THE HOST-PARASITE RELATIONSHIPS AND CONTROL OF *Paratylenchus projectus* ON *Iris germanica*

By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The tall-bearded iris, *Iris germanica*, is considered to be one of the most commonly grown perennials in the United States. The number of iris growers in the United States is in the millions (12). The Oklahoma Iris Society has a membership of approximately 5,000 (12). This number represents only a small percentage of the people of Oklahoma who grow iris for enjoyment and for the flowers' aesthetic value. The dollar value of irises in Oklahoma would be difficult to estimate.

The purpose of this study was to make an initial attempt to define the host-parasite relationships of *Paratylenchus projectus*, commonly known as the pin nematode, on *I. germanica* and determine control procedures. *Paratylenchus projectus* was first discovered in Oklahoma on iris in a planting of *I. germanica* cv. 'Snow Goddess' located at the Horticulture Department Nursery at Oklahoma State University in Stillwater, Oklahoma (27). Populations of *P. projectus* of up to 3,900 per 50 ml. of soil were found associated with the root systems of this iris planting (27). 'Snow Goddess' is recognized by many growers as one of the most disease resistant cultivars (12). *Paratylenchus projectus* was extracted from numerous samples of *I. germanica* plantings taken throughout northern Oklahoma during the initial phases of this study, indicating an ubiquitous distribution at least in the region sampled. The number found varied from few to as many as 4,000 per 50 ml. of soil. No specific plant symptoms could be related to the number of P. projectus found; however, there are apparent decreases in vigor of irises and in production of blooms in older plantings. This is an unexplained phenomenon well known to the growers of irises (3, 12). It is possible that this may be attributed in part to parasitism by P. projectus

It is hoped that this study will stimulate research to further define the relationships between P. projectus and I. germanica.

CHAPTER II

REVIEW OF LITERATURE

Taxonomy

Tylenchus macrophallus, collected from Dutch meadows, was described by de Mann in 1880 (33). Micoletzky, according to Thorne (33), erected the genus *Paratylenchus* and described the new species *Paratylenchus bukowinensis* in 1922. Subsequently, T. Goodey redescribed *Tylenchus macrophallus* from an English population and transferred it to the genus *Paratylenchus* (9). The great variability found in the English population caused Goodey to synonymize four other known species with *P. macrophallus* (9). The four species were *P. bukowinensis*; Micoletzky, 1922; *P. nanus*, Cobb, 1923; *P. anceps*, Cobb, 1923; and P. *besoekianus*, Bally and Reydon, 1931 (9). In 1953 Oostenbrink re-established species differences, broke the synonymy and re-established *P. bukowinensis* as the genotype (21). This was the first comprehensive treatment of the genus. An additional six species, including *P. projectus*, Jenkins, 1956 (14), have been described since 1953 which brought the total number of species to sixteen by 1959 (1). Two new species were added to *Paratylenchus* in a 1960 review of the genus by Tarjan (31). Raski erected the genus *Gracilacus* to contain those *Paratylenchus* spp. with stylet lengths greater than 48μ (23). Great variability is frequently encountered in the stylet lengths of the nematodes in this group and the description of *Gracilacus* was based almost entirely on this character. As a result Gracilacus was reduced to a junior synonym of Paratylenchus by Bryeski (28) and Lidiqi and Goodey (2). The most recent taxonomic treatment of the genus Paratylenchus is a 1965 review by Geraert in which he attempted to evaluate the diagnostic value of the morphological characteristics of the group (7). Geraert's treatment differed from those of previous workers in that he did not attempt to distinguish between all species but placed the most closely aligned species into "family-groups" and started that definitive diagnosis of any Paratylenchus population is ". . . almost impossible."

The morphological characteristics upon which species identification is based are so subtle that the taxonomy of the group is confused and Paratylenchus spp. remain among the most difficult of the Nemata to differentiate (7).

Bionomics

The study of the bionomics and pathogenicity of the pin nematodes, Paratylenchus spp., has been limited. This is due in part to their being the smallest of the plant parasitic nematodes, 180μ to 600μ in length, resulting in their passing through even the fine mesh screens used in standard extraction techniques and being overlooked in samples. Of the Paratylenchus spp. studied to date P. projectus, P. hamatus and P. dianthus have received the most attention.

The host range of P. projectus is apparently quite wide, as in one study (4) populations were either maintained or increased on 89 of 101 potential host plants tested. Under field conditions pin nematodes often occur in large numbers on both perennial and annual plants (16). They appear to be more prominent in the temperate zones rather than in the more tropical regions (4). Faulkner (6) reported that maximum increase in populations of *P. projectus* occurred at 29°C while lower numbers occurred at 26°C and declined further at temperatures of 23 and 17°C. Faulkner concluded that periods of higher temperatures favor the reproduction of *P. projectus* and may be an important factor determining its distribution.

The life cycle of *P. projectus* consists of four larval stages. The fourth "resistant" or "survival" stage apparently does not feed (26). This preadult or resistant stage is common only to the genus *Paratylenchus* among the plant parasitic nematodes (26). *Paratylenchus* spp. have been found to be resistant to temperature and moisture extremes as well as to have the ability to withstand long periods in the absence of food (16, 17, 26). Rhoades and Lindford, 1961, showed that *P. projectus* existed four years, seven months in fallow soil (26). This suggests that *P. projectus* could not effectively be controlled by fallowing techniques. Reuver, 1959, determined that the longevity of *P. amblycephalus* was reduced in soils of high moisture content (24). A study conducted by Norton, 1959, suggests that increased hatching of *P. projectus* eggs occurs after periods of rainfall (19). Greenhouse studies of *P. projectus* on tall fescue and tobacco resulted in stunting of top growth, increased root system, and increase of tillering of fescue (4). Populations of 40,000 per plant produced no observable histological evidence of feeding (4).

General Associations of *Paratylenchus* spp.

Paratylenchus are primarily ectoparasites of roots. They have been observed to occasionally feed as endoparasites, entering the exit points of lateral roots (26). The feeding habits of Paratylenchus were first observed by Lindford, et al. (26), but the first evidences of pathogenicity were reported by Lownsbery, et al., in 1952 (18). They found that stunting and extreme chlorosis of celery was associated with high populations of *P. hamatus*. Lownsbery, et al. (18), also found that the *P. hamatus* population proportionately decreased as the root system declined. *Paratylenchus hamatus* has also been reported associated with the development of chlorosis and undersized fruit of figs (32).

Paratylenchus populations have been found to cause significant reductions in plant growth and reduction of the length of flowering duration in spearmint and peppermint (6).

Paratylenchus dianthus has been associated with the decline of carnation (15).

