inoculated with P. penetrans was affected in a similar manner to ryegrass but to a lesser extent. The infested creeping red fescue plants tillered more but the clipping weights did not increase with each cutting compared to the controls (Table 1).

Neither annual ryegrass nor creeping red fescue plants

were fertilized after the inital application in order to place a stress on the plants and favor nematode infestation. Additional stress was placed on the grasses by growing them for two weeks in the dark. Even with these stresses, there did not appear to be a difference in plant growth between the nematode-infested boxes of grass and their controls. From this experiment, it would appear that P. penetrans did affect an increase in average green clipping weights of annual ryegrass but did not affect its root weight.

Oven-dried top weights from both annual ryegrass and fescue inoculated with P. penetrans were comparable to the oven-dried weights of their controls (Table 3). Instead of root weights, nematode yields were obtained from the roots of these two grass species. Ryegrass roots yielded about seven times more P. penetrans than fescue roots. The number of nematodes extracted from the soil surrounding fescue was much greater than from the ryegrass rooting media. The total number of P. penetrans associated with the roots of each grass was approximately the same (Table 3).

T. claytoni reduced roots of creeping red fescue significantly and may be considered pathogenic to fescue (Table 4). Kentucky bluegrass supported the largest number of nematodes and appears to be the best host of the three grasses. However, T. claytoni showed no adverse effects on Kentucky bluegrass; instead the top weight of the nematode infested plants was about twice that of the controls (Table 4).

The results from Experiment Three, particularly with

T. claytoni, did not duplicate those in Experiment Two probably because fewer nematodes were recovered in Experiment Three at the end of the tests (Tables 5, 6).

The top weight from T. claytoni infested annual ryegrass was significantly greater than the weight from nematode free plants. Perhaps a smaller number of nematodes could stimulate top growth (Table 5).

Creeping red fescue did not appear to be affected by T. claytoni. In Experiment Three, the roots of Kentucky bluegrass controls outweighed the infested roots; top weight difference was small. In Experiment Two, the tops of the controls outweighed infested tops but there was little difference between root weights.

The pH and fertility were maintained in Experiment Two but not in Experiment Three. These factors could cause varying results.

In Experiment Three, the top and root weights of both Kentucky bluegrass and creeping red fescue did not appear to be affected by P. penetrans. The root weight of nematode-infested annual ryegrass was significantly lower when compared to the controls. The greater number of nematodes recovered from ryegrass roots in both experiments compared to that obtained from the roots of the other grasses indicates that ryegrass is a better host.

The greenhouse experiments indicate that both nematode species are pathogenic or at least affect grass growth. The three grass species seem to be affected by the amount of

inoculum and environmental factors. These effects are slight particularly when grass is growing well.

Potassium, calcium, magnesium and phosphorous uptake by the turfgrass species did not appear to be affected by either P. penetrans or T. claytoni (Tables 7, 8, 9, 10).

Some of the nematocides applied at each dosage rate did reduce the nematode population. Most of the nematocides were more effective when applied at double the recommended dosage rate. The oven-dried clippings from each of four areas receiving double doses of Niagara 9227, Nemagon, Diazinon A1619 and Diazinon 500 weighed less than the clippings from those treated at the recommended rate.

Bayer 25141 applied at the recommended and at double the dosage rate was the only nematocide that significantly increased the average clipping weights (Tables 12, 14). The mean clipping weight from all plots was significantly increased by fertilizer, although the mean number of parasitic nematodes recovered from these plots was higher than those from most nematocide-treated areas. Under the conditions of this experiment, a reduction of parasitic nematodes by nematocides did not necessarily increase turf clipping weights while fertilizer applications always increased grass growth.

Nemagon, Diazinon A1619, Diazinon 500 and SKF - which reduced the number of parasitic nematodes when applied at double the suggested rate did not appear to effect an increase in grass top growth but did appear to increase turf root weight (Table 14).

Plots treated with Diazinon 500 at one-half the suggested rate yielded fewer nematodes and lighter root weights than plots to which the nematocide was applied at twice the rate. The number of parasitic nematodes recovered from plots treated with Diazinon A1619 at the recommended rate was less than from plots with one-half dosage, yet root weights taken from these plots were about the same. Bayer 25141, Meta Systox R and Shell SD 7727 at one-half the dosage rate yielded some of the highest root weights but not the lowest number of parasitic nematodes.

There was also no correlation between the nematode counts and root weights from the plots which received fertilizer. Apparently the fertilizer was used by the plants for the production of top growth rather than root growth. These inconsistencies in nematode counts, root weights and top growth indicate that the stimulation of grass growth was not entirely related to a decrease in parasitic nematodes.

