SELECTED PLANT PATHOGENS ASSOCIATED WITH BENTGRASS PRODUCTION AND PLANT GROWTH RESPONSE TO CERTAIN CHEMICAL TREATMENTS

By

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CHAPTER I

INTRODUCTION

Oklahoma has approximately 150 golf courses with millions of dollars worth of bentgrass (Agrostis spp.) putting greens. An average sized putting green alone has a value in the vicinity of ten thousand dollars. The enormous value of bentgrass makes it imperative that research on pathogenic organisms be carried out in such a manner that statistically valid data can be obtained. Sampling prior to chemical applications is necessary to determine the extent and locus of population variation within the test area. Such preliminary sampling is a prerequisite to determining the most suitable experimental design for extracting maximum statistically valid data from the study.

During the previous year, 1975, research on control of the nematode-soil fungus disease complex on bentgrass, using a nematicide and soil fungicide, showed a very beneficial response. Following a short period of drought in September, bentgrass not receiving treatment completely died out as did an adjacent non-treated plot. This study was set up on the non treated plot for the purpose of identifying the relationship of the various nematodes and fungi to the total disease complex.

The objectives of this study were:

1. To determine if the nematode population in 'Penncross' bentgrass (Agrostis palustris Huds.) was uniform enough within the test area to permit a statistically valid pesticide study.

2. To attempt to correlate nematode populations with density of 'Penncross' bentgrass.

3. To determine the fungi present within the test area.

4. To determine if a commonly used soil fungicide, nematicide, or a combination of the two elicit any growth response from 'Penncross' bentgrass in the absence of pathogenic organisms.

5. To attempt to define the host-parasite relationships of the various nematode genera recovered from the field uniformity study.

6. To determine at what soil horizons parasitic nematode populations occurred on 'Penncross' bentgrass.

CHAPTER II

REVIEW OF LITERATURE

Nematodes

Ornamental turf producers are becoming increasingly aware of nematode damage to turf. Nutter (72) pointed out that nematode damage to turfgrasses was already widespread in 1955.

Although the pathogenicity of various nematodes has been demonstrated repeatedly, a consistent, clearly defined correlation of nematode populations with turf damage is difficult to obtain (74). It is unlikely that it will ever be possible to assign a universal virulence index to any turf nematode due to varying test conditions. Factors which interfere with uniform evaluation of pathogenic response include: dissimilar nematode extraction techniques, soil types, climatic conditions, different nematode species, biotypes within a species, and the differential influence of consociate pathogenic species.

Tylenchorhynchus spp.

Tylenchorhynchus spp. have been found repeatedly to be one of the most abundant nematodes on turf (62, 71, 77, 91, 98, 99, 101). Taylor et al. (99), in surveying bentgrass putting greens in Illinois, found that in all but two samples from 26 greens, Tylenchorhynchus spp. were the most abundant of the parasitic genera. Using a modification of the

Christie and Perry extraction method (11), the average number recovered was 284 per 125 cc soil sample with the highest population greater than 1000/125 cc (99).

Troll and Rhode (102) found that creeping red fescue, inoculated with 5000 nematodes of T. claytoni Steiner per pot, had a significantly reduced root weight. However, there appeared to be considerable (although not statistically significant) increase in foliage weight of infected Kentucky bluegrass as compared to the controls. The average dry root weight was about half that of the controls.

Johnson (45) reported significant (.05 level) reduction in root weight of four varieties of bermudagrass inoculated with 1800 nematodes of T. martini Fielding per pot.

Laughlin and Vargas (54) demonstrated the pathogenic potential of T. dubius (Butschli) Filipjev on 'Toronto' creeping bentgrass. Both foliar and root weights were significantly reduced at inoculum densities of 500 and 1000 nematodes per pot. Sikora et al. (90), showed that T. agri Ferris reduced root growth of 'Toronto' creeping bentgrass, but only when co-inoculated with Pratylenchus penetrans was top growth adversely affected.

Criconemoides spp.

In assays of turf from Georgia, showing typical nematode damage, Johnson (47) consistently recovered high populations of Criconemoides spp. Parris (77) reported several genera of nematodes, including Criconemoides spp., to be the cause of turfgrass decline. Somerville (91) examined turf samples from 20 states and reported Criconemoides spp. as the sixth most prevalent nematode found. Good et al. (37, 38), stated

that Criconemoides spp. were found frequently on turf in Florida and Georgia, but seldom in large numbers.

Taylor et al. (99), reported only three samples from 26 bentgrass greens in Illinois had high populations of Criconemoides spp. However, Lucus et al. (62), recovered high populations of C. ornatus Raski from several bentgrass putting greens in North Carolina.

Johnson (47) demonstrated reduced root growth of Centipedegrass and Tifgreen bermudagrass inoculated with C. lobatum Raski. C. ornatus has been shown to reduce root weight of bermuda also, although not statistically.

Paratylenchus spp.

Paratylenchus has been found frequently in turf, however, there is little information in the literature to substantiate its influence on declining turf. Parris (77) did include Paratylenchus spp. as one of several genera associated with turf decline. Coursen and Jenkins (20) demonstrated shortening of internodes, underdeveloped lateral roots, and 31% greater tillering of fescue inoculated with 10,000 nematodes of P. projectus Jenkins. Other workers (37, 62, 64, 77, 99) have reported the occurrence of Paratylenghus spp. on turf, but pathogenicity has been been proven only in a few instances.

Other Genera

Additional genera which have been found to parasitize turf include: Heliocotylenchus (62, 64, 72, 91, 98, 99); Hoplolaimus (24, 53, 64, 77, 91, 99); Pratylenchus (62, 64, 77, 91, 99, 101); Trichodorus (38, 62, 64, 72, 77, 81, 82, 101); Xiphinema (37, 62, 64, 77, 91); Longidorus (64);

Belonalaimus (24, 62, 72); Tylenchus (64); and Psilenchus (64).

Troll and Rhode (102) stated that root weight of annual ryegrass inoculated with Pratylenchus penetrans was significantly less than controls.

Rhodes (81, 82) demonstrated that both Trichodorus christiei Allen and T. proximus Allen caused stubby root symptoms and significantly reduced the root weight of St. Augustinegrass in greenhouse pathogenicity trials.

Perry (79), in greenhouse experiments, found that decline and root injury of Kentucky bluegrass in Wisconsin was caused by Helicotylenchus spp.

Xiphinema spp. have been found in several samples from declining turf, but have not been implicated as serious parasites of turf.

Although plant parasitic nematodes have been frequently associated with turf injury, very little experimental evidence has been published on damage caused by specific nematode species parasitizing specific grasses.

Nematode Control in Turf

Nematode control enables grass to develop and maintain more vigorous root systems which during periods of stress can greatly influence the amount of damage incurred. Powell (80) demonstrated on a nematode infected lawn that vigor did not improve following fertilization, however, marked improvement was noted following treatment of DBCP (Nemagon). Nutter (73) noted improved turf condition after treatment with the following nematicides: DBCP, Nemakril, and VC-13. There was no clearcut correlation between decline of nematode populations and

increase in plant vigor.

Johnson (44) using Dasanit, significantly suppressed populations of Pratylenchus spp. for five months and Xiphenema americanum Cobb for two months. The most significant reduction of Trichodorus christiei occurred after application of a high rate of Dasanit. Wolford and Sturgeon (110) and Sturgeon and Jackson (96, 97) reported reduced nematode populations in turf following applications of Dasanit.

Troll and Rhode (103) demonstrated a significant increase in clipping weights of a Kentucky bluegrass-creeping red fescue mixture using Dasanit. Dasanit, applied at manufacturer's suggested rate and at double dosage rate, was the only nematicide that significantly increased the average clipping weights. The plots were infested with nematodes belonging to the genera: Pratylenchus, Tylenchorhynchus, Paratylenchus, Criconemoides, and Tylenchus. There was no correlation between the nematode counts and root weights from the plots that received fertilizer. They concluded that the stimulation of grass growth was not entirely related to a decrease in parasitic nematodes.

Hollis (41) proposed that stimulation of turfgrass growth may have been brought about by the effects of soil fertility and its interaction with the nematicides. Brodie and Burton (5) have also indicated a growth response of turf following application of organophosphate compounds was evident before significant nematode population reduction occurred. DiSanzo (25, 26) recovered higher populations of endoparasitic nematodes from soil treated with carbofuran (Furadan) than from untreated soil. Further study suggested that carbofuran inhibited the plant-parasitic nematodes activity primarily by affecting the orientation and feeding mechanisms.

In Vitro Feeding

Difficulties in the examination of nematodes in association with their host are obvious in the case of rhizosphere nematodes. Root observation boxes were first described by Dean (22) in 1929 in which roots could be observed quite readily through a glass side panel. However, this technique does not lend itself to close microscopic observation. In 1940, Linford (58) described a miniature root observation box which proved more effective where frequent microscopic studies were required. This allowed better observation of nematode activities in the rhizosphere than had previously been possible. However, observation at high magnifications was not possible, and little detail other than nematode body position could be observed due to the above stage lighting required.

Byars (9), trying to determine why certain plants are resistant to Heterodera radicicola (Greef) Muller, developed a technique involving an agar medium poured into test tubes and subsequent addition of a seedling tomato or cowpea. After the seedlings had grown for a day or more, H. radicicola larvae were transferred to the test tube. Higher magnifications were possible using this technique than with the root observation box and substage lighting permitted more detailed observations of the nematode's activities. Magnifications greater than 100x were not possible using this technique due to distortion caused by test tube thickness and tube curvature.

Since Byar's description of this technique, many modifications have evolved (55, 56, 59, 60, 70, 78, 86, 87, 107). The most widely used techniques are those described by Krusberg (53) and Loewenberg et al. (61), which involve petri dishes of hard agar inverted on the stage of

the microscope and viewed through the dish bottom. This technique has the disadvantage of viewing through the thick glass which does not allow observation at greater magnifications than 100x. Rhoades and Linford (83) developed the techique of observing the namatodes directly through the agar, using a 40x water immersion objective, as opposed to viewing them through the petri dish bottom. Bacterial contamination greatly reduced the longevity of the cultures and they were of little value after about one week.

Russell and Morrison (86) developed a technique in which a thin layer of agar in a petri dish was covered with a plastic film. The film was sealed in place with wax and the plate was inverted to promote root growth against the plastic film. The use of the plastic film reduced both contamination and dehydration of the agar and allowed direct observation of the nematodes in association with the root at magnifications up to 1000x.

In vitro study has accommodated the researcher in determining the feeding habits of nematodes among many other things. Christie (10) reported the histological study of the development of root-knot nematode galls which Linford (56) later, through in vitro study, determined to be in error. This is only one such example in which in vitro studies have aided the researcher.

Fungi

Root and foliage systems inhabit two quite different environments. Both environments together affect the health and vigor of the plant. Because these two environments are so different, root-infecting fungi constitute a natural ecological group of pathogens that are distinct

from air-born fungi causing foliar diseases. Thatch has a very great influence on both root and foliar diseases. Thatch, which is the accumulation of partially decomposed stems and leaves, is an extremely good inoculum source for many of the diseases affecting turf.

Pythium spp.

Pythium disease ('damping off,' 'grease spot,' or 'pythium blight') caused by species of the Pythium fungus is one of the most destructive of the cool season grasses, especially bentgrass (Agrostis spp.) (36, 66). Pythium blight appears as circular spots ranging in size from less than an inch to several inches (16, 17). This fungus is capable of entirely destroying established turf within 24 hours from the onset of environmental conditions favorable to disease development (17). In the early morning hours and during periods of high humidity, the leaves of diseased plants may be covered with the white cobwebbed mycelium of the fungus (17, 52). Generally, pythium blight is regarded as a hot weather disease with extremely rapid killing of turf at temperatures of 85 to 95 F (2, 66, 67, 68, 69).

Although extensive research has been undertaken on foliar blighting of bentgrass, little has been worked out on the disease of underground plant parts. Pythium spp cause severe damage to the root systems of cereals and grasses, as reviewed by Sprague (93, 94). The most important fungus causing root rot of grasses in North Dakota was P. arrhenomanes Drechsl (93). There are many reports of root damage and root rots to cereals and grasses caused by several Pythium spp. (6, 7, 8, 88, 92, 95, 104, 105).

A number of studies have shown Pythium-nematode interactions.

Inoculation of corn roots with Tylenchus agricola Steiner or Tylenchorhynchus claytoni Steiner and P. ultimum Trow resulted in less damage to the roots than when P. ultimum Trow alone was used (49). Neither Criconemoides quadricornus Raski nor P. irregulare Buisman caused disease of pecan roots at 27 C., but, when combined, reduced root weight by 50% (42). Root rot of chrysanthemum caused by P. aphanidermatum increased when Belonolaimus longicaudatus Rau or Meloidogyne incognita (Kofiod and White) Chitwood was present (46).

Schmitthenner (89) stated that Pythium spp. are capable of causing disease only when (assuming suitable host and temperature) the propagules are active, there is a sufficient amount of readily available nutrients, and the host is in a susceptible condition. All these conditions are met under high soil moisture (decreased oxygen) conditions. Moore et al. (68, 69), reported that of all environmental factors studied, variations in calcium nutrition, particularly a calcium deficiency, had the most pronounced influence on susceptibility of Highland bentgrass to pythium blight.

Fusarium spp.

Fusarium blight of turfgrass incited by Fusarium roseum (LK.) emend. Snyd. and Hans. F. sp. cerealis and Fusarium tricintum (Cda.) Snyd. and Hans. F. sp. poae was initially described by Couch and Bedford (18). Characteristic symptoms of the disease are dead areas which may be crescent-shaped, streaked, or in circles with a patch of live grass in the central portion.

Bean (1) stated that the fungus has both a crown rot and leafspot phase. He postulated that destruction of the crown area was the

important phase.

Couch and Bedford (18) reported that, in greenhouse studies, the bentgrasses are the most susceptible followed by Kentucky bluegrass and red fescue; however, Bean (1) found that fusarium blight is primarily a disease of bluegrass fairways and generally not a problem on bentgrass, and is probably due to more frequent fungicide applications to bentgrass greens. Endo (32) stated that Fusarium spp. were frequently associated with species of Curvularia, Helminthosporum, and Rhizoctonia on turf in Southern California. Disease severity has been positively correlated with high light intensity, high soil temperature, and drought stress (1, 2, 15, 18, 22).

Nematodes have frequently been associated with fusarium blight (13, 18, 32, 109); however, Couch and Bedford (18) obtained many samples from turf that were apparently free of plant parasitic nematodes in Pennsylvania. Vargus and Laughlin (106) reported that Tylenchorhynchus dubius may play a dominant role in the development of fusarium blight in Michigan. From greenhouse studies, Fusarium-nematode combinations did not increase severity significantly over the nematodes alone. Thus, the nematodes seemed to be the dominant pathogen.

Curvularia spp.

'Fading out' caused by Curvularia spp. is seldom a problem of vigorous growing turf. The disease occurs more frequently in warm weather characterized by a general yellowing or fading-out of the turf in small areas and can be confused with Helminthosporium diseases, chemical injury, drought, and nutrient deficiencies (109).

Helminthosporium spp.

The helminthosporium disease is caused by several different species. Wise (109) stated that the disease usually occurs in two stages, one affecting the leaf, the other the crown and roots. The early symptoms of the disease are small, purplish-brown specks on the leaf blade which enlarge into oblong spots or lesions. The tissue in the center dies, becoming tan or straw-colored with a dark border around the periphery. In severe cases, the infection spreads to the stem and roots causing a very destructive root rot.

Bean and Wilcoxon (3) demonstrated that *Helminthosporium vagans* Drechs., H. *sativum* P. K. & B., and *H. dictyoides* Drechs. were pathogenic on roots of bluegrass. In 1923, *H. stenacrum* Drechs. was reported to be associated with a withering of creeping bentgrass blades of *Agrostis stolonifera* L. (28). Also, in 1923, Drechsler (27) reported that *H. giganteum* Heald and Wolf was observed parasitizing creeping bentgrass. Leaf blighting of bentgrass caused by *H. giganteum* was again referred to in 1928 (29) and further confirmed in 1929 (30).

In 1935, Drechsler (31) described a leaf spot on bentgrass caused by *H. erythrospilum* Drechs. In dry weather, local lesions are less evident when infected leaves often wither such that would suggest drought. Couch (16, 17) has also attributed leaf withering to *H. erythrospilum* especially during July and August. Klomparens (51) reported *H. sativum* to be extremely destructive to creeping bentgrass. Endo (32) found *H. sorokinianum* (*H. sativum* P. K. & B.) to be the most common and destructive fungus isolated from turfgrasses in southern California. Healy and Britton (40) reported *H. sorokinianum* to be the

only species of Helminthosporium isolated from bentgrass putting greens in central and northern Illinois during 1963 and 1964, in which, both blighting and leaf withering occurred.

Sclerotinia homoeocarpa F. T. Bennett

Dollar spot, as the name implies, occurs as dead or bleached spots in turf about the size of a silver dollar. The dead areas rarely enlarge beyond their original size; however, they may become so numerous that overlapping occurs and produces a large area of dead turf.

Dollar spot is widespread in the United States with the exception of the coastal region of the Pacific Northwest (39). Bennett (4) in 1937, described the dollar spot disease caused by Sclerotinia homoeocarpa F. T. Bennett. The wide temperature range (20 to 30 C.) in which dollar spot is active is, in part, responsible for its wide distribution (32, 33).

Most varieties of bentgrass are susceptible to dollar spot (12, 14, 32); however, Cole (12) found that Colonial bentgrasses were partially resistant. Low fertility has been demonstrated by several workers to increase disease severity (19, 34, 35, 65).

Rhizoctonia solani Kuhn

Brown patch caused by Rhizoctonia solani Kuhn is one of the major diseases of turfgrasses in the United States, especially in areas characterized by extended periods of high temperatures and high atmospheric humidities (16, 17). Symptoms appear as light brown areas of dying grass, irregular and circular in shape, a few inches to several feet in diameter. This fungus, under proper conditions, can completely destroy a bentgrass putting green within a very short time.

Close mowing as practiced on putting greens is an important factor in the development of the disease. Rowell (85) showed that uncut bentgrass had very little disease as compared to closely cut bentgrass which showed severe disease.

Dahl (21) reported the occurrence of brown patch varied directly with temperature. The disease occurred 82% of the days that the minimum temperature was above 21 C. Dickinson (23) stated that infection appears to require a rather rapid rise in air temperature from 17-20 C. to between 26-29 C. Lukens and Stoddard (63) observed leaf wilt on Connecticut golf greens during hot summer days, although adequate soil moisture was present. The wilted grass was found to be heavily infected with R. solani.

CHAPTER III

GREENHOUSE TEST ON PLANT RESPONSE TO CHEMICALS

The purpose of this study was to determine if a nematicide, fungicide, or a combination of the two had a growth effect on Agrostis palustris, 'Penncross' creeping bentgrass. The pesticides chosen for this study were O, O Diethyl O [p - (methylsulfinyl) Phenyl] Phosphorothidate (Dasanit), a nematicide, and Pentachloronitrobenzene plus 5 - Ethoxy - 3 - (trichloromethyl) - 1, 2, 4, - thiadiazole (Terraclor Super-X), a fungicide.

Methods and Materials

Penncross creeping bentgrass was seeded uniformly in 1,000-ml plastic pots and covered to within 1 cm of the top with heat sterilized Kirkland Silt Loam soil. The pots were then placed in trays of water until the moisture reached the surface by capillary action. After the seeds had germinated and the seedlings were growing, 52 pots of approximately uniform density were selected for the test. The design was a randomized block utilizing four replications of each treatment.