Paratylenchus spp. have been found to be associated with many different higher plants and in substantial numbers (8, 29, 30). Evidence of pathogenicity is limited to a few plant types. Lindford, et al., found up to 900 individuals of *P. minutus* per gram of soil around pineapple roots in Hawaii without obvious detriment to the vigor of the plants (17).

CHAPTER III

HOST-PARASITE RELATIONSHIPS OF

Paratylenchus projectus ON *Iris germanica*

The objective of this study was to determine: (a) the pathogenicity, (b) the population in relation to soil and root depth, and (c) the preference of feeding areas of *Paratylenchus projectus* on *Iris germanica*.

METHODS AND MATERIALS

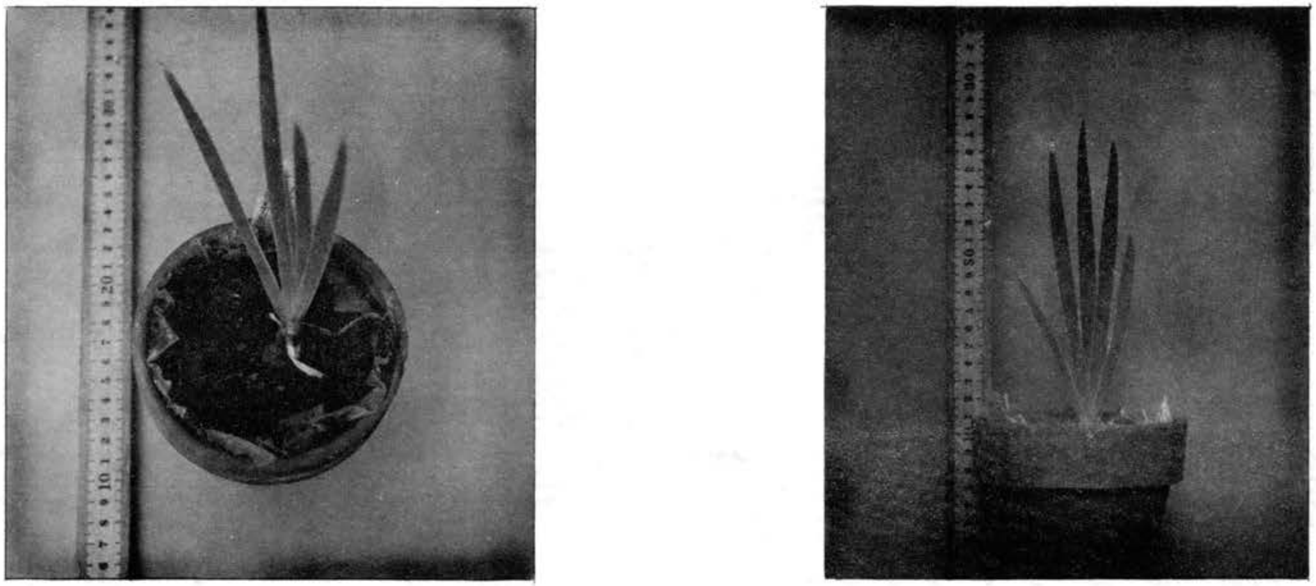
Experiment I

Pathogenicity

Current season offshoots were obtained from an *I. germanica* cv. 'Snow Goddess' planting at the Horticulture Department Nursery at Oklahoma State University in Stillwater, Oklahoma. The offshoots selected were approximately 0.5 cm. in diameter and weighed 0.3g. to 0.8g. Each offshoot was washed clean of soil and the dead material removed. If roots were present on the offshoots, they were removed at the point of emergence from the surface of the offshoot. Offshoots were disinfected by soaking them for one minute in ten percent "Chlorox" solution.

Offshoots were placed in a plastic container 11 cm. in diameter and 8 cm. in depth. Approximately one-half inch of sterilized builders sand was used as a potting medium. The containers were covered with plastic film which was secured by means of a rubber band placed around the top. The offshoots were allowed to root and grow in the containers for six weeks to deplete stored food in the rhizome. The *P. projectus* used for inoculum were obtained from the same planting as the offshoots. Large quantities of soil were processed by using a modification of the Christie-Perry technique (5, 20). The nematodes extracted were concentrated and stored at 1.5°C until sufficient inoculum was obtained. Aliquots of 10 ml. were taken from the concentration and placed in counting dishes. All plant parasitic nematodes except *P. projectus* were removed by the use of a dental pulp canal file with the aid of a stereoscopic scope. The cleaned *P. projectus* suspension was concentrated and stored at 1.5°C until sufficient inoculum was obtained. Each inoculum level was determined by aliquot. Water from the suspension containing no nematodes was collected following concentration and stored for use on plants of the check plots.

The offshoots were transplanted after six weeks to standard four-inch clay pots lined with plastic film containing a sterilized soil mixture of three parts soil to one part sifted builders sand. The plastic film (Figure 1) was used to prevent the root system from drying and inhibiting nematode activity next to the sides of the clay pot. The offshoots were inoculated at the time of transplanting. A 10 ml. water suspension of the desired nematode concentration was pipetted onto the roots and covered with the soil mix. Inoculum levels of 1,000, 10,000, 20,000 and 40,000 nematodes per plant were used. Water from the *P. projectus* suspension was pipetted onto the roots of the check plants in each plot. Each inoculum level was duplicated and randomized. The plants were placed in a greenhouse in which temperatures varied from 19°C night and 26°C day during the test period. The plants remained in



A B

Figure 1. Plastic film lining of clay pots. A. Top view of clay pot. B. Side view of clay pot.

the greenhouse for five months.

Measurement of top growth was recorded each week for the duration of the study.

At the termination of the study, each plant was washed free of soil. The soil and water were retained from the washing and processed by a modification of the Christie-Perry technique (5, 20). After 48 hours the number of P. projectus nematodes were counted. The plants were blotted with paper towels and total fresh weights recorded. The roots were then removed from the rhizome and weighed. A 10 cm. segment was removed from each of three different main roots originating from the rhizome. Each root segment was placed on filter paper in a pan filled with sufficient water to cover the root segment. A piece of plate glass was placed over each root segment, which was then photographed with a 35 mm camera. Positive transparencies were prepared and mounted. Tracings of each root segment and its laterals were prepared by projecting a positive transparency at 2.5x magnification with a "Kodak Carousel Projector." All measurements were taken from these tracings. Diameters and lengths of the primary, secondary, tertiary, quaternary and pentanary lateral roots were measured. Diameter measurements were taken; (a) close to the point of derivation; (b) at approximately one half the length; and (c) near the tip end. The three measurements were averaged for determination of the surface area. The length of all lateral roots of each type was determined by calibrating a map measure and following the tracing of each and converting the map measure units to centimeters. The square cms. of surface area of each root type was determined by converting the average diameter to circumference and multiplying by the total length.