There are a number of possible reasons for the discrepancies in the results. At the lowest dosage rate, some of the nematocides may stimulate the growth of roots and increase their weight. Certain nematode species in some turf plots may not be affected by the nematocides and thus cause differences in root weight. Still another possibility, as proposed by Hollis (19), is that stimulation of turfgrass growth may have been brought about by the effects of soil fertility and its interaction with the nematocides. The fertility of all the plots was fairly high and the applica-

tion of some of the chemicals may have increased the availability of nutrients for plant utilization. It is also possible that the nematocides reduced parasitic nematodes and at the same time increased nutrient availability both of which could have stimulated plant growth. The difficulty encountered in trying to determine the effect of nematodes on turfgrass grown in the field points out the need to control many variables if proof of nematode effects on turf is to be established.

IX. Summary

Experiments were conducted to investigate the pathogenicity of Pratylenchus penetrans and Tylenchorhynchus claytoni on annual ryegrass, creeping red fescue and Kentucky bluegrass.

Grass hosts were selected from fourteen species on the basis of their reaction to an application of diazotized sulphanilic acid (DSA) and to pectinol.

P. penetrans were observed at all root regions except the root cap of the three grasses. Fescue and ryegrass roots grown aseptically were readly penetrated and supported large numbers of P. penetrans. Root lesions and discoloration of the root tissues of the three grass species were not apparent. Applications of DSA to nematode infested roots failed to indicate the presence of large concentrations of phenols. Microtome sections of fescue and ryegrass roots infested with P. penetrans showed nothing more than the tearing of cortical parenchyma cells by the nematode.

T. claytoni was observed probing all root areas of the three grass species but nematode injury to the roots was not discernable.

Neither P. penetrans nor T. claytoni affected the uptake of potassium, magnesium, calcium and phosphorous.

The top growth of creeping red fescue, annual ryegrass and Kentucky bluegrass inoculated with either P. penetrans or T. claytoni did not appear to be affected by the nematodes.

The lower dried root and top growth weight of inoculated plants compared with non-inoculated controls indicated that T. claytoni was pathogenic to fescue. P. penetrans significantly reduced annual ryegrass root weight; T. claytoni appeared to increase ryegrass top growth.

Nematocides applied to turf containing a mixture of Merion bluegrass and creeping red fescue reduced the number of parasitic nematodes.

The clipping weights from plots receiving Bayer 25141, at the recommended rate and at double the dosage, were significantly increased. The top growth from all plots receiving fertilizer outweighed the clippings from any of the nematocide treatments. Root growth also was stimulated by some of the nematocides.

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XI. Appendix

A. The Ingredients of Nutrient Agar Used for Culturing Nematodes (Krusberg, 1960)

	Conc. stock solution	Per liter dilute agar
$Ca(NO_3).4H_2O$	25 gms/100 ml	2 ml
KNO_3	6.25 gm/50 ml	1 ml
$MgSO_4.7H_2O$	6.25 gm/50 ml	1 ml
KH_2PO_4	6.25 gm/50 ml	1 ml
H_3BO_3	0.05 gm/50 ml	1 ml
$FeSO_4.7H_2O$	330 mg/100 ml	1 ml
Micronutrient solution*		1 ml
Vitamin Mixture**		1 ml
Sucrose		20 grams
Agar		15 grams
2,4-dichlorophenoxy-acetic acid	200 mg/100 ml	1 ml

*Micronutrient solution	Per 1000 ml stock
1000 ppm $MnCl_2.4H_2O$	3.602 gm
100 ppm $ZnSO_4.7H_2O$	.443 gm
40 ppm $CuSO_4.5H_2O$	.175 gm
100 ppm $Na_2Mo_2O_4$	.254 gm

**Vitamin Mixture	Per 100 ml stock sol'n
Glycine	300 mg
Niacin(Nicotinic acid)	50 mg
Pyridoxine. HCl	10 mg
Thiamin. HCl	10 mg

B. Ingredients of Nutrient Agar for Growing Grass Species (Bredakis, 1959)

	Per liter water
CaCl	30 mg
$MgSO_4.7H_2O$	90 mg
$(NH_4)_2\ HPO_4$	80 mg
KCl	30 mg
Agar	20 grams
Micronutrient solution*	20 cc

Micro elements	Per liter stock sol'n	*Per liter diluted stock sol'n
Citric acid	2.20 grams	55.0 mg
$FeSO_4.7H_2O$	2.50 grams	62.5 mg
$MnCl_2.4H_2O$	1.80 grams	45.0 mg
H_3BO_4	1.44 grams	36.0 mg
$ZnSO_4.7H_2O$	1.10 grams	27.5 mg

C. Scientific Names and Synonomy of the Two Nematode Species Used in This Investigation.

Present Scientific Name	Synonym
*Tylenchorhynchus claytoni Steiner, 1937.	Tylenchus dubius Butschli, 1873.
**Pratylenchus penetrans (Cobb, 1917) Filipjev and Schuurmans Stekhoven, 1941.	Tylenchus penetrans Cobb, 1917.
	Auguillulina pratensis in Goodey, 1932 and 1933; W. Schneider, 1939.
	Pratylenchus pratensis in Filipjev, 1941; Goodey, 1951.

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