The bentgrass was planted January 23, 1976, and clipped weekly to ensure a dense growth. Beginning February 25, 1976, foliage clippings were taken every four days in which both the wet foliage and oven-dry foliage weights were obtained. Each pot of bentgrass was rated for color, using a scale from one to nine as shown in Table I.

TABLE I

CHART FOR RATING BENTGRASS COLOR

Color	Plate	Ref. No.	Rating
Dark Viridian Green	VII	37-K	9*
Viridian Green	VII	37-I	8
Vivid Green	VII	37--	7
Cendre Green	VI	35-B	6
Emerald Green	VI	35--	5
Neva Green	V	29--	4
Green-Yellow	V	27-B	3
Light Greenish Yellow	V	25-B	2
Oil Yellow	V	25-I	1**

*Top color rating.

**Low color rating.

SOURCE: R. Ridgway, Color standards and color nomenclature (1912), Washington, D.C., Plate V-VII.

Fifty three days after planting a noticeable drop in color rating and foliage weight indicated a nutrient deficiency. (In order to determine if the pesticides used could interact with the nutritional elements in the soil making them more available, as proposed by Hollis (41), low nutrient levels were necessary.) On March 16, the chemicals were applied to the various treatments which were then drenched with 1.27 cm. of water using a squeeze bottle held to a height that would simulate sprinkler irrigation. Chemicals, rates of formulation, and corresponding treatment numbers are presented in Table II. Eight days after chemical application, when no significant increase in foliage weight or color rating was observed, fertilizer (Ortho-Gro Liquid Plant Food, 12-6-6) was applied at the rate equivalent to 226.8 grams per 93.025 square meters (0.5 lb./1,000 sq. ft.). A second application of fertilizer was made at the same rate 12 days after the first application.

Results and Discussion

A uniformity trial was carried out prior to chemical application to determine if there were any extraneous affects from the greenhouse environment. There was no significant difference among clipping weights at the .05% level, although significant differences were observed between sample dates in both wet and dry weights (Figs. 1 and 2). Greater differences were observed in dry weights than wet weights of foliage between cutting dates. These differences did not seem to present a problem and were thought to be due to growing conditions such as moisture, temperature and/or variation in clipping times.

The coefficient of variation (C.V.) was less in the wet foliage

TABLE II

FORMULATION AND RATES OF CHEMICALES USED
IN GROWTH RESPONSE TEST

Treatment	Chemical and Formulation	Rate/93.025 sq. m	
		Formulation	Active Ingredient
	Terraclor Super-X		
1	TSX 10-2.5 G	2.27 kg	.227 kg
2	TSX 10-2.5 G	4.54 kg	.454 kg
3	TSX 10-2.5 G	9.08 kg	.908 kg
4	TSX 10-2.5 G	13.62 kg	1.362 kg
	Dasanit		
1	Das 15 G	.754 kg	.113 kg
2	Das 15 G	1.49 kg	.224 kg
3	Das 15 G	2.99 kg	.448 kg
4	Das 15 G	4.49 kg	.673 kg
	Terraclor Super-X/Dasanit Combination		
1	TSX/Das 10-2.5-15 G	3.02 kg	.340 kg
2	TSX/Das 10-2.5-15 G	6.03 kg	.678 kg
3	TSX/Das 10-2.5-15 G	12.07 kg	1.356 kg
4	TSX/Das 10-2.5-15 G	18.11 kg	2.035 kg

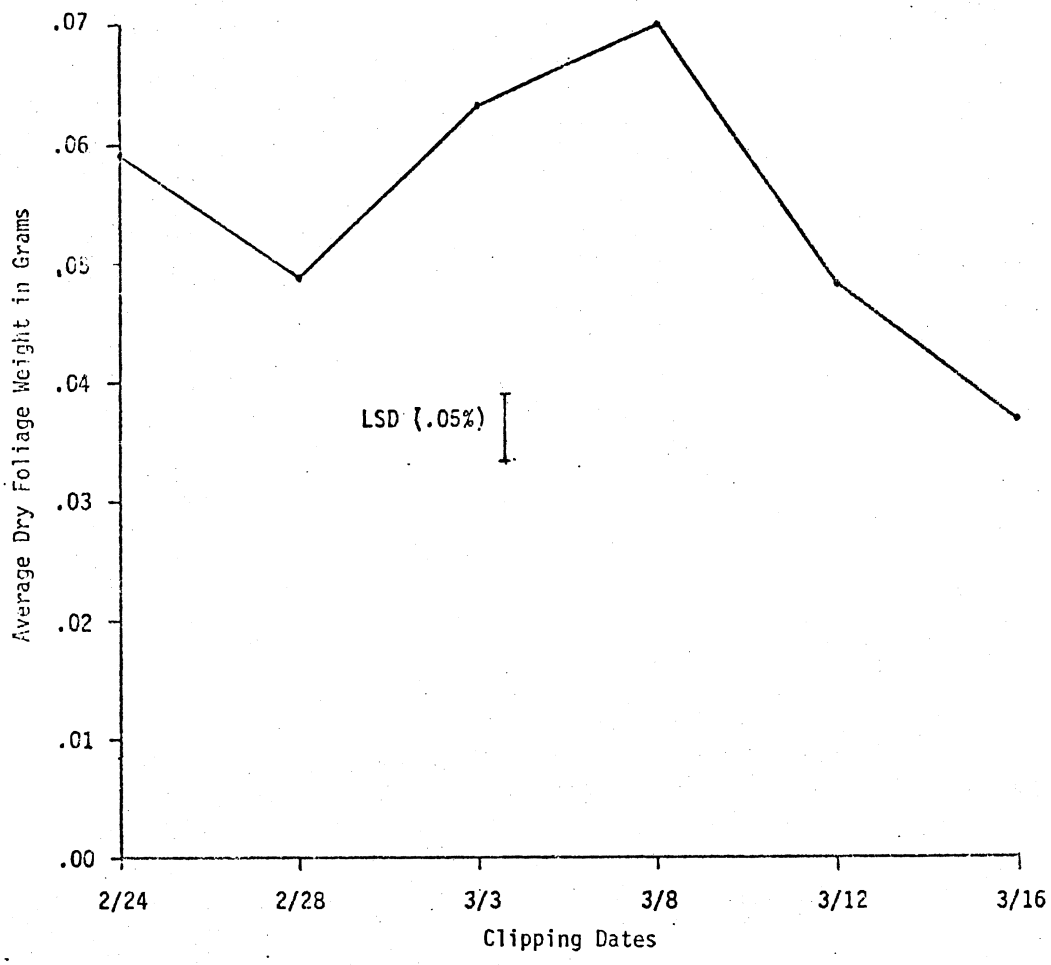


Fig. 1. Average Dry Foliage Weight Showing Variation Between Cutting Dates

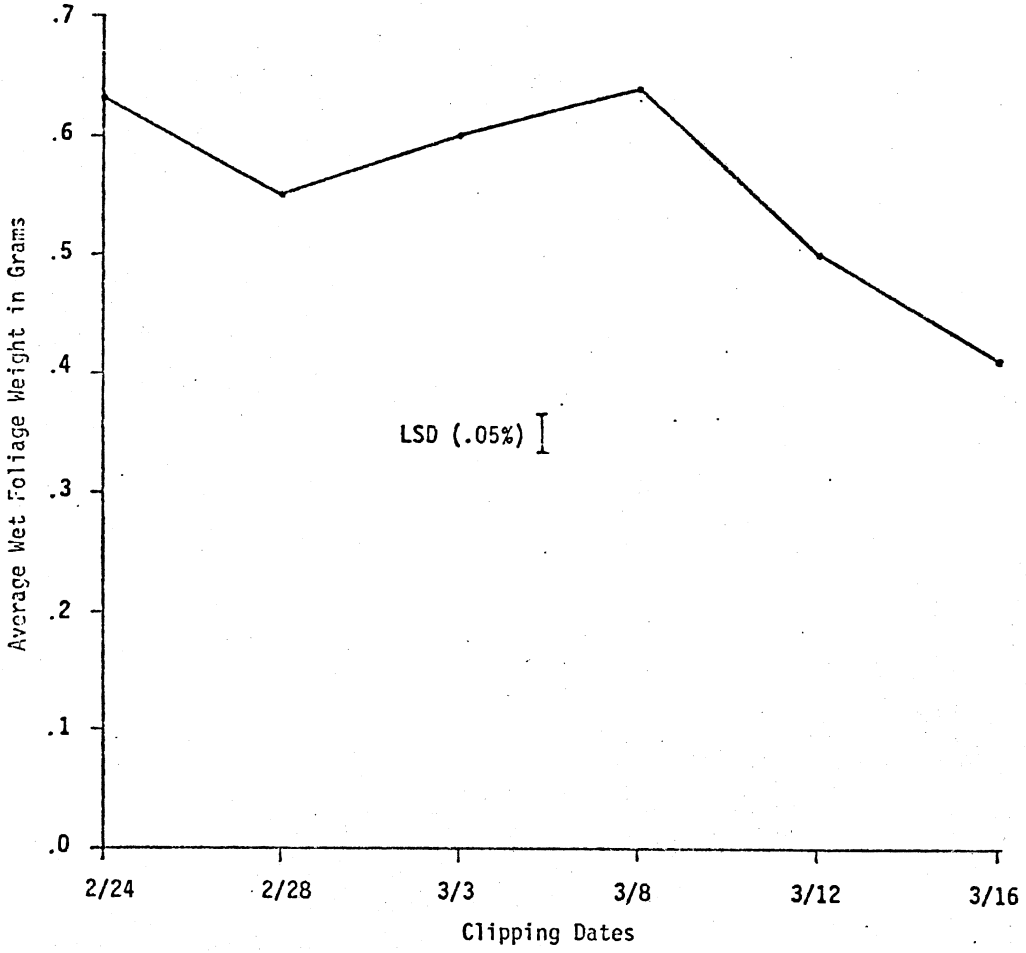


Fig. 2. Average Wet Foliage Weight Showing Variation Between Cutting Dates

clippings than in dry foliage, however, both generally decreased with time (Fig. 3). Because the C.V.'s were lower in the wet foliage weights, analysis was run only on the wet weights for the remainder of the study.

The color rating decreased with time (Fig. 4) which was one of the criteria, along with decreased clipping weights (Figs. 1 and 2), that dictated when the chemicals were to be applied.

Four days after application of the chemicals, wet foliage weight from bentgrass receiving Terraclor Super-X generally decreased with increasing rates of chemicals. However, treatment #3 had a substantial increase over the other Terraclor Super-X treatments as well as the check (Fig. 5). Bentgrass receiving the various rates of Dasanit generally decreased with increased chemical rates. However, foliage weights from treatment #2 increased notably over the other Dasanit treatments and the check. Increased wet foliage weights, over the check, were obtained from treatments #1 and 2, which received the combination of Terraclor Super-X and Dasanit, but decreased in treatments #3 and 4. Dasanit alone, suppressed the growth of the bentgrass more than Terraclor Super-X alone, and the combination of Terraclor Super-X and Dasanit, except for treatments #1 and 2, had an even greater suppression effect than either chemical alone at comparable rates.

Increased clipping weights were obtained from all treatments receiving Terraclor Super-X except for treatment #2, which decreased eight days after chemicals had been applied (Fig. 6). Of the treatments receiving Dasanit, only treatment #4, which was the high rate, had a slight decrease in wet foliage weight over the check. Treatments #1 and 2, which received the combination of Terraclor Super-X and Dasanit, had increased wet foliage weights over the check with decreases in treatments

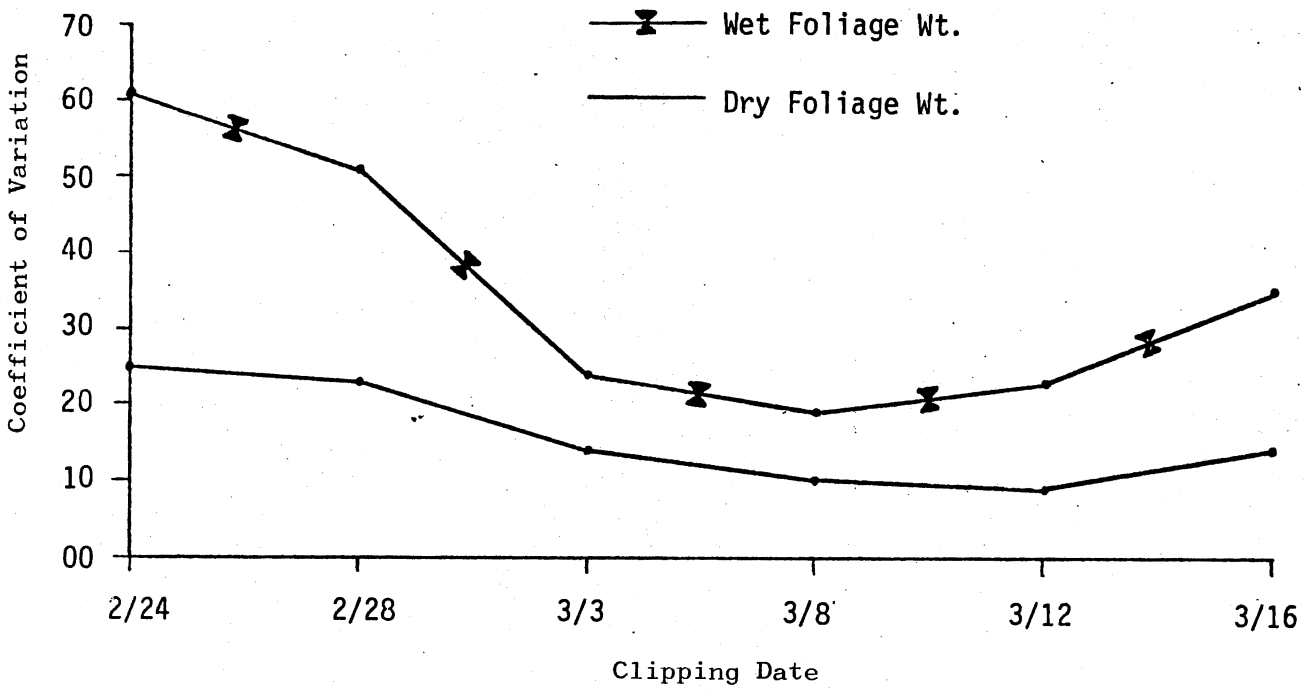


Fig. 3. Decline in Coefficient of Variation With Respect to Time

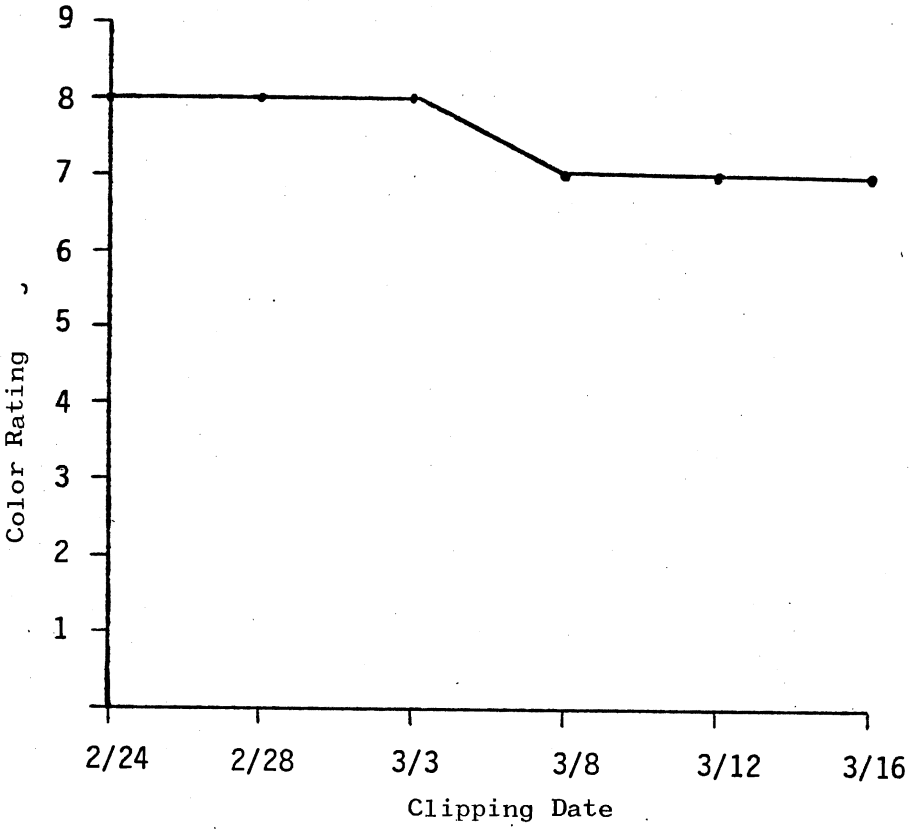


Fig. 4. Decline in Color Rating With Time on 'Penncross' Bentgrass

#3 and 4. Bentgrass treated with Terraclor Super-X or Dasanit alone, generally increased in wet foliage weights, even at the higher rates of chemical application. It was only at the higher rates (treatments #3 and 4) of the combination that suppression of growth was observed.

Twelve days after chemical application and four days after fertilizer [226.8 gm. (12-6-6)/93.025 sq. m.] was applied, all treatments had a notable increase in wet foliage weights (Fig. 7). Wet foliage weights decreased with increased rates of Terraclor Super-X except for treatment #3 which was still considerably less than the check. All treatments receiving Dasanit and the combination of Terraclor Super-X and Dasanit had progressively less foliage weight as the rates of chemicals increased. Compared to the check, all chemical treatments suppressed growth, especially at the higher rates.

The same trend was indicated by the foliage weights taken 16 and 20 days after chemical application and eight and 12 days after fertilizer had been applied (Figs. 8 and 9). Foliage weights of all treatments decreased with the increased rates of the chemicals.

Recovery from the suppression effect of Terraclor Super-X was indictated by the wet foliage weights 24 days after the chemicals had been applied and 4 days after the second fertilizer application (Fig. 10). Treatment #1, receiving the low rate of Terraclor Super-X, had a substantial increase in wet foliage weight over the check. The treatments receiving the higher rates of Terraclor Super-X were only slightly lower than the check. Treatments receiving Dasanit alone, did not show any signs of recovery. However, the treatment receiving the low rate of Terraclor Super-X and Dasanit combination (treatment #1) had an increase in foliage wet weight over the Dasanit alone, at the low rate.

Suppression of growth was still quite evident using the higher rates of Dasanit and the combination of Terraclor Super-X and Dasanit.

The same trends were noted twenty-eight and thirty-two days after the chemicals had been applied and eight and twelve days after the second application of fertilizer (Figs. 11 and 12). All treatments generally held their relative position with respect to wet foliage weights. All treatments receiving Terraclor Super-X alone had almost completely recovered from the suppression evidenced earlier. The Dasanit treatments did not recover as fast as the Terraclor Super-X treatments, although there was some recovery relative to the check. The low rate of Terraclor Super-X and Dasanit combination (treatment #1) was only slightly lower than Dasanit (low rate) alone, however, the higher rates of both were still substantially lower than the check.