Root segments from plants of each of the inoculum levels were stained in acid fuchsin and stored in lactophenol for observation of possible feeding sites.

Experiment 2

Soil Depth

This experiment was conducted at the Iris germanica cv. 'Snow Goddess' planting in the Horticulture Department Nursery at Oklahoma State University.

A two-foot length of an infested row of plants was selected. Trenches approximately two feet deep were dug around the row segment. A sharp machette was used to remove the tops of the plants, rhizomes, and soil in increments. Seven 2 in. soil increments were removed and placed in non-vented polyethylene bags. Each composite sample was mixed thoroughly and two 50 ml. aliquots taken. The samples were processed by a modification of the Christie-Perry technique (5, 20). The number of P. projectus in each aliquot was recorded and the two averaged.

Experiment 3

Feeding Area Preference

Soil from infested I. germanica cv. 'Snow Goddess' was placed in a "modified root observation box" (13). Current season offshoots of 'Snow Goddess' were planted in the first inch of soil and the root observation boxes placed at an approximate 35 degree angle with the glass observation surface down to promote root growth on the observation surface. The plants were placed in the greenhouse with varying temperatures as described in Experiment 1. The plants remained in the greenhouse for four weeks. The treatments were replicated four times.

At the termination of the experiment the sides of the root observation boxes were carefully removed. Tapping on the glass before removal helped to eliminate sticking of the soil to the glass and disturbing the position of the root system. A razor blade was used to separate the lateral roots from the more mature portion of the root system. Ten lateral roots of the same length (2 cm.) and the soil within 0.3 cm. of the root were removed from each replication. Likewise, ten segments of the mature roots and adjacent soil were removed. Each mature root segment, lateral root, and adjacent soil were removed by the use of a microspatula making sure to obtain only the soil within approximately 0.3 cm. surrounding them. Roots and soil from each replication were placed in separate 50 ml. beakers and the contents processed by a modified Christie-Perry technique (5, 20). The number of P. projectus was recorded.

Results and Discussion

Experiment 1

Pathogenicity

Plant height measurements indicated no differences among treatments nor were any visual differences in foliage color noted (Figure 2). Above ground symptoms resulting from plant parasitic nematode feeding on the root system are mostly nonspecific. In some instances plants can be severely damaged by high populations without producing above ground symptoms and particularly under optimum greenhouse conditions (18). Above ground symptoms other than reduced size and vigor have not

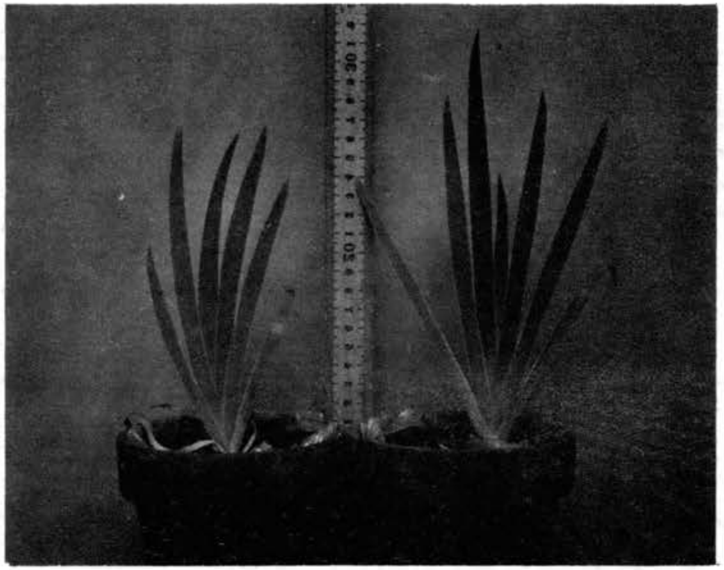


Figure 2. A comparison of the effect of an inoculation of 40,000 *Paratylenchus projectus* per plant on plant height.
(Left): 40,000 inoculum level.
(Right): Check plant.

been associated with P. projectus in Oklahoma iris plantings.

Plant fresh weight (Table 1) indicated no differences among treatments. The check plants weighed slightly more than did the inoculated plants, even though the data of root surface area yielded significant differences at the 0.05 percent level (Figure 3). Figure 3 shows that there was a significantly greater production of root surface in the check plants than in the inoculated plants. Figure 4 shows the average amount of root surface area produced by lateral, secondary lateral, tertiary, quaternary and pentanary roots. Root diameter of inoculated plants was greater than that of check plants. This may be the reason fresh weight of inoculated plants was approximately the same as the fresh weight of the check plants. The larger diameter of the inoculated roots increased the root weight, but due to the shorter length and lack of lateral branching (Figure 5, 6, 7, 8, 9), the inoculated roots had a reduced surface area. The production of root hairs is primarily on the lateral roots rather than the main root. The efficiency of the root system was reduced by the loss of epidermal surface area and root hair production. These could be factors contributing to the reduced size and vigor of I. germanica when in association with high populations of P. projectus, despite the reputed disease resistance of the cultivar 'Snow Goddess' (12). Paratylenchus projectus has been shown to be a primary pathogen in the reduction of growth in celery (18); on the other hand it has been associated with increased growth of tall fescue (4). The reduced efficiency of the root system of I. germanica cv. 'Snow Goddess' caused by P. projectus may not be evident in the parent rhizome. The deleterious effect should be more evident in the new offshoots produced by the parent rhizome. The vigor of the offshoots

TABLE I

EFFECT OF VARIOUS *Paratylenchus projectus* INOCULUM LEVELS ON *Iris germanica* cv. 'SNOW GODDESS' FRESH ROOT WEIGHT

Inoculum Level	Plant Number	Initial Plant Weight	Initial Av. Weight	Final Plant Weight	Av. Final Weight	Av. Weight Increase
40,000	1	0.60g.	0.63g.	8.52g.	7.44g.	6.81g.
40,000	2	0.65g.		6.36g.		
20,000	1	0.50g.	0.50g.	4.92g.	4.92g.	4.42g.
20,000	2	0.50g.		0.00		
10,000	1	0.50g.	0.40g.	8.45g.	7.15g.	6.75g.
10,000	2	0.30g.		5.84g.		
1,000	1	0.30g.	0.35g.	8.87g.	6.88g.	6.53g.
1,000	2	0.40g.		4.99g.		
CHECK	1	0.60g.	0.70g.	12.06g.	10.51g.	9.81g.
CHECK	2	0.80g.		8.96g.		

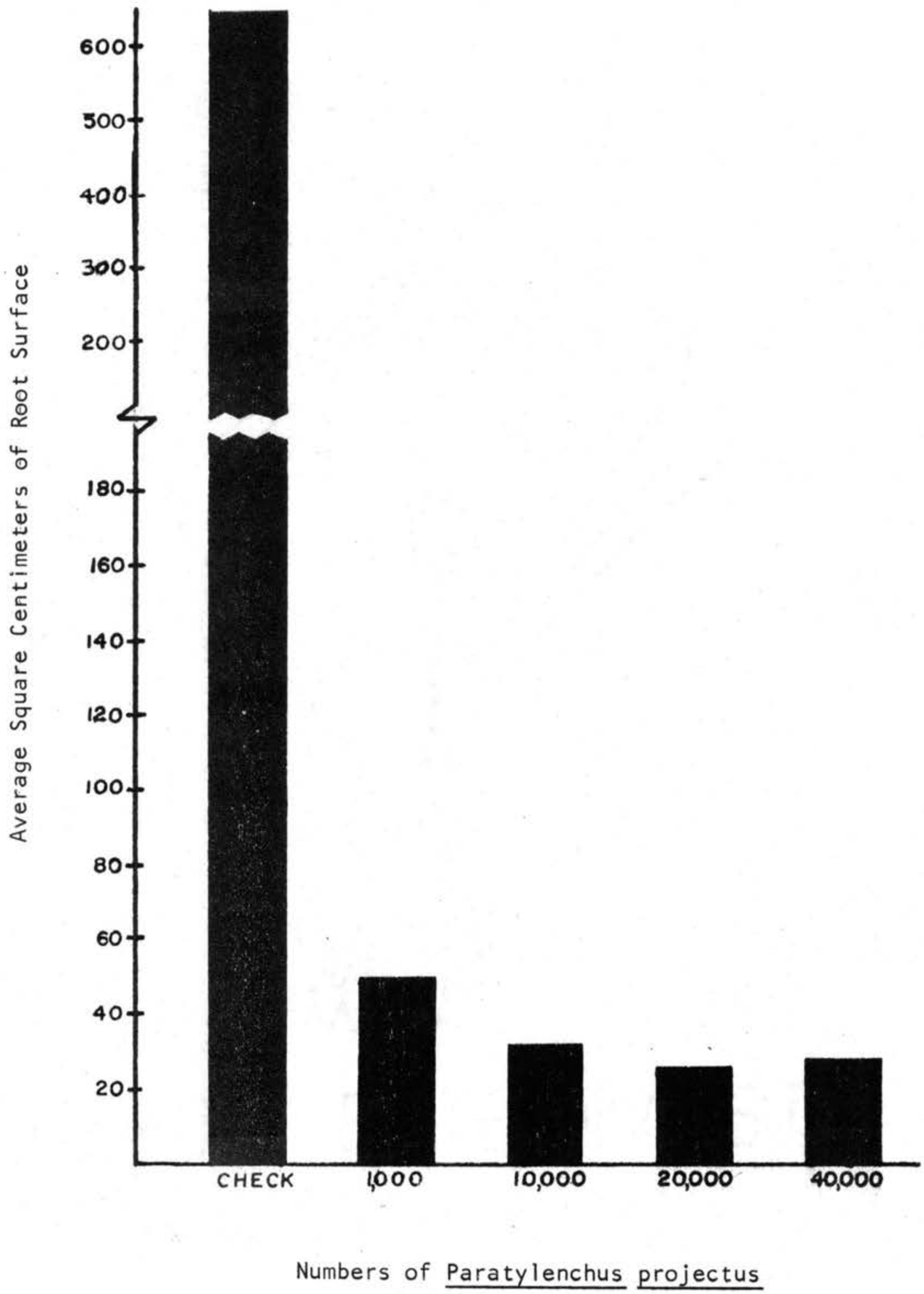


Figure 3. Effect of _Paratylenchus projectus_ on the total root surface area produced by _Iris germanica_ cv. 'Snow Goddess'. (Values significant at .05 percent)