Results of the wet foliage weights, before fertilizer applications were made, indicate that there may be an interaction between not only Dasanit, but also Terraclor Super-X and the nutritional elements in the soil making them more available for uptake by the plant. Although a slight increase in wet foliage weight, as compared to the check, was produced by bentgrass receiving the lower rates of Terraclor Super-X, Dasanit, and the combination, the increase was not significant. After the first fertilizer application, there was no indication of a growth stimulus over the effect of the fertilizer alone and, in fact, there was a notable suppression of growth in all treatments as compared to the check. Dasanit suppressed growth of the bentgrass more than Terraclor Super-X and the combination of both suppressed growth more than either alone. However, the treatments receiving Terraclor Super-X had all but completely recovered from the suppression by 32 days after

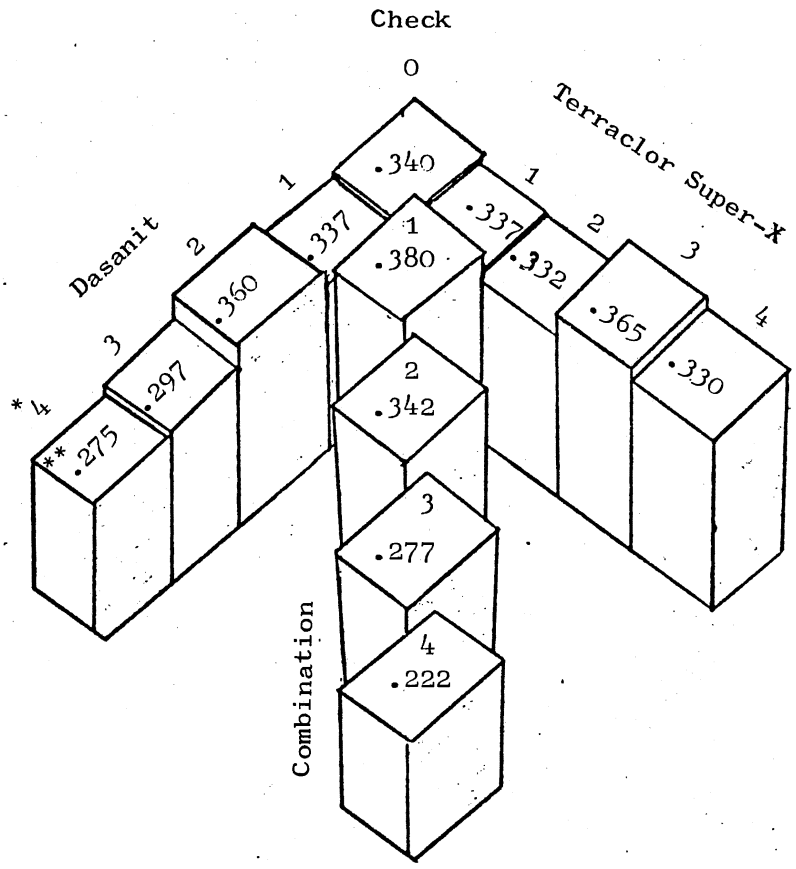


Fig. 5. Effect of Chemical Treatments on Wet Weight of Foliage After Four Days

*Treatment number for corresponding chemical. See Chart II for rates.

**Weight in grams wet foliage.

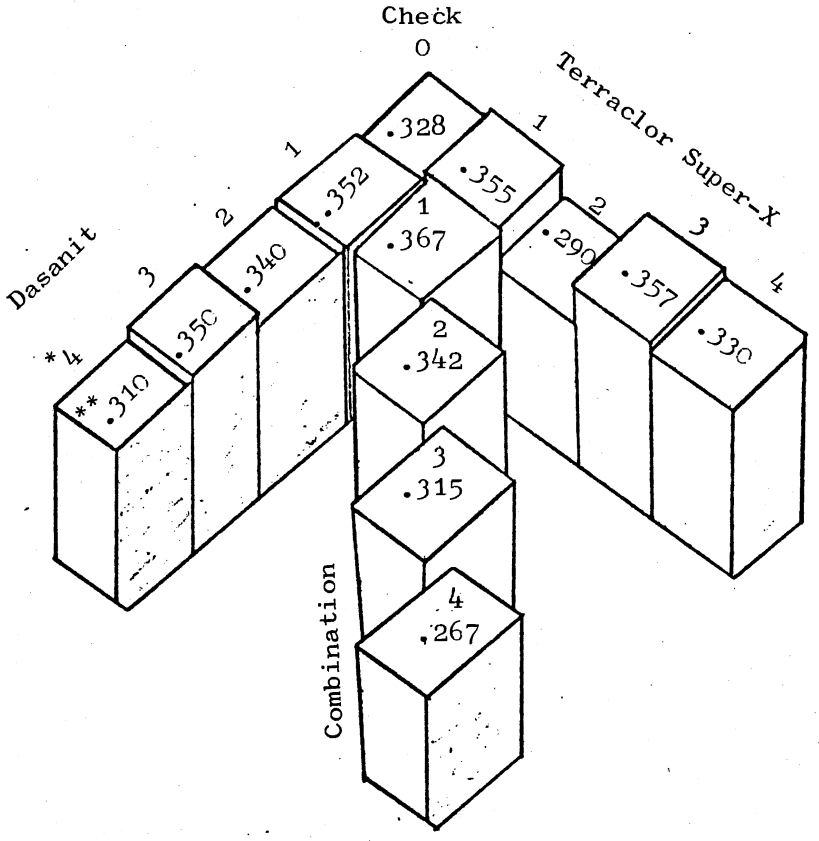


Fig. 6. Effect of Chemical Treatments on Wet Weight of Foliage After Eight Days

*Treatment number for corresponding chemical. See Chart II for rates.

**Weight in grams wet foliage.

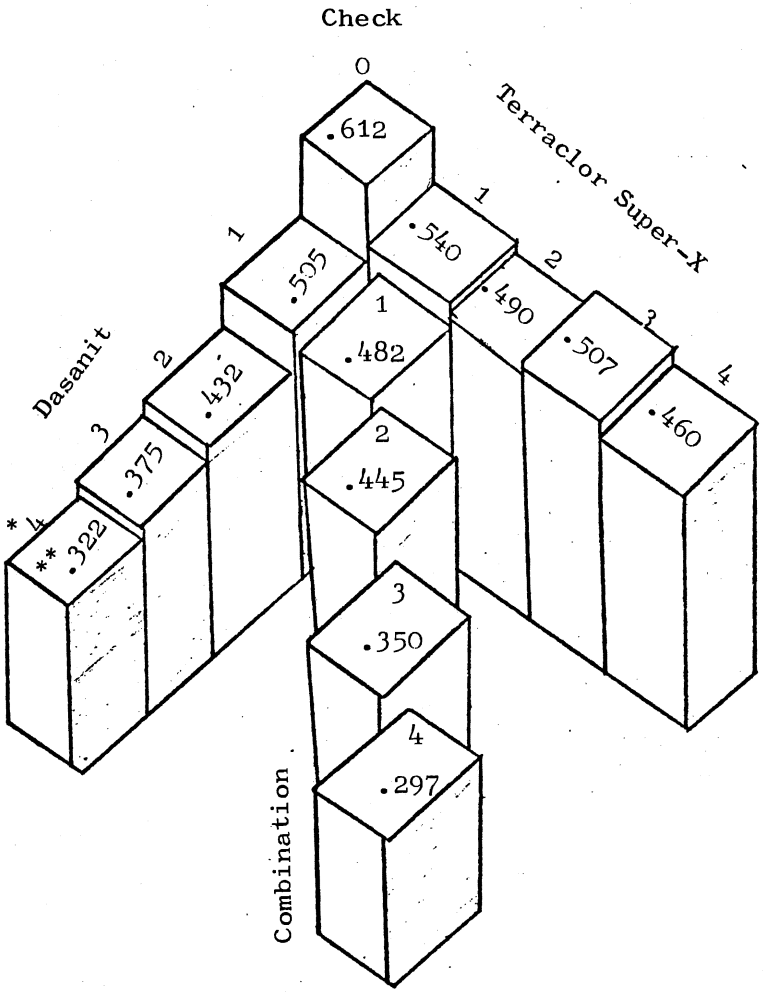


Fig. 7. Effect of Chemical Treatments on Wet Weight of Foliage After 12 Days. Four days after first application of fertilizer [226.8 gm (12-6-6)/93.025 sq. m].

*Treatment number for corresponding chemical. See Chart II for rates.

**Weight in grams wet foliage.

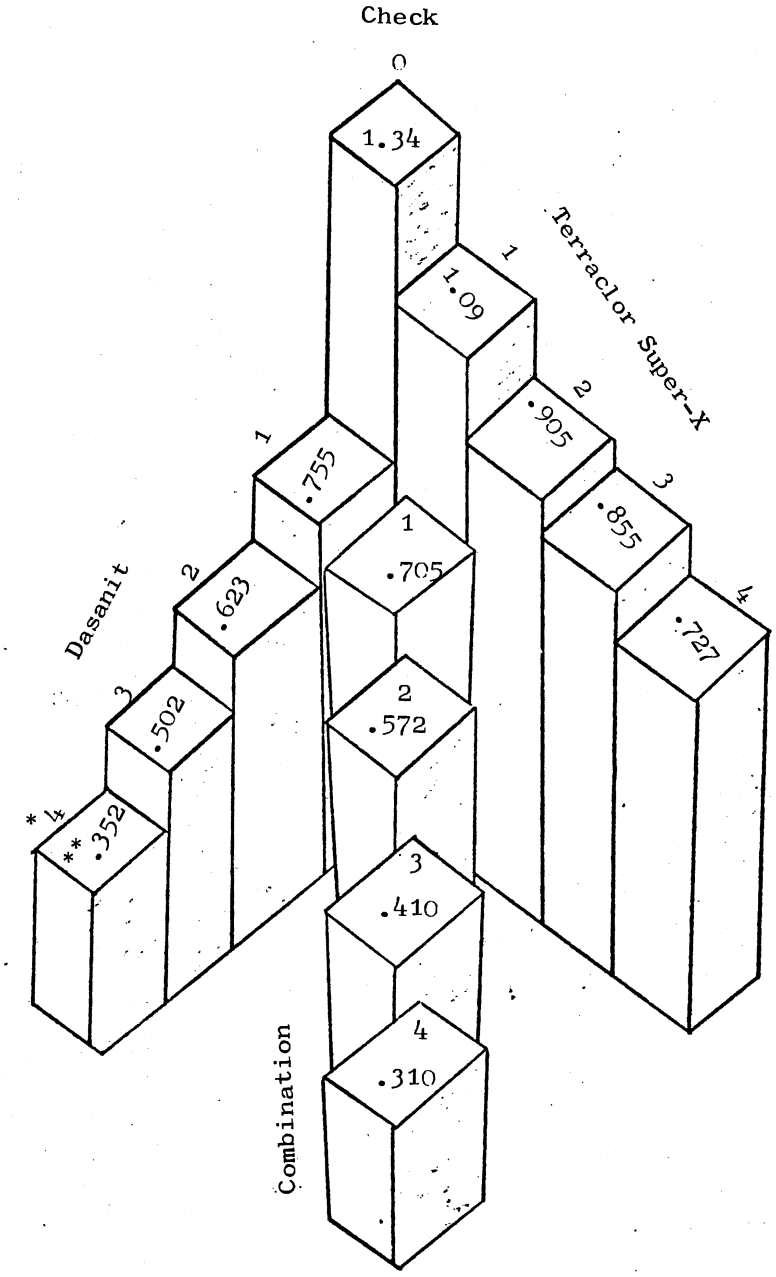


Fig. 8. Effect of Chemical Treatments on Wet Weight of Foliage After 16 Days. Eight days after first application of fertilizer [226.8 gm (12-6-6)/93.025 sq. m].

*Treatment number for corresponding chemical. See Chart II for rates.

**Weight in grams wet foliage.

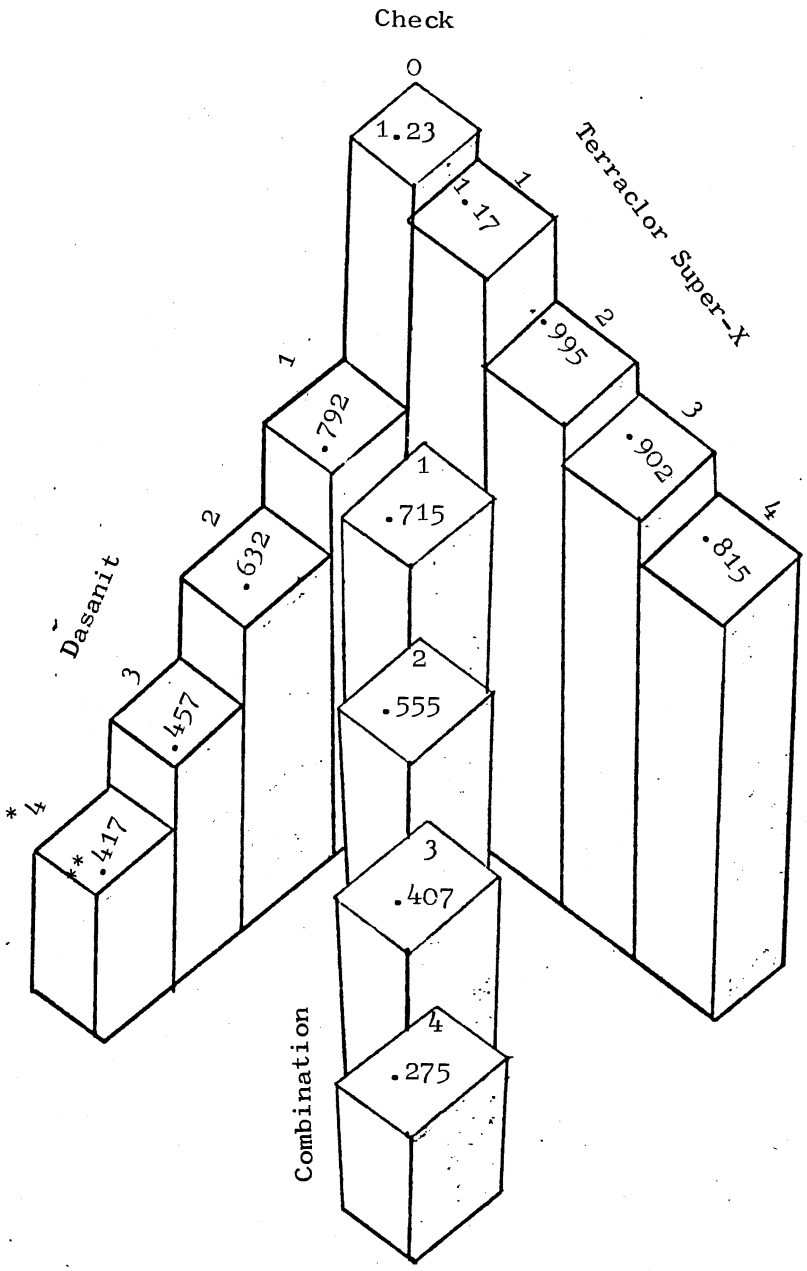


Fig. 9. Effect of Chemical Treatments on Wet Weight of Foliage After 20 Days. Twelve days after first application of fertilizer [226.8 gm (12-6-6)/93.025 sq. m].

*Treatment number for corresponding chemical. See Chart II for rates.

**Weight in grams wet foliage.

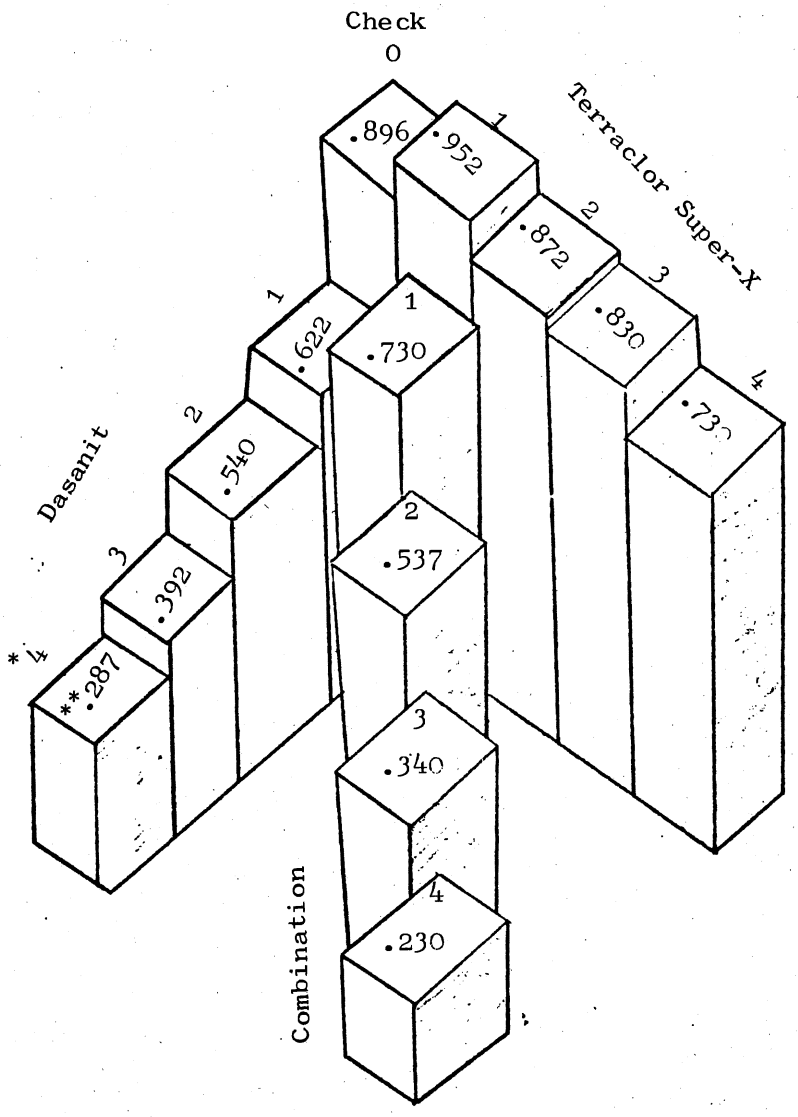


Fig. 10. Effect of Chemical Treatments on Wet Weight of Foliage After 24 Days. Four days after second application of fertilizer [226.8 gm (12-6-6)/93.025 sq. m].

*Treatment number for corresponding chemical. See Chart II for rates.