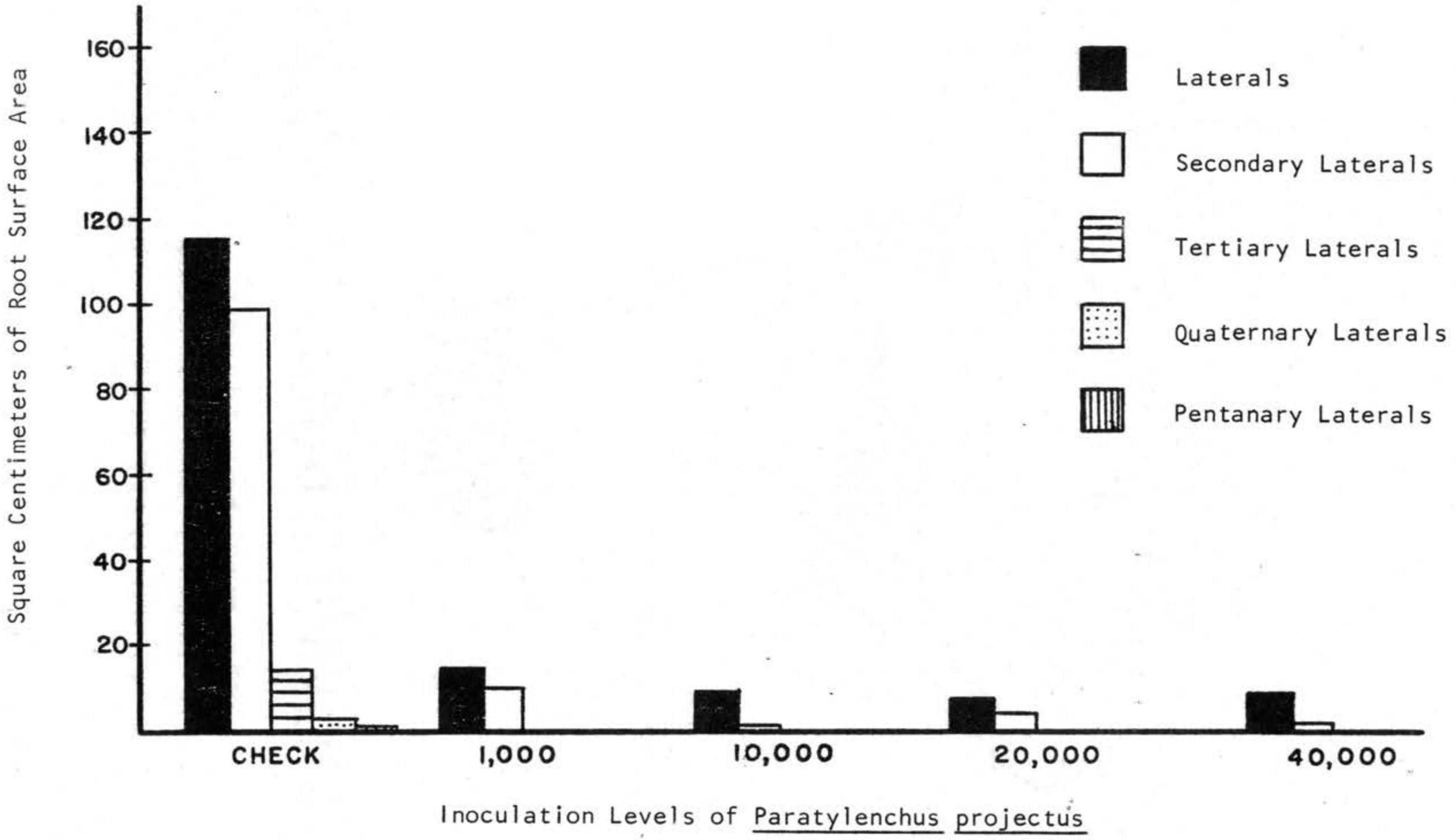
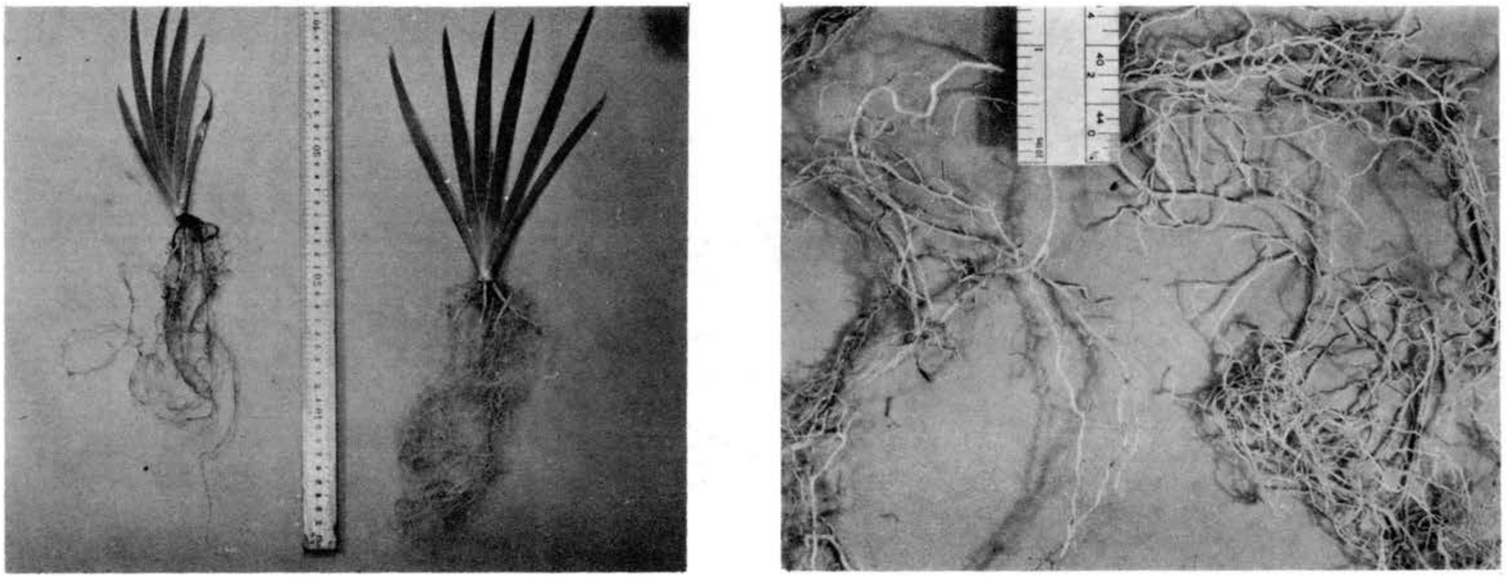


Figure 4. Effect of *Paratylenchus projectus* on lateral root development on *Iris germanica* cv. 'Snow Goddess'. (Values significant at .05 percent)



A B

Figure 5. Comparison of *Iris germanica* plants, inoculum levels of 40,000 and 0 *Paratylenchus projectus*. A. Entire plants; (Left): 40,000 and (Right): 0 inoculum levels. B. Enlarged view; (Left): 40,000 and (Right): 0 inoculum levels.

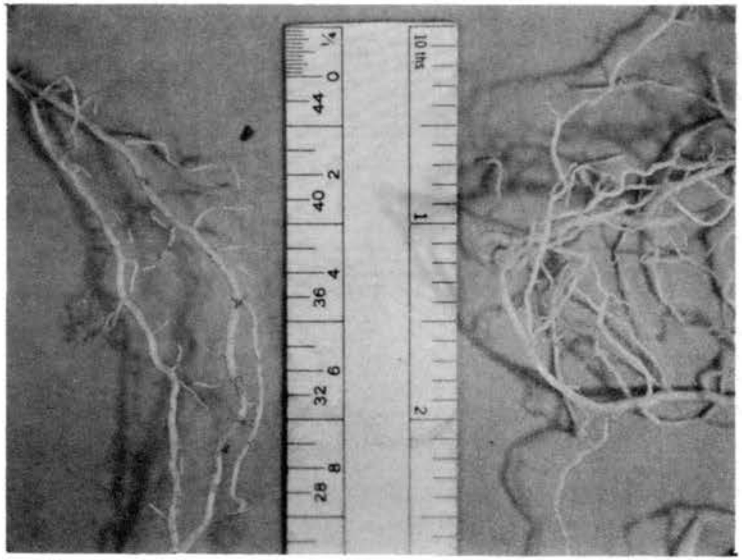


Figure 6. Enlarged view of Figure 5-B. Note: The lack of lateral branching in inoculated plant on the left.