**Weight in grams wet foliage.

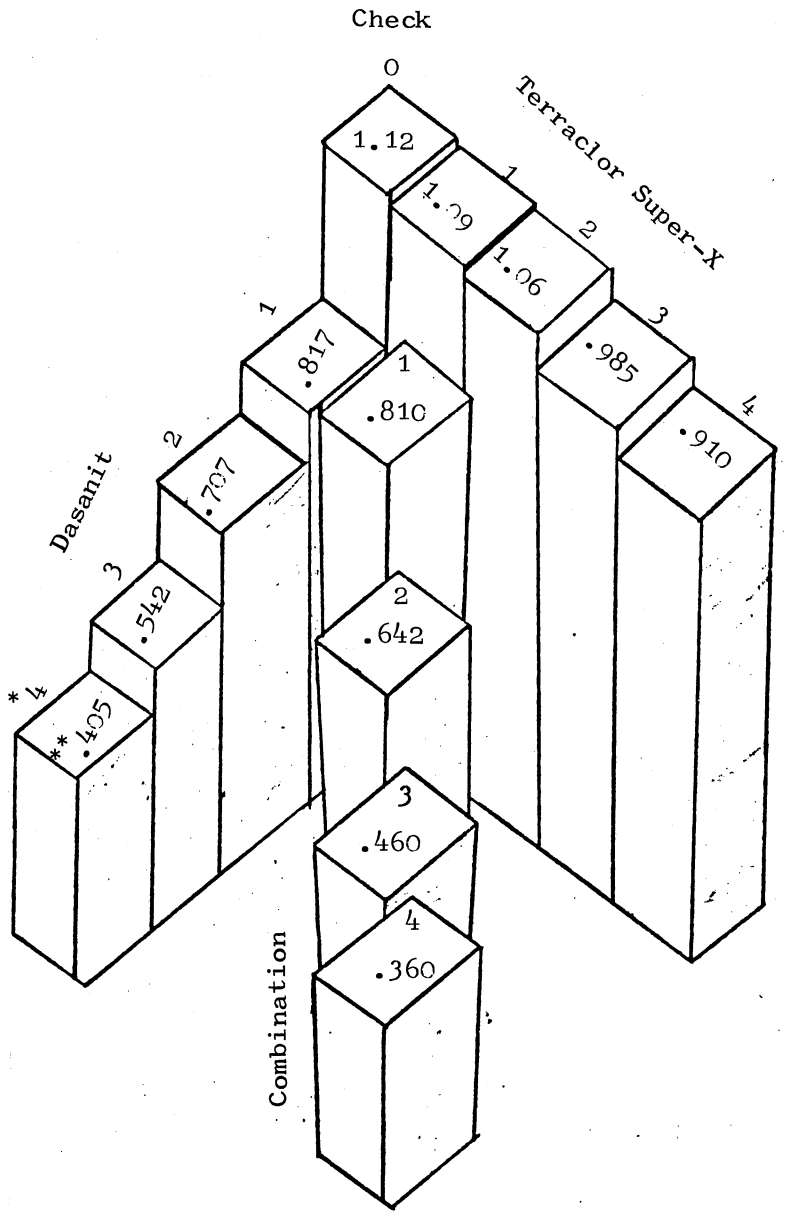


Fig. 11. Effect of Chemical Treatments on Wet Weight of Foliage After 28 Days. Eight days after second application of fertilizer [226.8 gm (12-6-6)/ 93.025 sq. m].

*Treatment number for corresponding chemical. See Chart II for rates.

**Weight in grams wet foliage.

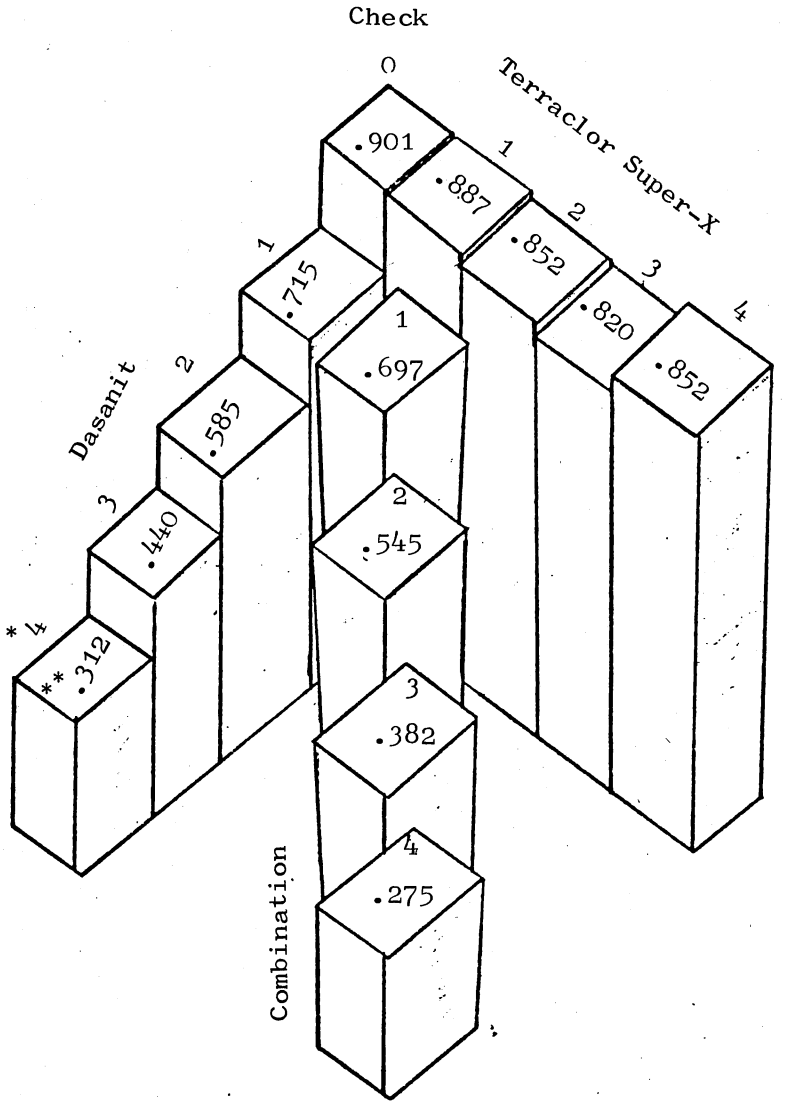


Fig. 12. Effect of Chemical Treatments on Wet Weight of Foliage After 32 Days. Twelve days after second application of fertilizer [226.8 gm (12-6-6)/ 93.025 sq. m].

*Treatment number for corresponding chemicals. See Chart II for rates.

**Weight in grams wet foliage.

chemical application. As evidenced by the growth suppressing effects of Terraclor Super-X, Dasanit, and the combination, applications at the higher rates could be injurious for field application. However, under field conditions, there are many factors that can influence the extent to which phytotoxity occurs. Such examples are: amount of thatch buildup, organic matter, clay content, and vigor of the turf.

CHAPTER IV

FIELD NEMATODE POPULATION AND ASSOCIATED FUNGI

The objective of this study was to determine the nematode population uniformity, the fungi present, and their relation to turf density.

Methods and Materials

The study was carried out on Agrostis palustris 'Penncross' creeping bentgrass, located at the Agronomy Research Station in Stillwater, Oklahoma. The bentgrass was an established plot approximately 10 years old in which there had been no nematicidal applications. The soil type was a Kirkland Silt Loam.

Twenty sampling areas were laid out in a randomized block design and replicated four times. Each sampling area was approximately .8 by 3.1 m in size. Using a soil probe, 40 soil plugs were taken at random and placed in previously numbered plastic bags. Each soil plug measured approximately one centimeter in diameter and four centimeters in length. The soil was mixed and a 100 ml sample from each of the 80 plots was processed using a modification of the Christie-Perry Extraction Method (11). Nematode samples were taken every two weeks beginning December 29, 1975, up until April 12, 1976. Color of the turf, as previously described, and density (0 to 10, 10=100%) ratings were taken on each sampling date.

Because Criconemoides spp. require more time to penetrate the Scotty

tissues, the samples were left for 48 hours instead of 24. Extremely high populations of nematodes made reading very difficult, therefore, counts were determined by aliquot. Each sample was poured into a 50 ml beaker, the water level adjusted to 20 ml, swirled vigorously and a 10 ml sub-sample extracted and poured into a counting dish. The nematodes were then identified with the aid of a steroscopic microscope (30X).

On April 12, to determine the fungi present, 15 soil plugs were taken from each of the 80 sampling areas as previously described for nematode analysis. The four replications in each of the 20 sampling numbers were combined, mixed thoroughly, and a sub-sample taken for fungal isolations.

To recover the wide range of fungi encountered in the rhizosphere, selective media were used. These included a selective medium for Pythium spp. as described by Kerr (48), Rhizoctonia spp. as described and modified by Papavizas (76) substituting beet seed for buckwheat stem pieces, and Ohio agar medium for the isolation of general fungi (108). Dollar spot, caused by Sclerotinia homoeocarpa F. T. Bennett, readings were made visually.

Results and Discussion

A uniformity trial was conducted to determine the seasonal fluctuation of the nematode population, and determine the experimental design required to obtain valid data. The plot was set up so that the replications ran north and south because of a slight slope toward the south. The greatest variation in nematode population was expected to occur in a north to south direction because of a soil moisture gradient across the plots with the southern most plots having the highest moisture level.

This positioning of the replications was expected to reduce the effects of variation from that source.

Statistical analysis of the data proved the expected variation in population running from north to south; however, there were greater variations in population west to east across the replications. Fig. 13 shows the layout of the plot with the four replications in which the first row of all four replications has been designated as column 1 and the second row of the four replications as column 2, the third row as column 3, and the fourth row as column 4. From this point, discussion will be directed to columns.

As shown in Fig. 14, the total population of Criconemoides sp. rose substantially from December 29, 1975, to January 12, 1976, and dropped again on January 26, 1976. The population thereafter did not fluctuate greatly. A prominent increase occurred in column 3 which, except for December 29, 1975, is consistently high throughout all sampling dates. In fact, the number of nematodes in all columns consistently showed a similar pattern at each sampling date.

The population of Tylenchorhynchus sp. at the respective sampling dates, shows a substantial increase from December 29, 1975, up to February 9, 1976, at which time the population began to decrease and then leveled off (Fig. 15). The number of Tylenchorhynchus sp. within each column shows a very direct column effect as was evidenced in Criconemoides sp. (Fig. 14). Although Tylenchorhynchus sp. populations show a column effect, it is almost the exact reciprocal of that found in the Criconemoides population. The number of Tylenchorhynchus sp. in column 3 was consistently lower at all sampling dates except for December 29, 1975, in which the population in column 4 was lower.

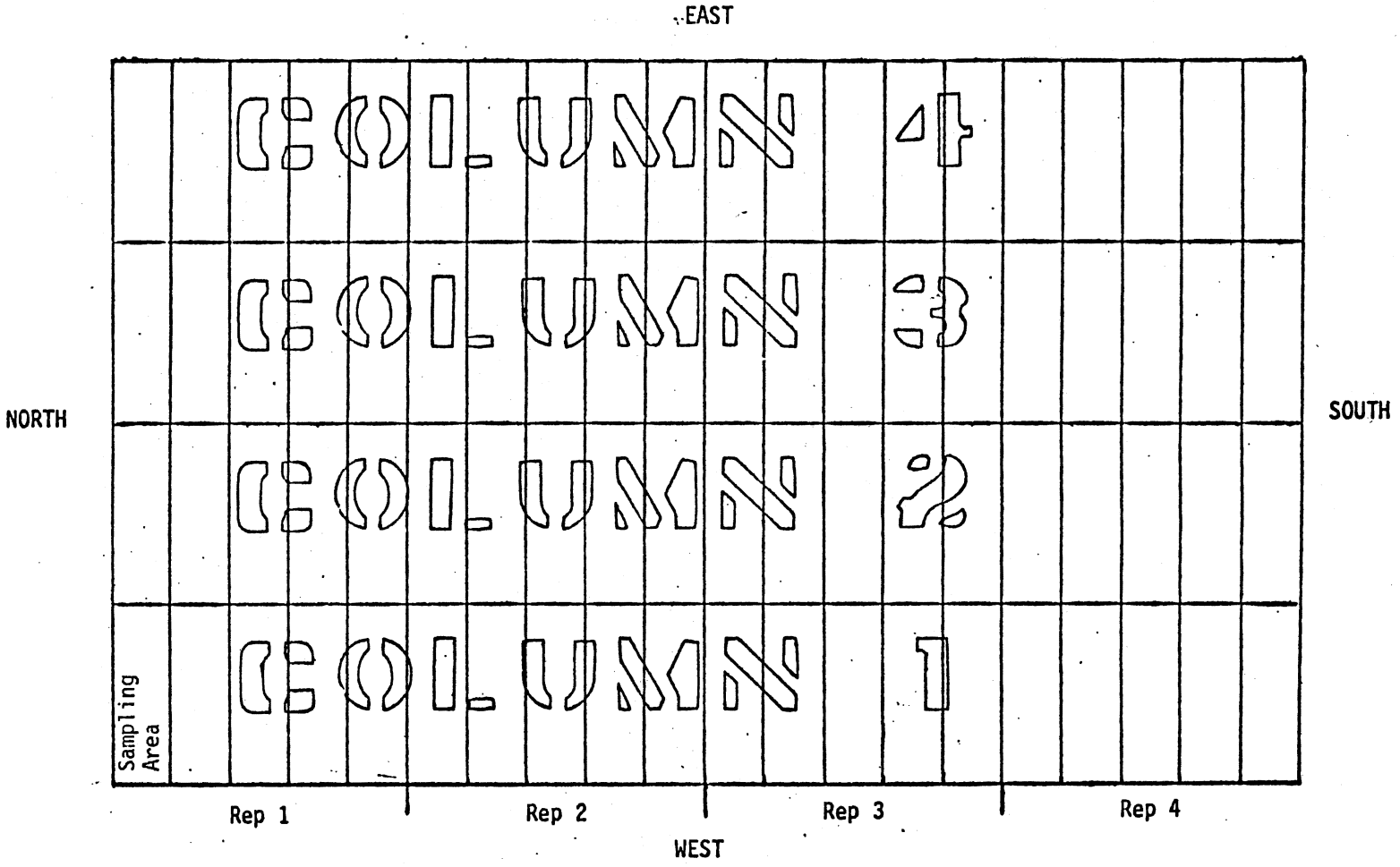


Fig. 13. Plot Design Showing Replications and Columns With Reference to Direction With 20 Sampling Areas (.8 by 3.1 m) in Each Replication and 20 Sampling Areas in Each Column

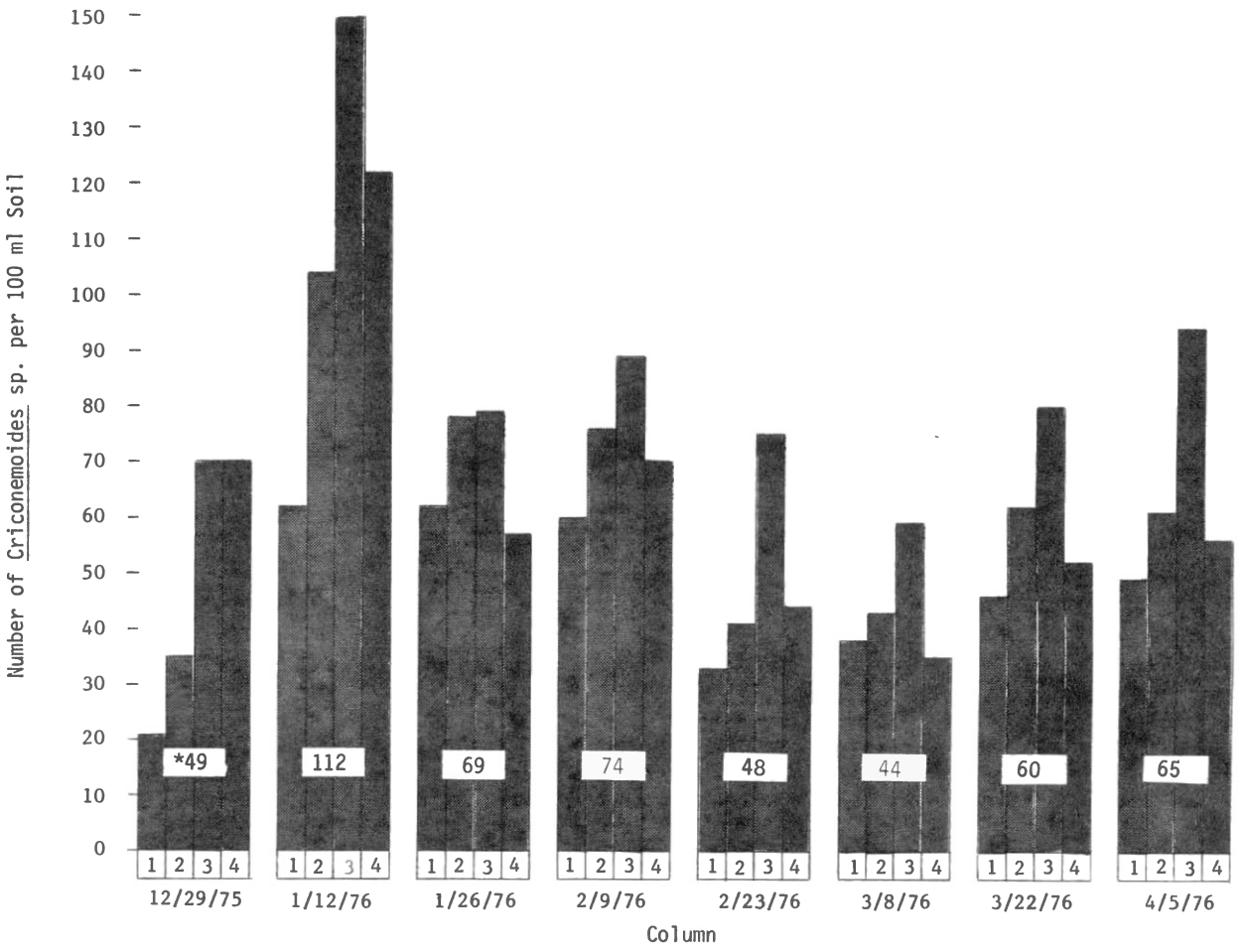


Fig. 14. Average Number of Criconemoides sp. in Each Column and Population Trend Among Respective Sampling Dates

*Average of four columns.

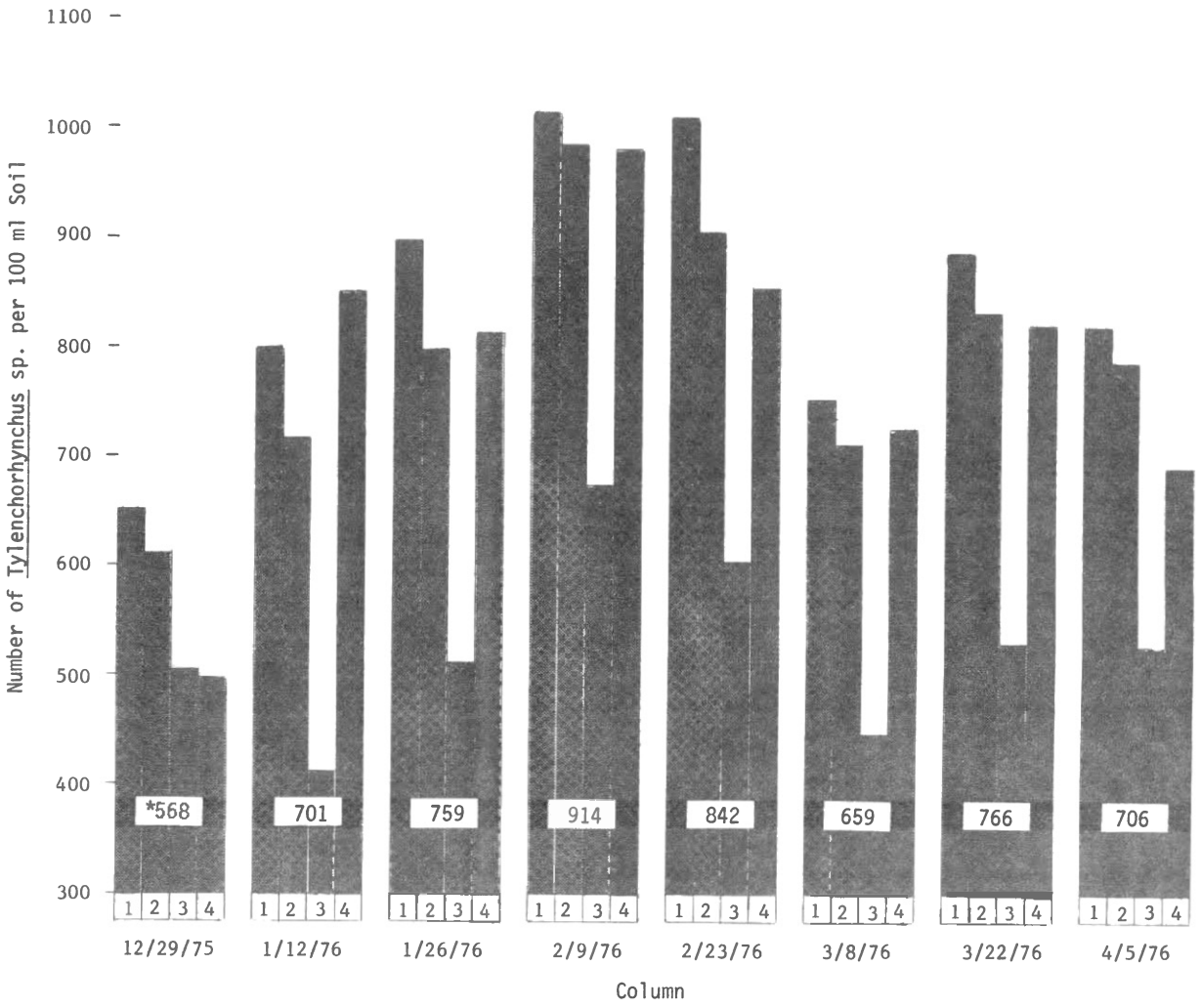


Fig. 15. Average Number of Tylenchorhynchus sp. in Each Column and Population Trend Among Respective Sampling Dates

*Average of four columns.

Paratylenchus sp. populations did not have the variation among columns as did Criconemoides sp. and Tylenchorhynchus sp., although column 2 was considerably higher on the first two sampling dates (Fig. 16). The population, as a whole, increased on January 1, 1976, over the previous sampling date and then dropped sharply on January 26, 1976. The population for the remainder of the test period did not fluctuate greatly.

The data indicate a possible interaction between Criconemoides sp. and Tylenchorhynchus sp., however, as the population of one genus increased, the population of the other did not necessarily decrease. There was no indication that an interaction occurred between Paratylenchus sp. and Criconemoides sp. and Tylenchorhynchus sp. Some factor had a definite effect on the population of Tylenchorhynchus sp. and Criconemoides sp. in column 3. The effect of moisture levels could hardly be the cause of variation since the moisture gradient went from north to south within each column. The soil type and texture was uniform throughout the plot. The effect of temperature gradients would also seem unlikely, since temperature and moisture content are positively correlated, provided the soil type is uniform. Soil temperatures taken were determined to be of no value and could not be used since each sampling area was not measured. The possibility of the effect from the vegetative growth outside the plot can also be discounted since 'Colonial' bentgrass was growing on the east side and "Seaside' creeping bentgrass on the west side. Records indicate there has never been a nematicide applied to the plot. The precise sampling and processing procedures used should have eliminated chances of variation introduced by the researcher.

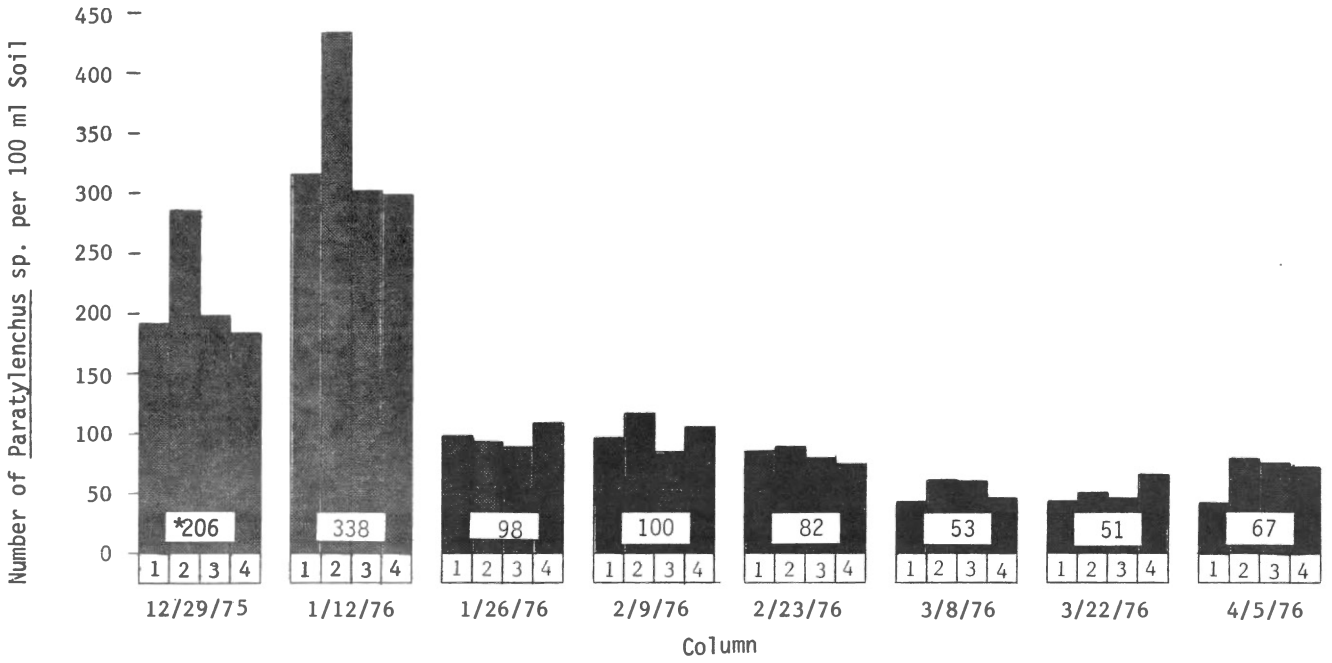


Fig. 16. Average Number of Paratylenchus sp. in Each Column and Population Trend Among Respective Sampling Dates

*Average of four columns.

Color rating was of little value prior to the March 8, 1976, sampling date because the grass was in a semidormant state. It had been uniformly rated as a six on the color chart (Table I) up to March 8, at which time the bentgrass began to grow more vigorously, producing a darker green color rated as seven. On the following sampling date, March 22, the bentgrass was given a top color rating of nine, which continued throughout the balance of the test period (April 5).

Turf density of each sampling area was consistently heavy and uniform and rated at 100% throughout the period of the study. Although extremely high populations of nematodes were present, no evidence of decline in turf density was observed, hence, no visual correlation of nematode population to turf density could be made.

Other nematodes recovered infrequently and in very low populations were Pratylenchus sp., Helicotylenchus sp., Trichodorus sp., and Xiphinema sp.

The results of the uniformity trial have shown that a randomized block experimental plot design cannot be used on this plot. Other alternatives are to increase the number of replications substantially, use a Latin square design, or a split plot design. Increased replications are often impossible and the Latin square design limits the number of treatments to four. The split plot design increases plot size, but the benefits more than outweigh the disadvantages since each plot has an adjacent check plot. This design could eliminate many of the problems encountered in dealing with the numerous sources of variation.

Isolations were made only to determine the fungi present. Although 'brown patch' caused by Rhizoctonia solani is a very prominent and

destructive disease in Oklahoma, the fungus could not be isolated during the many attempts made. Papavizas (75) (personal communication) stated that the inability to recover Rhizoctonia solani in high organic soil with high populations of micro-organisms is not too uncommon. There were no disease symptoms observed during the course of the study other than a trace of dollar spot caused by Sclerotinia homoeocarpa.

The following fungi were isolated: Pythium spp, Fusarium roseum Link, Fusarium solani (Mart.) Appel and Wr., Helminthosporium sativum P. K. & B., Curvularia lunuta (Wakker) Boed., Sclerotinia homoeocarpa F. T. Bennett, Nigrospora spp., Rhizopus spp., Trichoderma spp., Alternaria spp., Verticillium spp., Aspergillus spp., Cephalosporium spp., Humicola spp., Penicillium spp., Leptosphaerulinia spp., Phoma spp., Sordaria spp., Neocosmospora spp., Scolecobasidium spp., Cladisporium spp.

On the basis of previous sampling, nematodes and fungi were determined to be involved in this complex soil disease. Because of the complexity of the disease, only the nematode portion of this problem was investigated.

CHAPTER V

IN VITRO FEEDING OBSERVATIONS

The objective of this study was to determine the feeding habits of the nematodes recovered from the field study.

Methods and Materials

The in vitro technique used in this study was described by Russell and Morrison (86), and is outlined in greater detail below.

A .75% agar solution was prepared, autoclaved, and cooled to approximately 50 C. Sufficient agar was then poured into a 60 mm polystyrene petri dish to completely cover the bottom. Excess agar was immediately withdrawn with a pipette until a .1 to .2 cm agar film remained. After the agar cooled, a pre-cut disk of .0254 mm plastic film was fitted to the agar surface and smoothed to remove any bubbles trapped beneath it.

The plastic disks were prepared by spreading sheets of Handi Wrap brand film over polystyrene petri dish covers and cutting around a metal template with a single edged razor blade. Handi Wrap brand plastic sheet was used because its plastic allows oxygen exchange more readily than the heavier mill plastic.

The top one-fourth of the agar and the plastic were then cut with a razor blade and removed (Fig. 17A). The plastic was lifted and 10 seedlings were positioned between the plastic and agar surface (Fig. 17B).

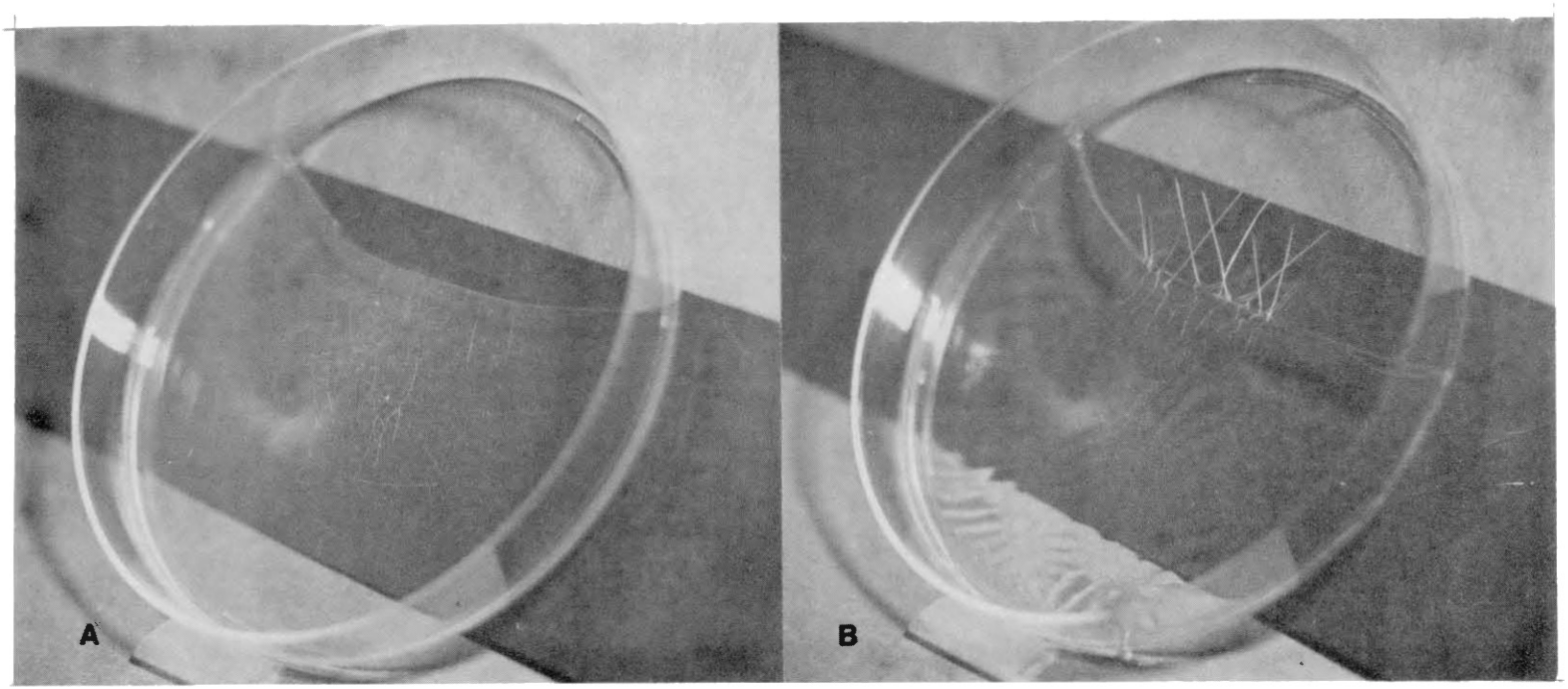


Fig. 17 (A-D). Steps in Preparation of Polystyrene Petri Dishes for Nematode In Vitro Observations.
(A) Petri dish with top one-fourth portion of agar and plastic removed.
(B) Petri dish after the plastic was lifted and 10 seedlings positioned between plastic and agar. Note the length of bentgrass roots.

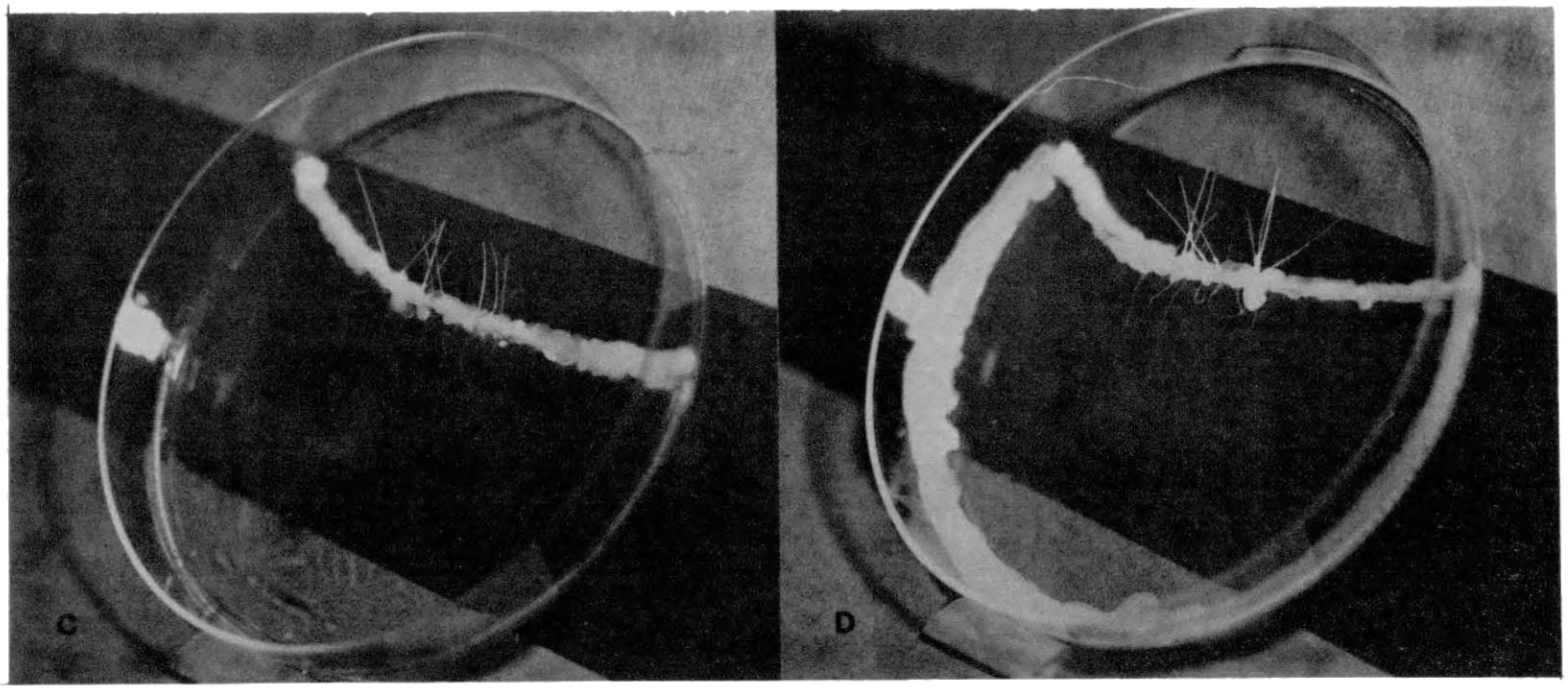


Fig. 17 (Continued). (C) Petri dish with 10 bentgrass seedlings in which a petroleum jelly and wax solution was spread across crown area as a sealant. (D) Petri dish after the bottom edge had been lifted to inoculate with nematodes and subsequently sealed.

Equal parts of petroleum jelly and wax were heated to 50 C and a film applied across the top as a sealant (Fig. 17C). The dishes were then elevated to approximately 45°, with the face down, to keep the roots growing against the plastic film. The petri dishes were kept under continuous fluorescent light for one to two days.

The nematodes were concentrated and drawn off with a pipette which was then suspended in order to let the nematodes settle to the orifice. The bottom end of the plastic was lifted and a very small droplet released. The plastic was carefully replaced and ringed with the wax-petroleum jelly solution (Fig. 17D).

The petri dishes were replaced in the position previously described to insure that the roots grew next to the plastic film. Observations were made periodically.

Results and Discussion

The feeding habits and parasitism of several genera of nematodes were simultaneously studied *in vitro*. Nematodes used in this study were those collected from the field uniformity study.

Direct observations of creeping bentgrass roots showed *Tylenchorhynchus* sp. feeding on epidermal cells (Figure 18). As the nematode moved, tentative jabs were made by the stylet at various epidermal cells. When an actual attempt was made to gain stylet entrance into the roots, stylet thrusts varied in number from 25 to 98. The average number of thrusts was in the range of about 50. Stylet penetration was extremely difficult to observe; however, immediately after stylet thrusts had subsided rapid metacorpal movement began. Duration of feeding times varied from only a few seconds to a maximum of 17 minutes. There seemed

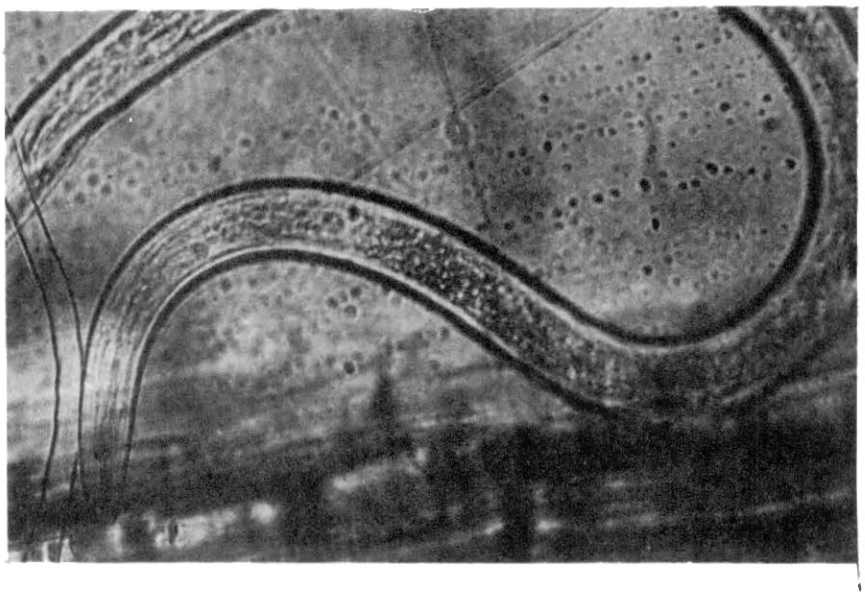


Fig. 18. Tylenchorhynchus sp. Feeding on Epidermal Cell. Note stylet position and the lack of detectible stylet penetration. (Magnification, 278X)

to be a definite attraction to the apical meristematic region, however. They fed readily on all portions of the root except for root hairs and the root cap and fed only as an ectoparasite. During feeding the nematode body generally laid parallel and close to the root surface. Nematode feeding sites on root epidermal cells did not indicate any injury had occurred. However, some stunting was observed when many Tylenchorhynchus sp. fed on one root. Observations were typical of those noted by Krusberg (53).