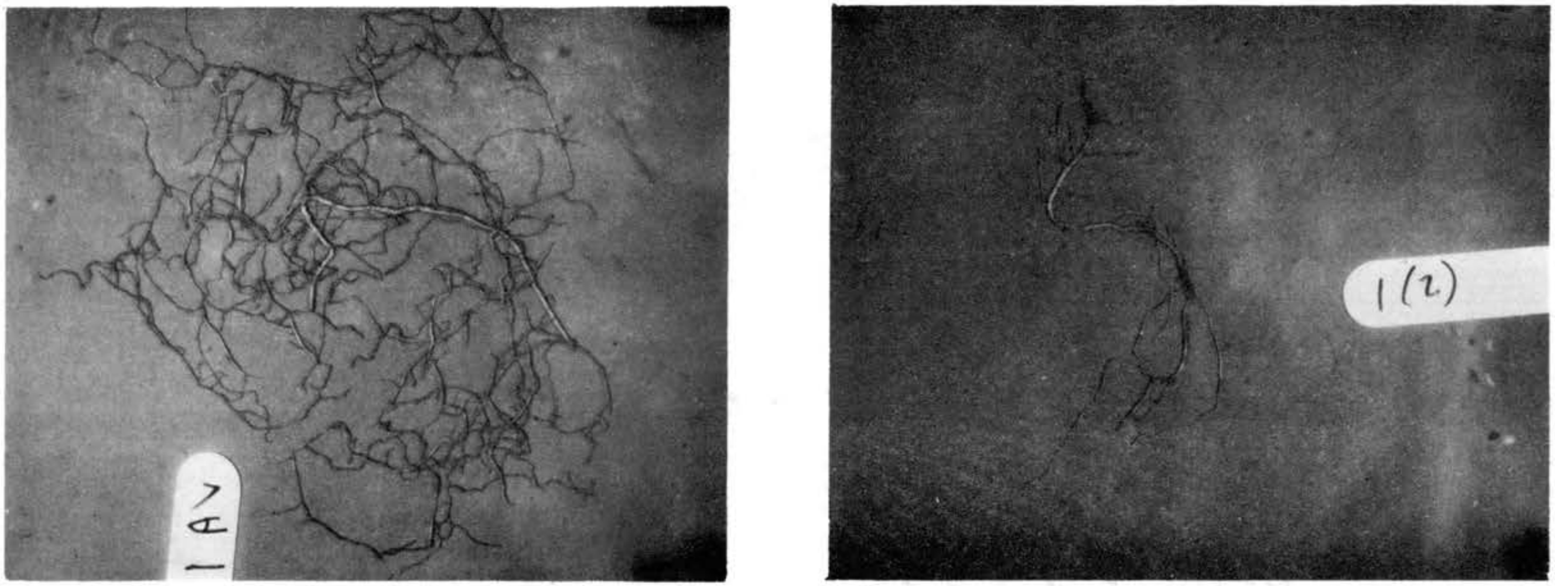


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Figure 7. Effects of Paratylenchus projectus on lateral branching of Iris germanica cv. 'Snow Goddess' roots. A. 10 centimeter section from non-inoculated plant. B. 10 centimeter section from 1,000 inoculum level.

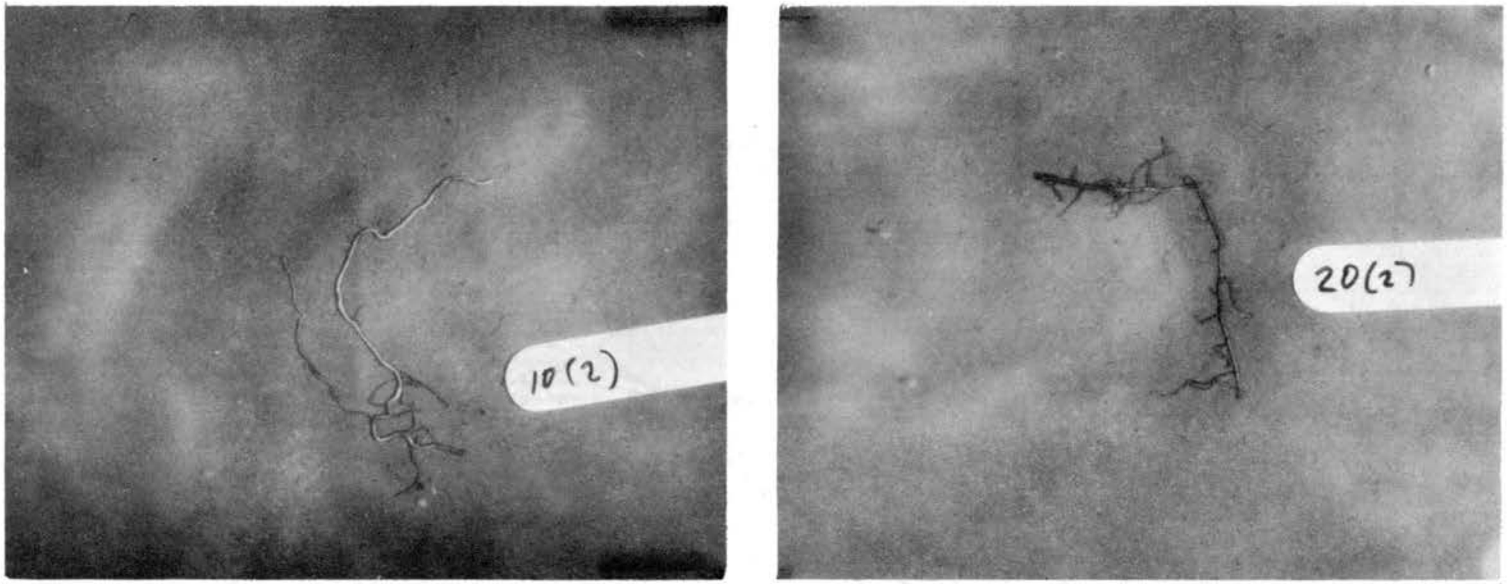

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Figure 8. A 10 centimeter section of main root. A. 10,000 inoculum level. B. 20,000 inoculum level.

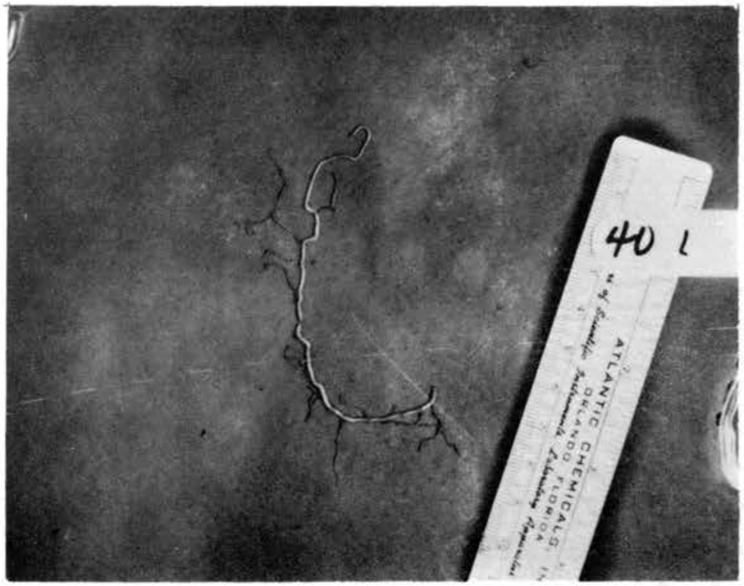


Figure 9. A 10 centimeter section from a main root of a 40,000 inoculum level.

produced by the parent rhizome is dependent upon the health and vigor of that parent (3). Due to the type growth of the rhizomes of iris, noticeable reduction in plant vigor may or may not be recognized until after several growing seasons. There has been an unexplained reduction in plant vigor in the I. germanica 4-5 years after planting (3). It is believed by many iris growers that iris is a "heavy feeder" and depletes the soil of nutrients in 4-5 years. To determine whether a portion of this reduction in vigor could be attributed to P. projectus would require further research.