Trichodorus spp. fed as migratory ectoparasites. At the inception of feeding, the stylet was thrust in and out at such a rapid rate that counts were difficult. Stylet thrusts ranged from approximately five to seven thrusts per second. After varying periods of time the rate of thrusts would slow and eventually increase in speed. The nematodes fed for relatively short periods; the longest being two minutes and 45 seconds. Often the nematode was observed feeding for only four or five seconds and then moving to an adjacent cell, only to return to the original feeding site. The feeding habits of the Trichordorus sp. parallel those stated by Russell (87) for the most part except that semi-endoparasitic feeding was not observed. A very strong attraction to the root cap and meristematic region was quite evident and no feeding occurred in the region of maturation as shown in Fig. 19. There was no stubby-root condition or necrosis at any of the feeding sights.

Direct observation of Criconemoides sp. feeding on the roots of creeping bentgrass showed they were capable of feeding at almost any point in or above the maturation zone. Feeding was not observed on the root tip, meristematic region, or root hairs. Penetration of the stylet was observed to be approximately 45% of the stylet length, as shown in Fig. 20.

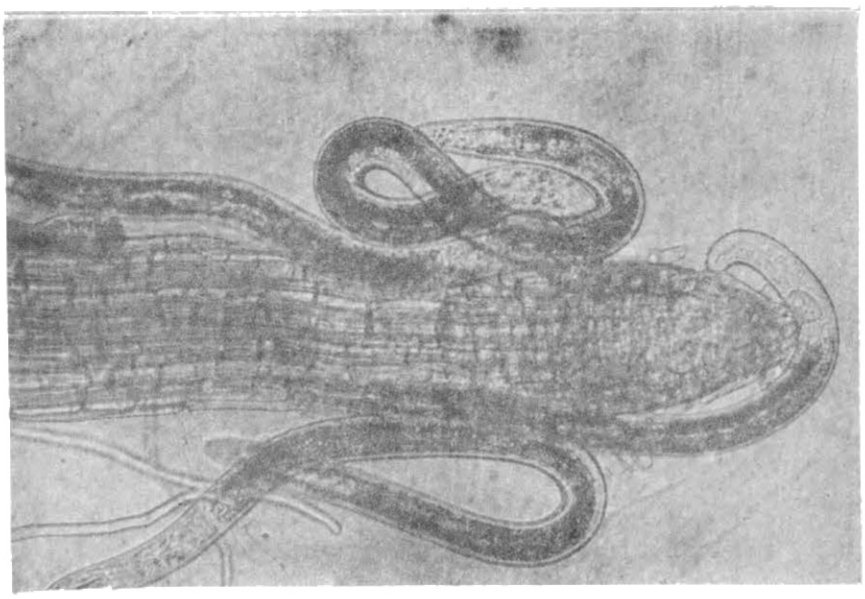


Fig. 19. Trichodorus sp. Feeding on Root Tip and Meristematic Region of 'Penncross' Bentgrass (Magnification, 263X)

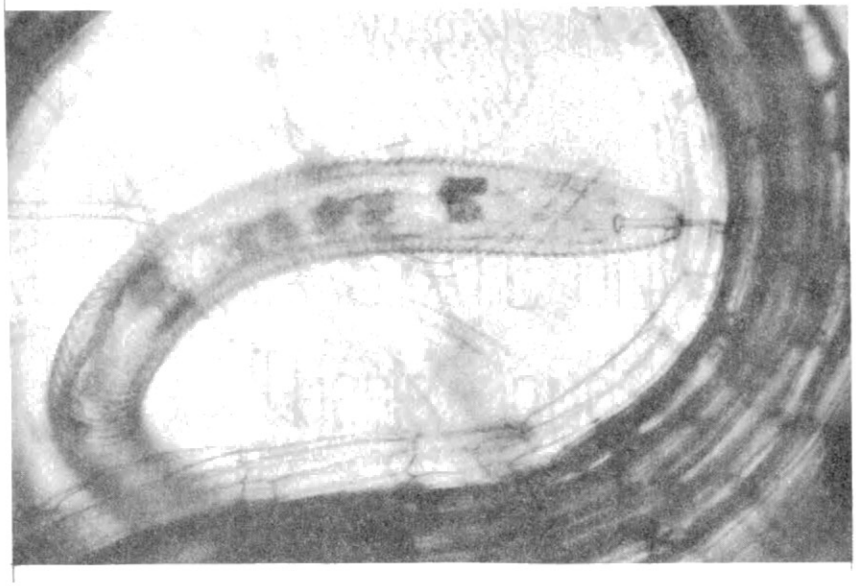


Fig. 20. Criconemoides sp. Feeding on Mature Bentgrass Root. Note the percentage of stylet penetration. (Magnification 313X)

Jenkins and Taylor (43) stated that C. curvatum had been observed to feed as an ectoparasite and at times as a semi-endoparasite. At least in a few cases, the nematode was observed entirely within the root cortex. The Criconemoides sp. observed in this study fed only as an ectoparasite.

Long periods of rhythmic pulsation of the metacorpus was demonstrated with only short intermittent pauses. The Criconemoides sp. fed for long periods of time. One nematode was observed feeding in the same position each time the plate was checked during a 36 hour period, however, no detectable injury or necrosis occurred.

Insufficient inoculum levels of Helicotylenchus sp. made detailed studies impossible. The nematode generally fed in the mature root zone; however, as evidenced by Fig. 21 feeding also occurred on root hairs. The Helicotylenchus sp. in this study fed ectoparasitically, although Jenkins and Taylor (43) stated that Helicotylenchus spp. were capable of ectoparasitism, semindoparasitism, and migratory endoparasitism on corn.

Stylet penetration of approximately 15% of the stylet length was observed. Although no necrosis occurred, cytoplasmic agglutinution was seen as shown in Fig. 22.

Tylenchus spp. fed almost exclusively on root hairs (Fig. 23); however, on one occasion feeding was observed in the region of the root cap. Insertion of stylet tip was impossible to detect, although movement of the esophageal bulb was confirmed. From observations on a limited number of specimens, feeding time was recorded, on the basis of metacorpal activity, to be two minutes for the shortest duration to 23 minutes for the longest.

Tylenchus spp. are generally thought to be almost insignificant as

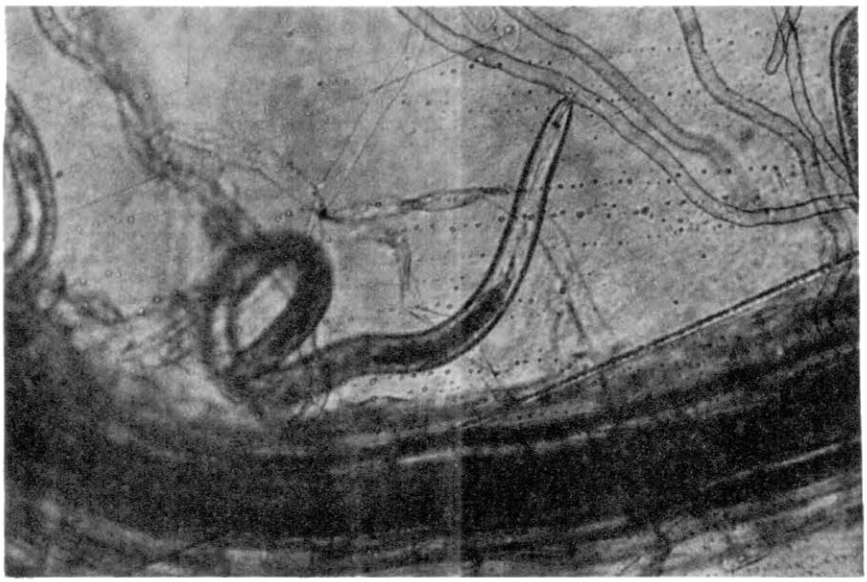


Fig. 21. Helicotylenchus sp. Feeding on Root Hair of Bentgrass. Note the spiral of the body. (Magnification, 206X)

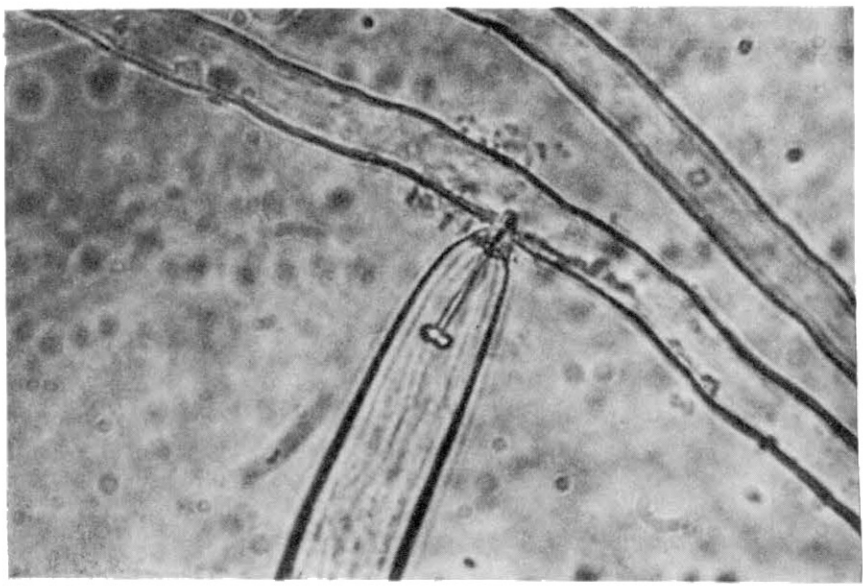


Fig. 22. Enlargement of Helicotylenchus sp. Feeding on Root Hair and the Cytoplasmic Agglutination (Magnification, 790X)

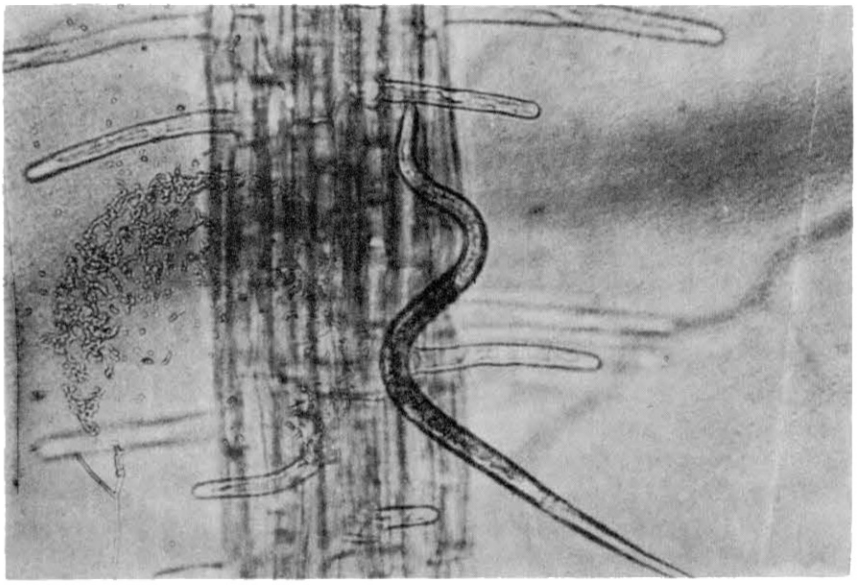


Fig. 23. Tylenchus sp. Feeding on Root Hair of Bentgrass. Note body position. (Magnification, 163X)

plant parasites. This is not surprising since little recognition has been extended to the Tylenchus spp. If cytoplasmic agglutination (Fig. 24) is any indication of virulence, the pathogenic potential of this nematode may be underestimated in previous studies.

Fig. 25 shows Pratylenchus sp. feeding in the mature root zone which is consistent with Linford's (57) observations. Again, only limited observations were made due to insufficient inoculum. Only one specimen was observed to feed and apparently no attempt was made to enter the root. Immediately after introduction, the nematodes began to move towards the bentgrass roots. Several Pratylenchus sp. were seen probing in the meristematic region although successful feeding was not observed. Klinkenberg (50) noted endoparasitism of P. penetrans on oats in vitro although most fed as ectoparasites.

Great difficulty was experienced in an attempt to observe Paratylenchus sp. feeding. Although inoculum levels from less than 50 to over 1000 were introduced, feeding was not observed. Agar concentrations of 1%, 1.5%, and 2% water agar were used. None seemed to enhance the ability of Paratylenchus sp. to feed in vitro. Plates were placed in complete darkness for several days in which the only light received was during brief examinations. In order to provide something that the nematode could use for body purchase during an attempt to insert its stylet, as many as 20 bentgrass seedlings were used. The roots grew close together, but still no feeding occurred. The nematode did migrate to the roots immediately upon introduction. High numbers of Paratylenchus sp. were seen aggregated in the meristematic region as shown in Fig. 26. Different temperatures were not used. As will be shown in the vertical distribution study, Paratylenchus sp. is primarily a nematode

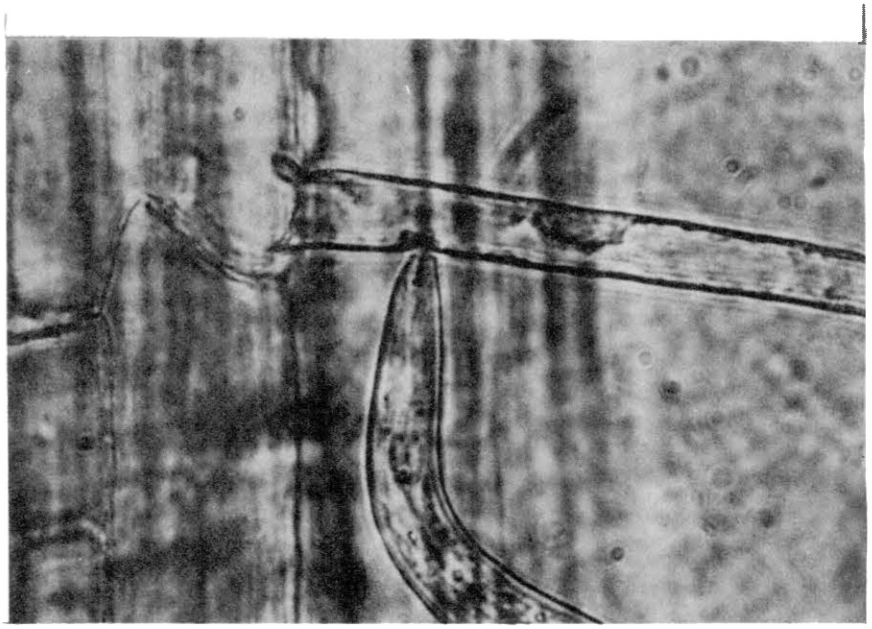


Fig. 24. Enlargement of Tylenchus sp. Showing Cytoplasmic Agglutination. Note the opposite side of root hair in which feeding has occurred. (Magnification, 695X)

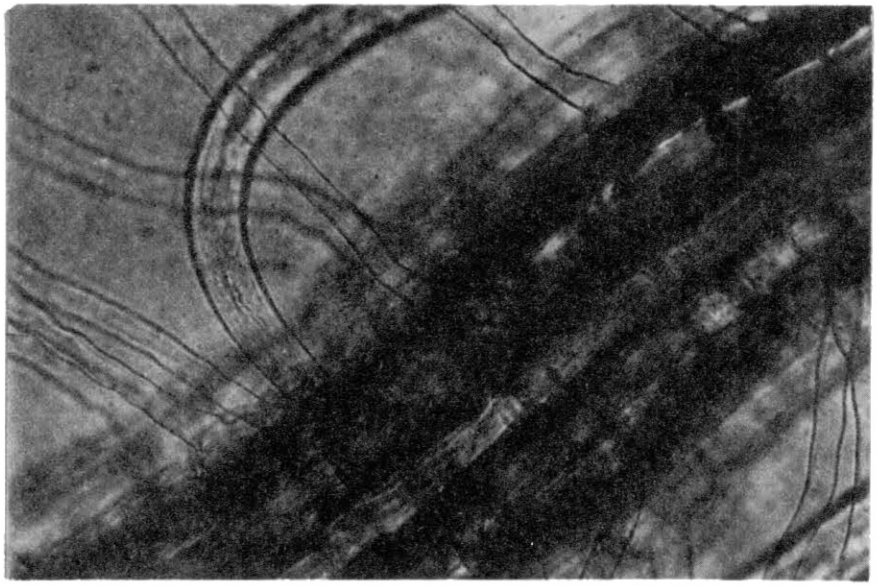


Fig. 25. Pratylenchus sp. Feeding in Mature Root Zone on Bentgrass (Magnification 290X)

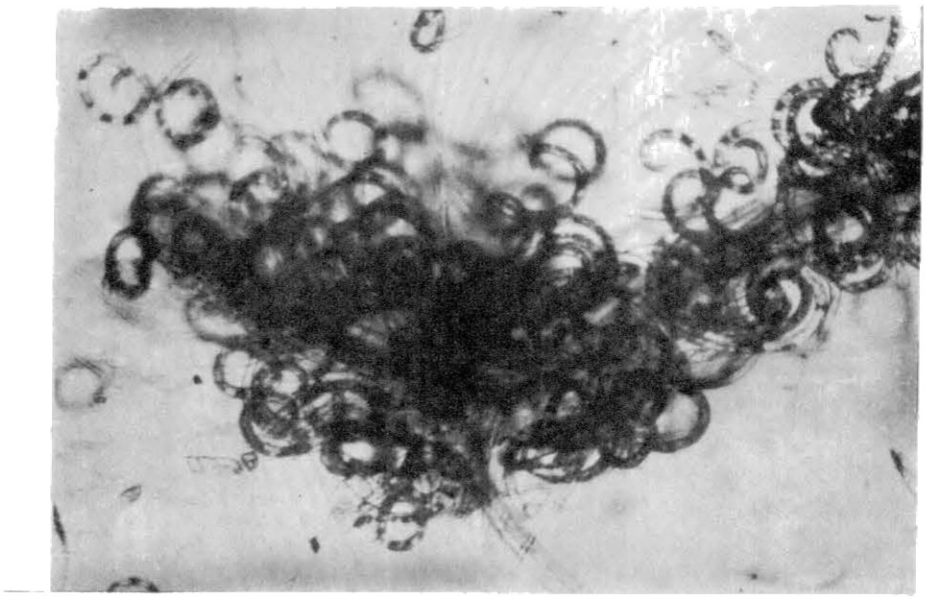


Fig. 26. Paratylenchus sp. Aggregated Around Root Showing Attraction (Magnification, 84X)

of deeper soil horizons. At the depths at which they occurred in the field, they would not be subjected to rapid temperature fluctuations.

Despite repeated attempts, as described for Paratylenchus sp., Xiphinema sp. was not observed to feed in vitro. Although, the nematodes were attracted to and remained in the vicinity of the root, no attempt to feed was observed.

Nematodes of the genera Tylencholaimellus and Psilenchus were recovered at times in moderate populations. Both are suspected plant parasites, however, little work has been done on their bionomics as noted by Jenkins and Taylor (43). Both were introduced along with the other genera and observed. Tylencholaimellus sp. appeared on two occasions to be feeding on a root hair in one instance and an epidermal cell the other. Due to the absence of esophageal activity and failure to observe stylet penetration, feeding could not be confirmed.