Stained roots from the different inoculum levels revealed no specific evidence of cellular reactions to the feeding of P. projectus, although very darkly stained epidermal cells at random locations were commonly found in the inoculated plants. Most of the P. projectus remaining with the roots through the staining process were found in the main roots under the epidermal layer. The principal point of access to the cortical region of the root was apparently through those areas of the epidermis ruptured by lateral root initiation. Few P. projectus were found under the epidermal layer of lateral roots.

Experiment 2
Soil Depth

Figure 10 shows the distribution of the P. projectus population around the root systems of I. germanica cv. 'Snow Goddess' at various soil depths.

This experiment was conducted on one planting of I. germanica, and the data obtained are not necessarily applicable to other situations. The time of year of sampling, plant vigor, and age of the planting are a

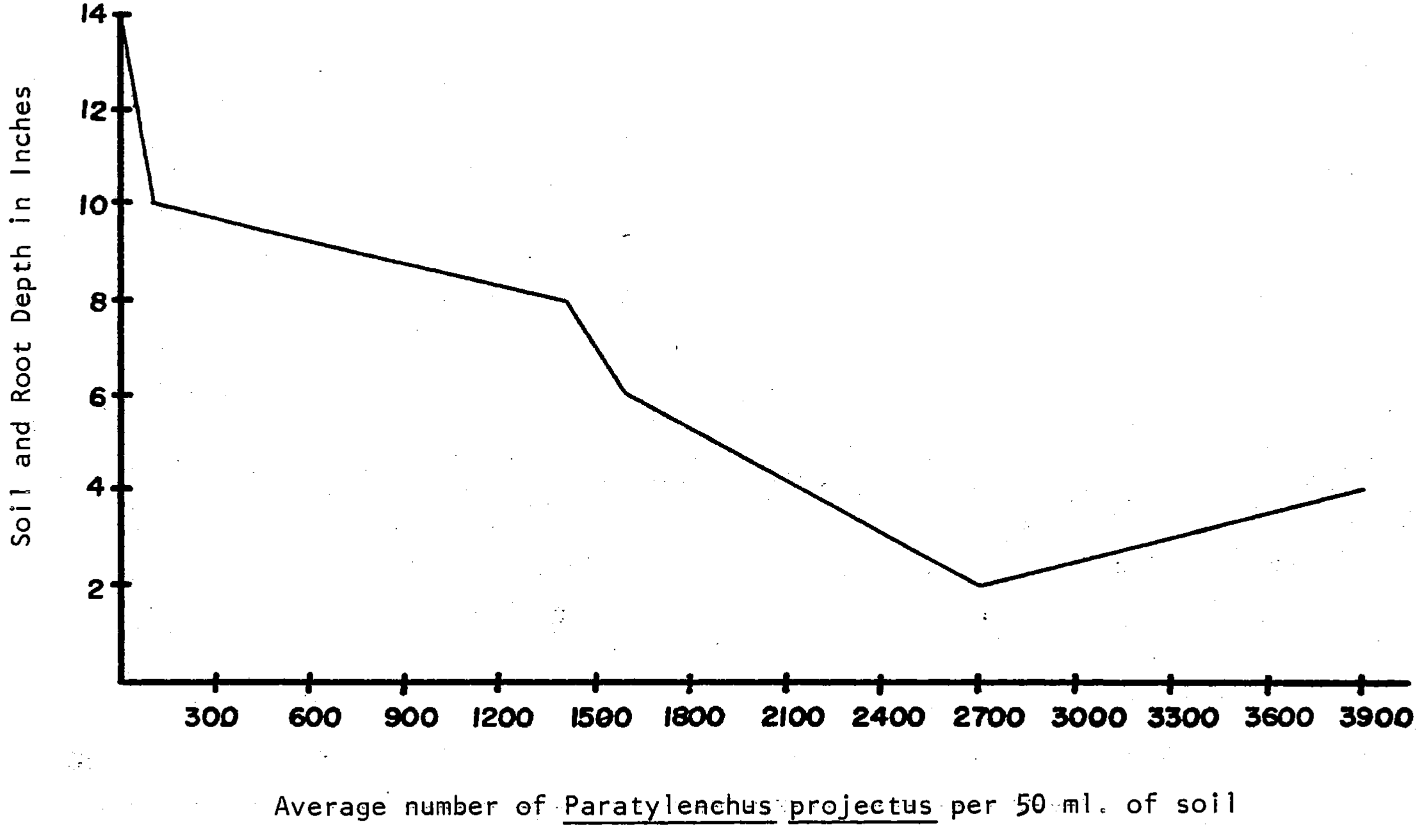


Figure 10. Number of *Paratylenchus projectus* at certain depths.

few of the complicating factors that might be expected to affect population distribution. Assumptions can be made about the population distribution in the soil, but to distinguish between the direct influences and the indirect effects on the plants is difficult.

The data collected from the planting show that at the 0 - 2 in. soil depth no P. projectus were found. This is the zone in which the more mature portions of the root system are found and may indicate a feeding area preference. At this depth the soil dries readily and may not provide enough moisture to permit activity. This depth is also subject to wide fluctuations in temperature and these extremes may inhibit nematode activity. The 2 - 4 in. soil depth contained the highest number of P. projectus. This zone contains the largest percent of the root system in which lateral branching of the roots occurs. Feeding areas are more prevalent and the soil atmosphere and temperature are more stable. There was a continued decline in the number of P. projectus recovered with each additional depth increment (4 - 6, 6 - 8 8 - 10 and 10 - 12 in.) as the amount of roots decreased. The lack of available feeding sites probably inhibits higher populations from occurring at the lower soil depths (8 - 12 in.).

Norton (19) found that P. projectus populations were lower during dry periods and that they increased immediately following rainfall. Periodic sampling of the 'Snow Goddess' plantings support Norton's findings in that P. projectus populations increase after periods of high moisture. Populations were observed to increase during the periods between late March and June and decreased during the drier months of July, August, and September. Increases in population occurred during October and November and remained relatively constant until March. It was also noted that between March and September the number of adults increased. Populations recovered during the winter consisted almost entirely of fourth stage larvae. Of the two periods in which P. projectus populations increased, the greatest increase occurred during October and November. This also supports the findings of Rhoades et al. (25). The 2 - 4 in. depth would contain adequate moisture and respond more rapidly to temperature changes than the deeper soil strata. This may be one of the reasons for the highest population occurring at the 2 - 4 in. depth. The 2 - 4 in. depth would have lower temperatures during the winter months. Rhoades et al., (26) found that the preadult larvae of P. projectus are tolerant to desiccation and low temperatures. This finding is supported by the greater number of adults recovered during the spring population increase and the greater number of preadults recovered during the fall population increase. The greater number of adults during the spring increase could possibly be attributed to the root diffusates secreted by the iris during active growth.