Psilenchus sp. was not observed to feed, however, it stayed in close association with the roots and at times would position its head against the root in a posture which was similar to that of the nematodes which were observed to feed successfully (see Figs. 18 and 25).

CHAPTER VI

NEMATODE VERTICAL DISTRIBUTION STUDY

The objective of this study was to determine at what soil horizons parasitic nematode populations occurred on 'Penncross' bentgrass.

Methods and Materials

The study was carried out on 'Penncross' creeping bentgrass, Agrostis palustris, located at the Agronomy Research Station in Stillwater, Oklahoma.

Soil samples were taken at random around the periphery of the uniformity plot using a soil probe with dimensions of 2 cm by 45 cm Twenty samples, taken to a depth of approximately 35 cm, were placed on a board and cut into 4 cm increments down to 28 cm. Each increment was placed in a plastic bag to be taken to the laboratory for processing. The soil was mixed and two 100 ml samples were processed the same as previously described for the nematode uniformity trial.

Root volume per 100 ml of soil was determined at the 6/22/76 sampling date. Soil plugs were soaked for two hours because of the heavy clay-type soil, and poured onto a U.S. Standard Sieve, series no. 40. Water was sprayed with sufficient force to separate soil from roots. The roots were then air dried at 21 C for three hours and were then weighed.

Results and Discussion

The largest population density of the nematode genera Criconemoides sp., Tylenchorhynchus sp., and Paratylenchus sp. occurred in the top four centimeters (cm) of the soil on the January 12, 1976, sampling date (Fig. 27). Tylenchorhynchus sp. populations declined substantially down to 16 cm as did the population of Criconemoides sp., although not as drastically. Populations of Paratylenchus sp. were consistently high at all depths (0-16 cm). Xiphinema sp. appeared in the four to eight cm zone and subsequent zones which is consistent with the theory that they are deeper rhizosphere nematodes. Conventional shallow sampling on turf may be responsible for underestimating the importance of Xiphinema spp. on turf.

By June 22, all plant parasitic nematodes had declined considerably in the zero to four cm zone relative to the previous sampling date (Fig. 28). Again, the number of Tylenchorhynchus sp. declined at each successive depth and none were recovered in the 24 to 28 cm zone. The Criconemoides sp. population increased slightly at the four to eight cm zone and then began to decrease in number at each successive zone. The Paratylenchus sp. population was considerably lower in the zero to four cm zone compared to the number recovered in January. However, they increased substantially in the four to eight cm zone and were consistently high all the way down to 28 cm. Populations of Xiphinema sp. were again recovered in the deeper soil profile, this time appearing in the eight to 12 cm range and continuing throughout. Pratylenchus sp. was recovered in the eight to 12 cm zone with a significant population occurring in the 12 to 16 cm zone. Trichodorus sp., Pratylenchus sp., and Xiphinema sp. were all present in the 16 to 20 cm zone down to 28 cm.

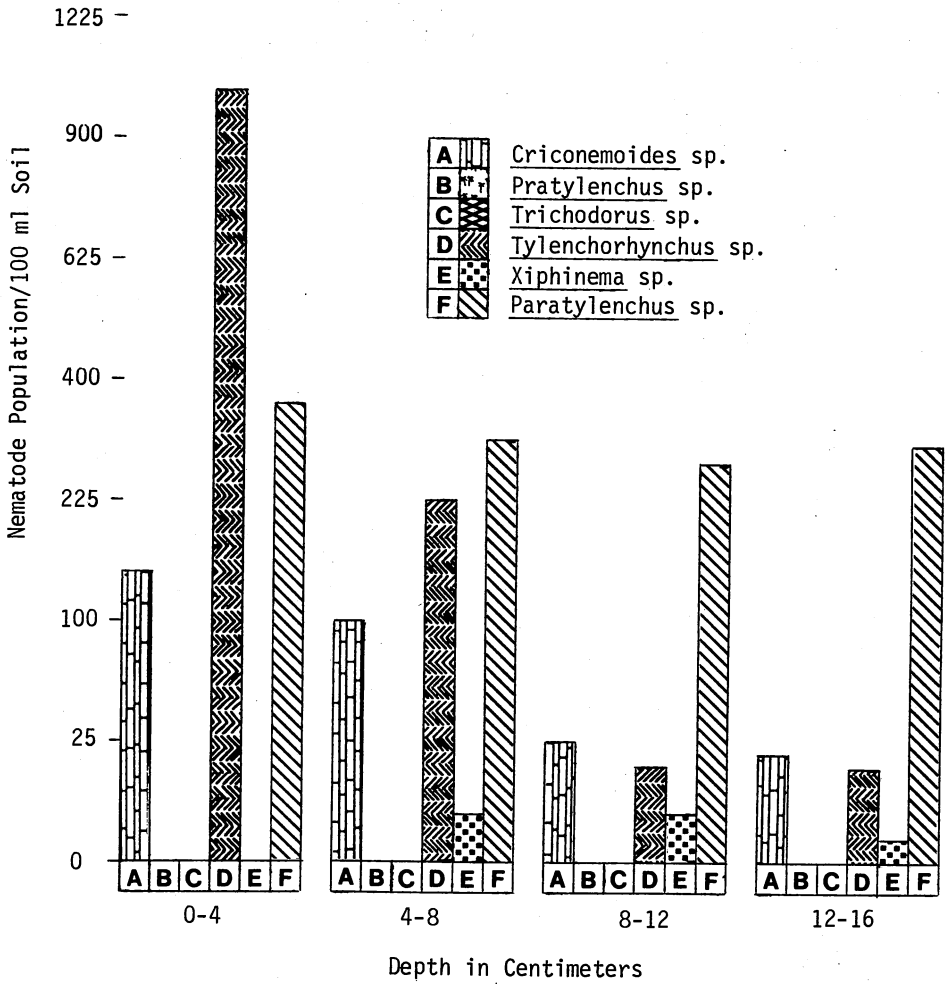


Fig. 27. Population Levels of Nematodes by Soil Depth on January 12, 1976

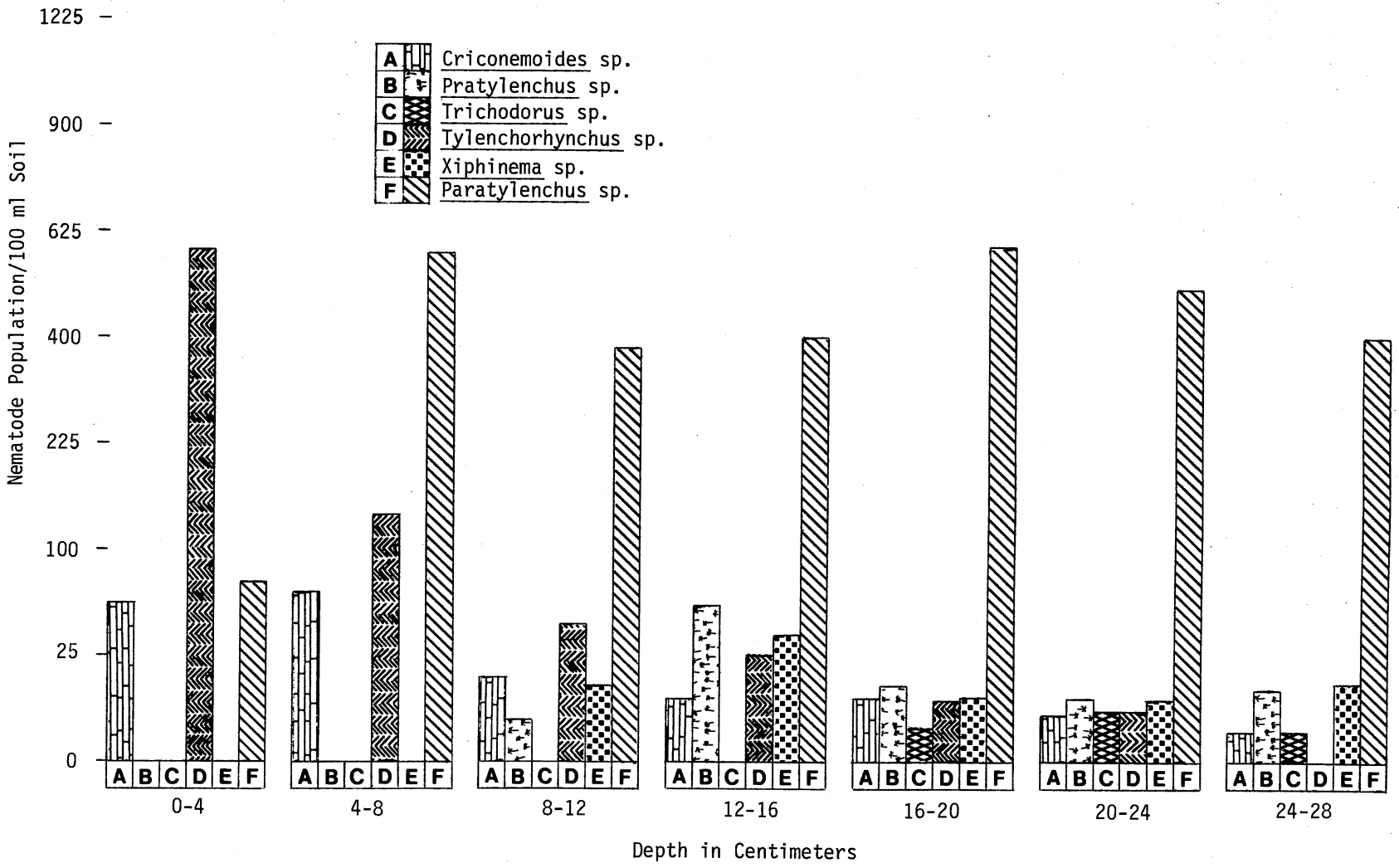


Fig. 28. Population Levels of Nematodes by Soil Depth on June 22, 1976

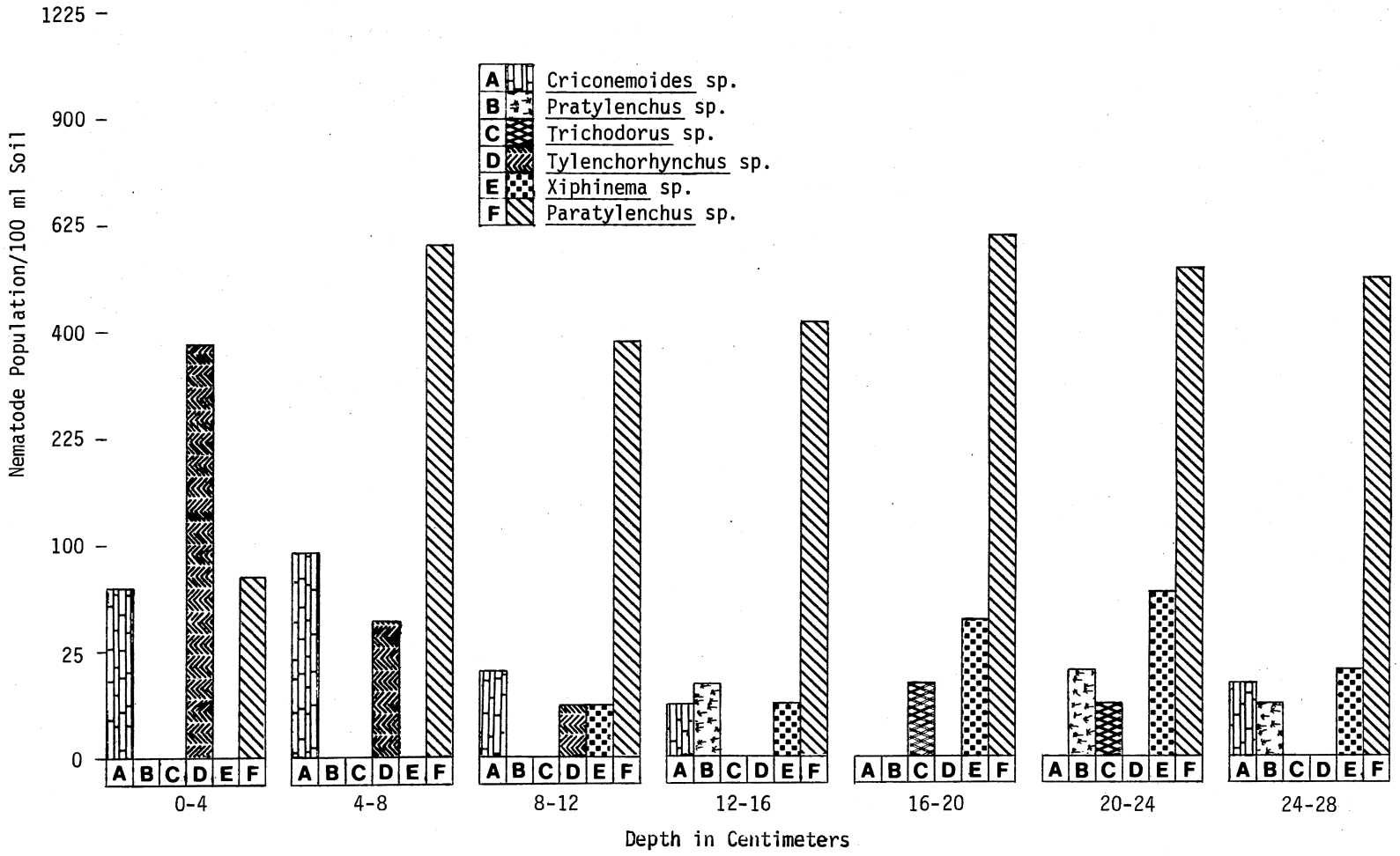


Fig. 29. Population Levels of Nematodes by Soil Depth on June 28, 1976

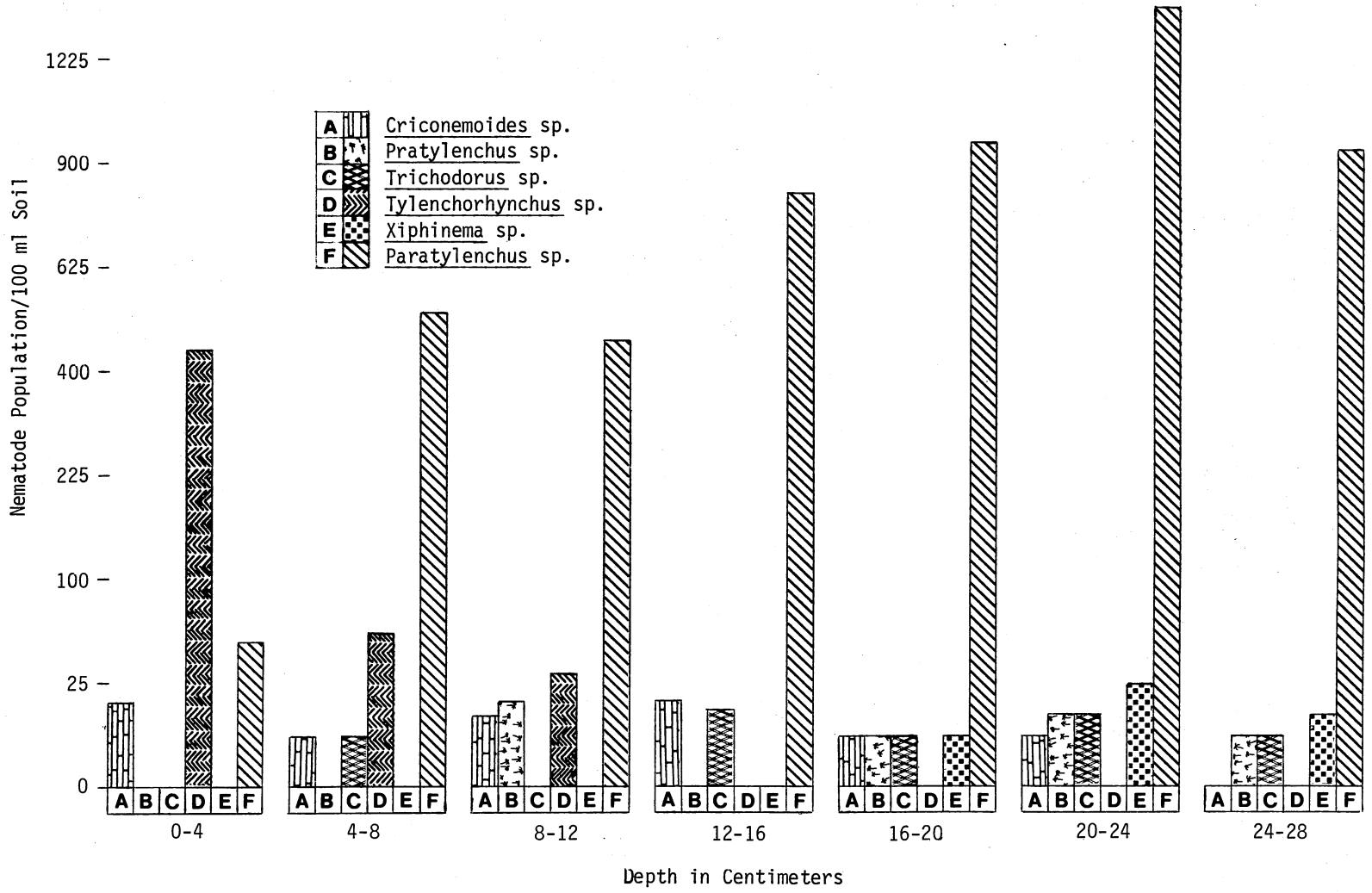


Fig. 30. Population Levels of Nematodes by Soil Depth on July 7, 1976

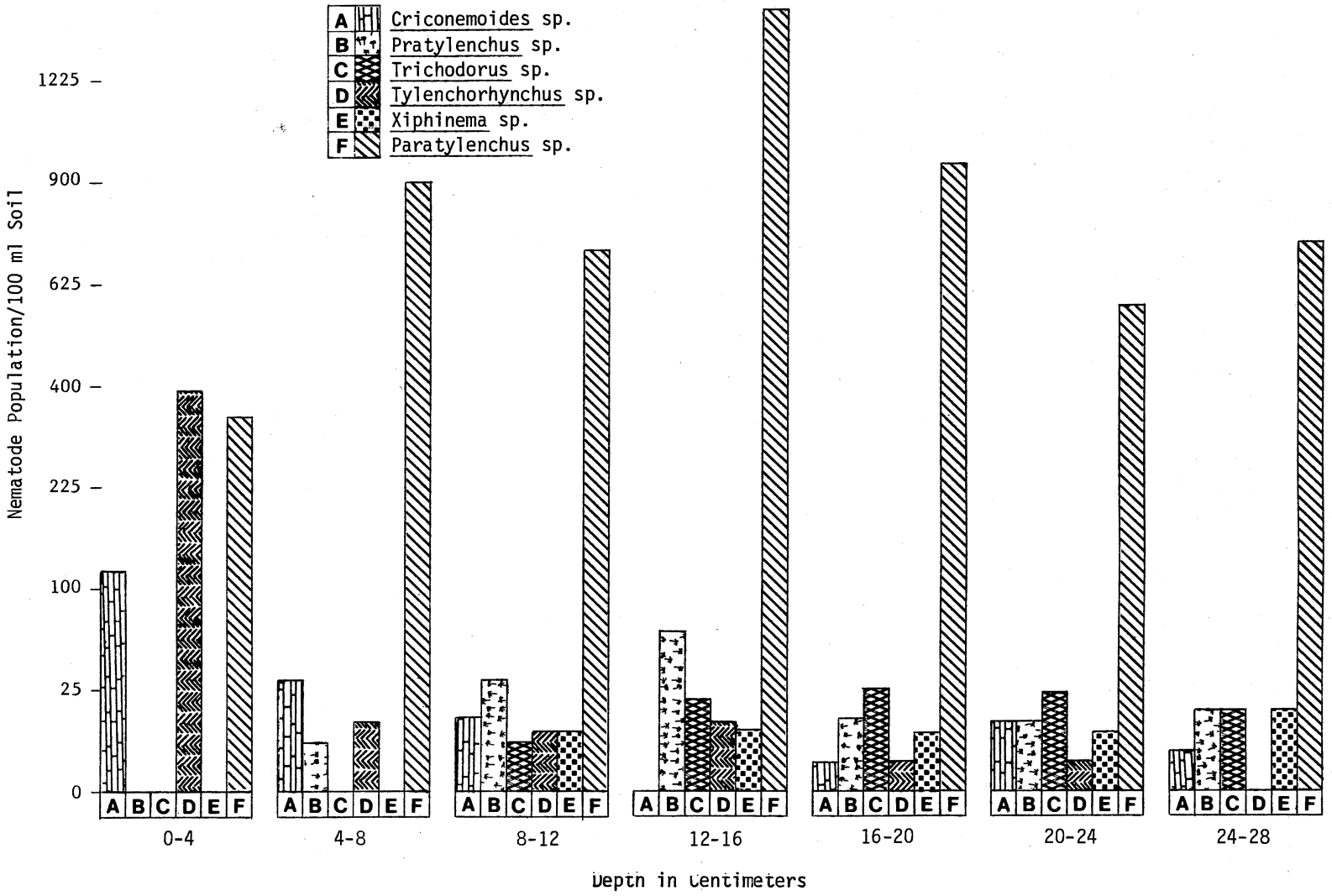


Fig. 31. Population Levels of Nematodes by Soil Depth on July 26, 1976

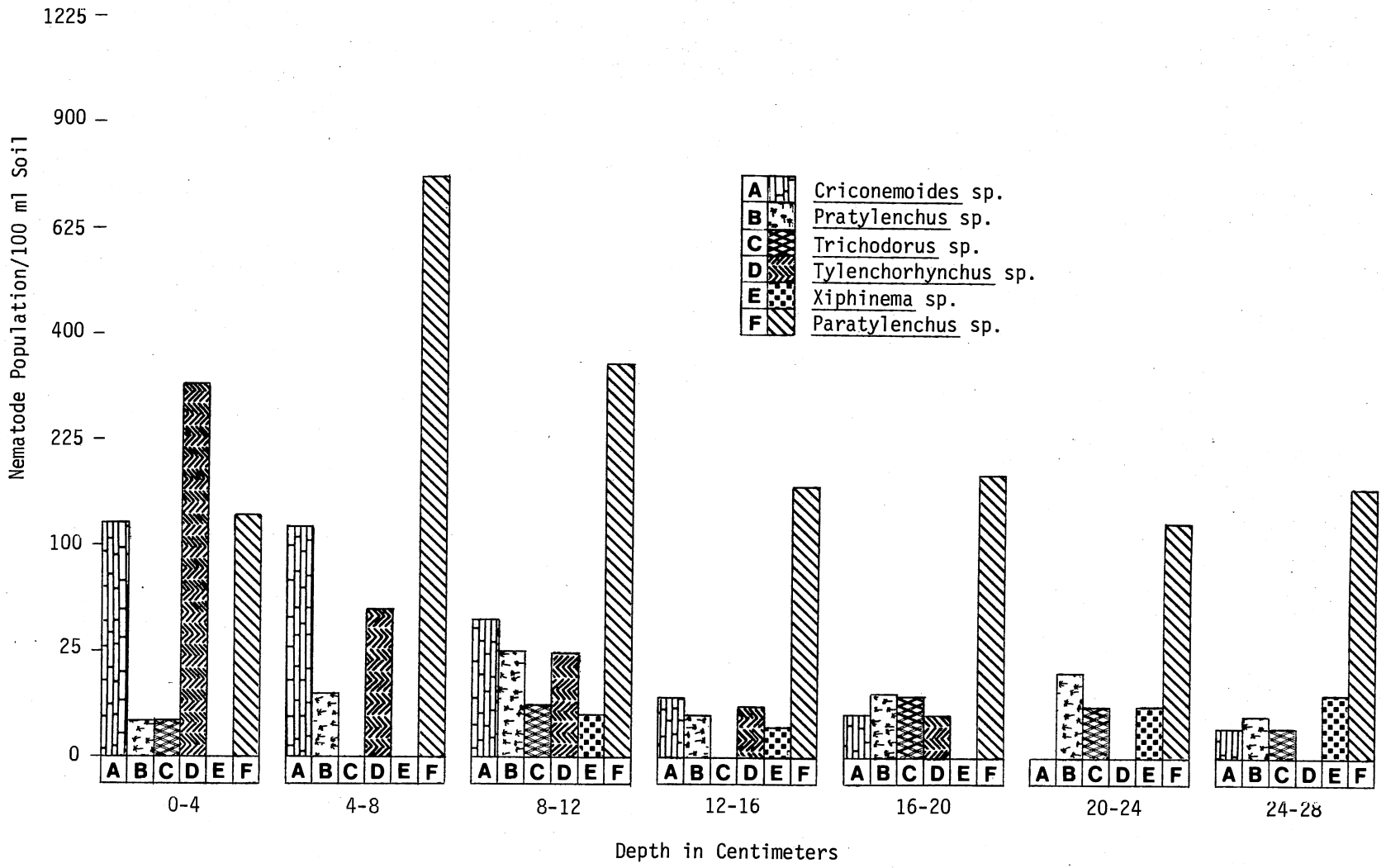


Fig. 32. Population Levels of Nematodes by Soil Depth on August 3, 1976

Although the population of each was not extremely high, when added together they made up a sizable population.

The same trend was noted for Tylenchorhynchus sp., Criconemoides sp., and Paratylenchus sp. at the June 28 sampling date (Fig. 29). There was a greater fluctuation at the deeper zones for all nematodes except Paratylenchus sp. The Xiphinema sp. population was relatively high in the 16 to 24 cm zone as compared to previous sampling dates.

Criconemoides sp. and Paratylenchus sp. populations declined in the zero to four cm zone on July 7, relative to the previous dates. However, the Paratylenchus sp. increased substantially in the four to eight cm zone and continued to increase down to 24 cm (Fig. 30). The Criconemoides sp. population level varied from one zone to the next and was not recovered below 24 cm. The Tylenchorhynchus sp. population decreased at each successive zone and was absent from the 12 to 28 cm zones. Pratylenchus sp. was recovered in the eight to 12 cm; Trichodorus sp. in the 12 to 16 cm zone; Pratylenchus sp., Trichodorus sp., and Xiphinema sp. in the 16 to 28 cm zones.

The Criconemoides sp. and Paratylenchus sp. populations increased notably on the July 26 sampling date as compared to the July 7 sampling date (Fig. 31). The population at the remaining soil depths did not deviate greatly from the previous trend. Again, Tylenchorhynchus sp. populations declined at successive depths. Pratylenchus sp., Trichodorus sp., and Xiphinema sp. were recovered in small to moderate numbers in the eight to 28 cm zones.

On August 3, Criconemoides sp. and Tylenchorhynchus sp. population levels follow much the same trend as previously noted (Fig. 32). Paratylenchus sp. populations increased in the four to eight cm zone;

however, a significant reduction occurred below this zone. Pratylenchus sp. and Trichodorus sp. were recovered in the zero to four cm zone, but they occurred in very low populations. The population of Pratylenchus sp., Trichodorus sp., and Xiphinema sp. fluctuated slightly in the deeper zones but generally followed much the same pattern as previously noted.

In this study zero to eight cm was considered as shallow; eight to 20 cm as intermediate, and 20 to 28 cm as deep rhizosphere zones. The Tylenchorhynchus sp. and Criconemoides sp. were shallow rhizosphere nematodes. The Paratylenchus sp. population beyond the zero to four cm zone was consistently high. Therefore, the Paratylenchus sp. in this was recovered in all zones. Pratylenchus sp., Trichodorus sp., and Xiphinema sp. were intermediate and deep rhizosphere inhabiting nematodes.

Temperatures at the various soil depths did not seem to be a significant factor as shown in Table III. The important aspect is the very low temperatures on January 12, 1976, in which the four to eight cm zone was partially frozen. Despite the frozen condition of the soil, the nematode population was higher than at the following sampling on June 22, 1976.

To determine the significance of the nematode population in the deeper rhizosphere, as opposed to those recovered in the zero to four cm zone, roots were extracted on the May 22 sampling date. The data presented here correspond to those shown in Fig. 27. The root weights as shown in Fig. 33 are not to be considered as precise measurements but only as approximations. The heavy clay-type soil made extraction of root fragments with precision impossible.

TABLE III

SOIL TEMPERATURE AT DIFFERENT DEPTHS WITH REFERENCE TO SAMPLING DATES

Depth (cm)	1/12/76	6/22/76	6/28/76	7/5/76	7/26/76	8/3/76
0-4	3.9	22.8	22.2	23.9	23.3	21.7
4-8	1.1*	22.8	21.7	21.1	22.8	21.1
8-12	1.7	22.2	21.7	20.0	22.8	20.0
12-16	2.2	22.2	22.2	20.0	22.8	20.0
16-20	**	22.2	22.2	20.0	22.8	20.0
20-24	**	22.2	22.2	20.0	22.8	20.0
24-28	**	21.7	21.7	20.0	22.8	20.0

*Soil frozen.

**Not taken.

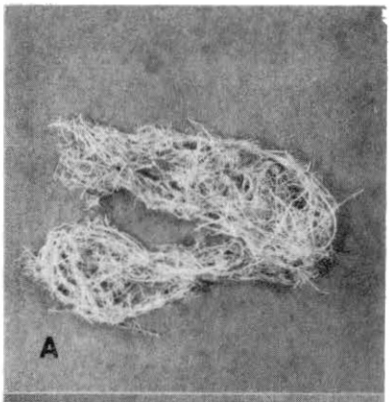
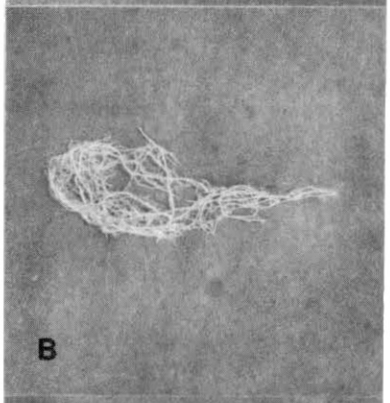
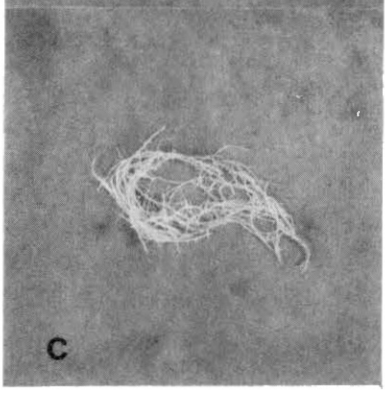
Fig. 33 (A-G). Roots Extracted From 100 ml of Soil at the Different Soil Horizons With Respective Nematode Genera and Population

(A) Roots from zero to four centimeter zone.
(Root weight: .1948 gm).

(B) Roots from four to eight centimeter zone.
(Root weight: .0117 gm).

(C) Roots from eight to 12 centimeter zone.
(Root weight: .0099 gm).

ROOT VOLUME VS. NEMATODE POPULATION

Depth (cm)		Population	Genus
0-4		57	Criconemoides
		0	Pratylenchus
		0	Trichodorus
		591	Tylenchorhynchus
		0	Xiphinema
		72	Paratylenchus
4-8		69	Criconemoides
		0	Pratylenchus
		0	Trichodorus
		138	Tylenchorhynchus
		0	Xiphinema
		591	Paratylenchus
8-12		18	Criconemoides
		4	Pratylenchus
		0	Trichodorus
		42	Tylenchorhynchus
		14	Xiphinema
		384	Paratylenchus

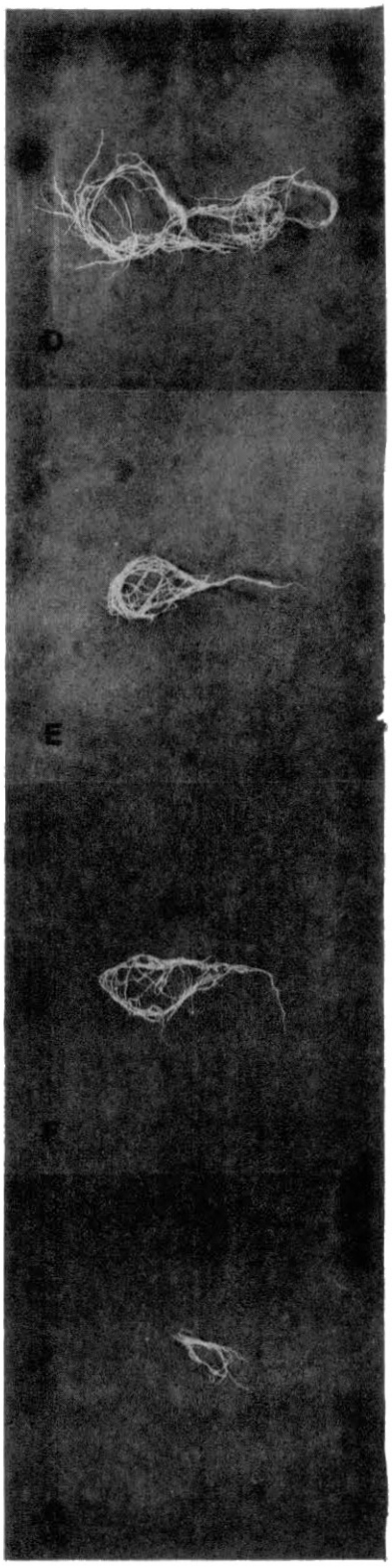
(D) Roots from 12 to 16 centimeter zone.
(Root weight: .0098 gm).

(E) Roots from 16 to 20 centimeter zone.
(Root weight: .0032 gm).

(F) Roots from 20 to 24 centimeter zone.
(Root weight: .0026 gm).

(G) Roots from 24 to 28 centimeter zone.
(Root weight: .0015 gm).

Depth (cm)	Population	Genus
12-16	10	Criconemoides
	54	Pratylenchus
	0	Trichodorus
	24	Tylenchorhynchus
	38	Xiphinema
	420	Paratylenchus
16-20	10	Criconemoides
	14	Pratylenchus
	3	Trichodorus
	8	Tylenchorhynchus
	10	Xiphinema
	598	Paratylenchus
20-24	6	Criconemoides
	10	Pratylenchus
	6	Trichodorus
	6	Tylenchorhynchus
	8	Xiphinema
	510	Paratylenchus
24-28	2	Criconemoides
	12	Pratylenchus
	2	Trichodorus
	0	Tylenchorhynchus
	14	Xiphinema
	396	Paratylenchus



Successively fewer roots were recovered in the deeper soil horizons (Fig. 33). As previously noted, a corresponding decline in populations of Criconemoides sp. and Tylenchorhynchus sp. also occurred. Paratylenchus sp., Pratylenchus sp., Trichodorus sp., and Xiphinema sp. were notable exceptions as their population generally increased with depth. Pratylenchus spp., Trichodorus spp., and especially Xiphinema spp. are recognized as highly pathogenic nematodes (100). The limited number of bentgrass roots in the deeper rhizosphere are presumed to be important to the survival of bentgrass under severe drought conditions. Due to the significant population levels of Pratylenchus sp., Trichodorus sp., and Xiphinema sp. and the limited number of roots in the deeper rhizosphere, these genera become extremely important. Although Paratylenchus sp. are not considered to be highly pathogenic on turf, the population occurring in the deeper rhizosphere is such that would inevitably cause some destruction to the root system. Only .7% of roots by weight occurred in the 24 to 28 cm zone as opposed to those in the zero to four cm zone. In view of the much reduced root system and significant nematode populations at the deeper zones, it is felt that the relationship of nematode population per gram of root weight at a given soil (rhizosphere) horizon might give a more accurate reflection of the potential importance of a given nematode than the customary number of nematodes per volume of soil. Table IV shows the nematodes per gram of root weight at the different soil depths. The data presented in Table IV correspond to that shown in Fig. 28.

TABLE IV

PLANT PARASITIC NEMATODES/GRAM ROOT WEIGHT RECOVERED AT VARIOUS DEPTHS IN 'PENCROSS' BENTGRASS

Depth (cm)	Gram Root Weight /100 ml soil	Total Nematode Population /100 ml soil	Nematodes/Gram Root Weight						
			Criconemoides	Pratylenchus	Trichodorus	Tylenchorhynchus	Xiphinema	Paratylenchus	Total Plant Parasitic Nemas /Gram Root Weight
0-4	.1948	720	292	0	0	3,031	0	369	3,696
4-8	.0117	798	5,892	0	0	11,785	0	50,471	68,149
8-12	.0099	462	1,818	404	0	4,242	1,414	3,878	46,667
12-16	.0098	546	1,020	5,510	0	2,448	3,877	42,857	55,714
16-20	.0032	643	3,125	4,375	937	2,500	3,125	186,875	200,938
20-24	.0026	546	2,307	3,846	2,307	2,307	3,076	196,153	210,000
24-28	.0015	426	1,333	7,999	1,333	0	9,333	263,999	284,000

CHAPTER VII

SUMMARY

1. A field uniformity trial on the nematode population in the field plot demonstrated that the randomized block design was an inappropriate statistical design for experimentation under the conditions of this test, however, a split plot design could alleviate the problem.
2. There was no visual correlation between nematode population and density of bentgrass.
3. The only fungal disease symptom observed during the test period was 'dollar spot' caused by Sclerotinia homoeocarpa.
4. A greenhouse study was used to determine the effects of a nematicide, soil fungicide, or a combination of the two on the growth of 'Penncross' bentgrass in the absence of pathogenic organisms. This study indicated, prior to fertilization, a slight increase, although not significant, in wet foliage weight of bentgrass receiving low rates of all chemicals.
5. In vitro feeding observations demonstrated:
   (A) Tylenchorhynchus sp. fed primarily in the meristematic region although feeding occurred on all root parts except root hairs and root tips.
   (B) Trichodorus sp. fed exclusively at the root tip and

meristematic region of the root.

(C) Criconemoides sp. exhibited little preference with respect to feeding on root system, however, feeding did not occur on root hairs or root tips.

(D) Helicotylenchus sp. fed in mature root zone and in one instance on a root hair. Some cytoplasmic agglutination was observed at the feeding sites of Helicotylenchus sp.

(E) Tylenchus sp. fed on root hairs and in one instance at the root tip. Large amounts of cytoplasmic agglutination were observed at the feeding sites of Tylenchus sp.

(F) Pratylenchus sp. was only observed to feed as a migratory ectoparasite in the maturation zone of the root.

(G) Paratylenchus sp. and Xiphinema sp. were not observed to feed in vitro. Upon inoculation, both genera immediately migrated toward the roots and remained in the vicinity throughout the observed period.

(H) Tylencholaimellus sp. feeding as an ectoparasite on root hairs and epidermal cells was not definitely confirmed.

(I) Psilenchus sp. stayed in close association with the roots, however, no probing of stylet was observed.

6. Vertical nematode distribution studies suggest that:

(A) Traditional bent sampling techniques may be inadequate as nematode populations in the deeper soil profiles are missed.

(B) Nematodes per gram root weight at different soil levels may be a more sensitive indicator of pathogenic potential than nematodes per volume of soil.

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