Experiment 3

Feeding Area Preference

The results of this experiment are shown in Figure 11. The number of P. projectus extracted from the lateral roots were 10 fold of that extracted from the main roots. The data in Experiment 1 indicates that P. projectus prefer lateral roots as feeding areas.

There is an indication that P. projectus enter the ruptured epidermis at the point of lateral root emergence when availability of feeding areas is limited. This could be a possible mechanism of

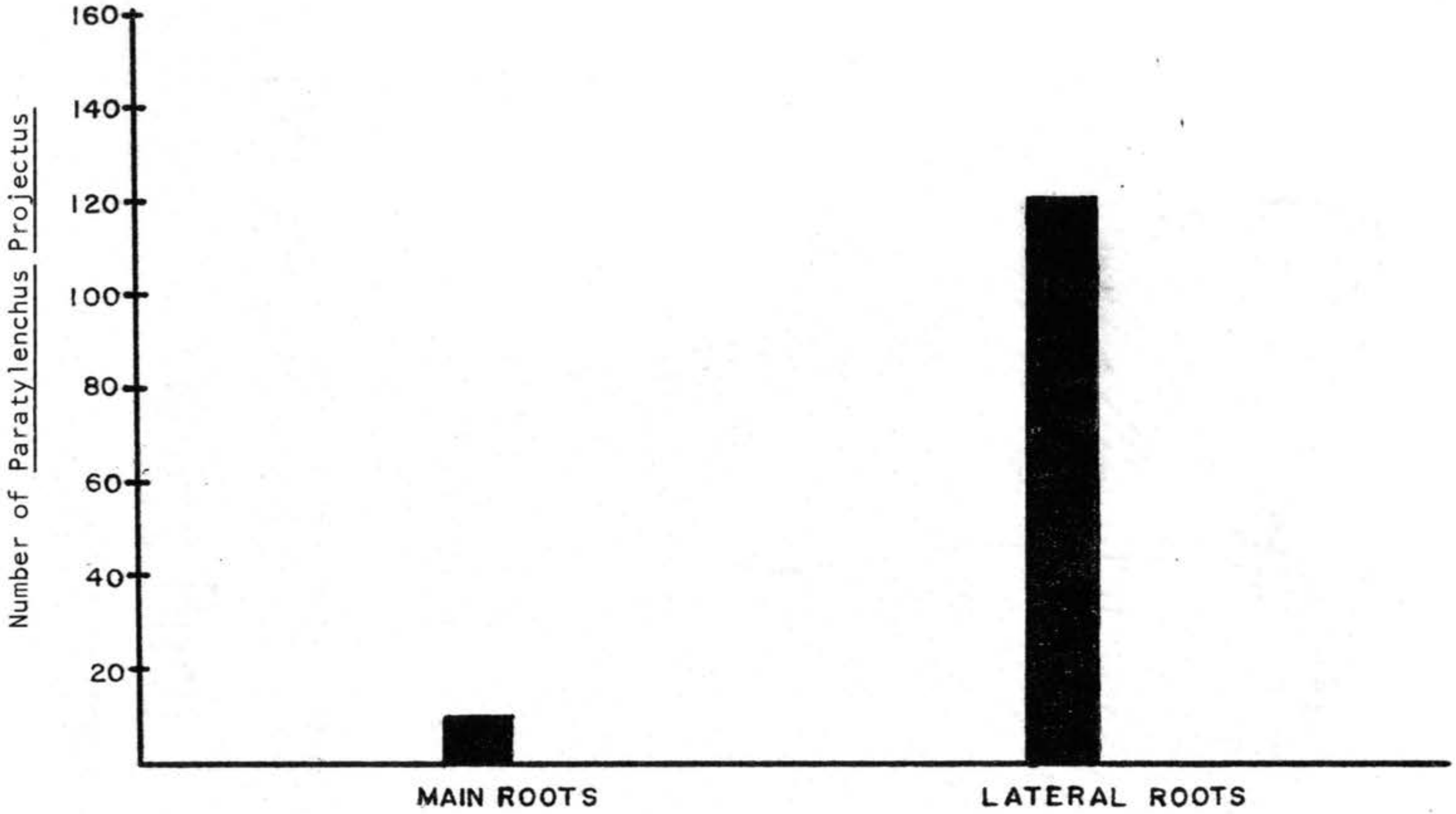


Figure 11. Average number of *Paratylenchus projectus* extracted from main roots and lateral roots of *Iris germanica* cv. 'Snow Goddess'. (See Methods and Materials, Experiment 3)

survival when the population increases beyond the carrying capacity of the roots. This was observed in the higher inoculum rates. Fewer *P. projectus* entered into the cortical layer in the main roots of the 1,000 inoculum level.

CHAPTER IV

IN-VITRO

The objective of this study was to determine the feeding habits of *Paratylenchus projectus* on *Iris germanica*.

METHODS AND MATERIALS

Offshoots of *I. germanica* cv. 'Snow Goddess' were removed from the parent rhizome. All offshoots were approximately 0.5 cm. in diameter and 1.5 cm. in length. All existing roots and loose debris were removed from the offshoot. The offshoots were immersed and washed in a beaker containing distilled water. They were then disinfected by immersing for five minutes in Rada's solution and rinsing in distilled water.

A 0.75 percent water agar was prepared and poured into a 10 cm. x 1.5 cm. plastic petri dish in a layer approximately 0.5 cm. in depth. The agar was allowed to cool and a small section of the agar approximately the size of the offshoot was removed. The offshoots were then placed in the depression formed. The petri dish covers were taped to prevent the lifting of the covers by shoot growth. The petri dishes were placed in a non-vented polyethylene bag to prevent evaporation and the plants allowed to grow under natural light supplemented with incandescent and flourescent light. After the roots reached approximately 1.0 cm. in length, the petri dishes were removed from the non-vented polyethylene bag and the roots inoculated. A series of dishes were prepared and the plants were allowed to grow for four weeks prior to their inoculation. This procedure was followed in order to obtain more lateral root development. The plants were not inoculated until the water film which encompassed the roots had evaporated to the point that the water film was not visible.

The plants were inoculated with P. projectus extracted from an existing population on I. germanica cv. 'Snow Goddess' by the use of a modification of the Christie-Perry technique (5, 20). Forty nematodes of varied developmental stages were placed directly above the root on the agar surface by the use of a dental pulp canal file. The covers of the petri dishes were retaped and the nematodes and roots were observed through the bottom of the dish with the aid of a compound microscope. Observations were made at regular intervals for sixteen months.

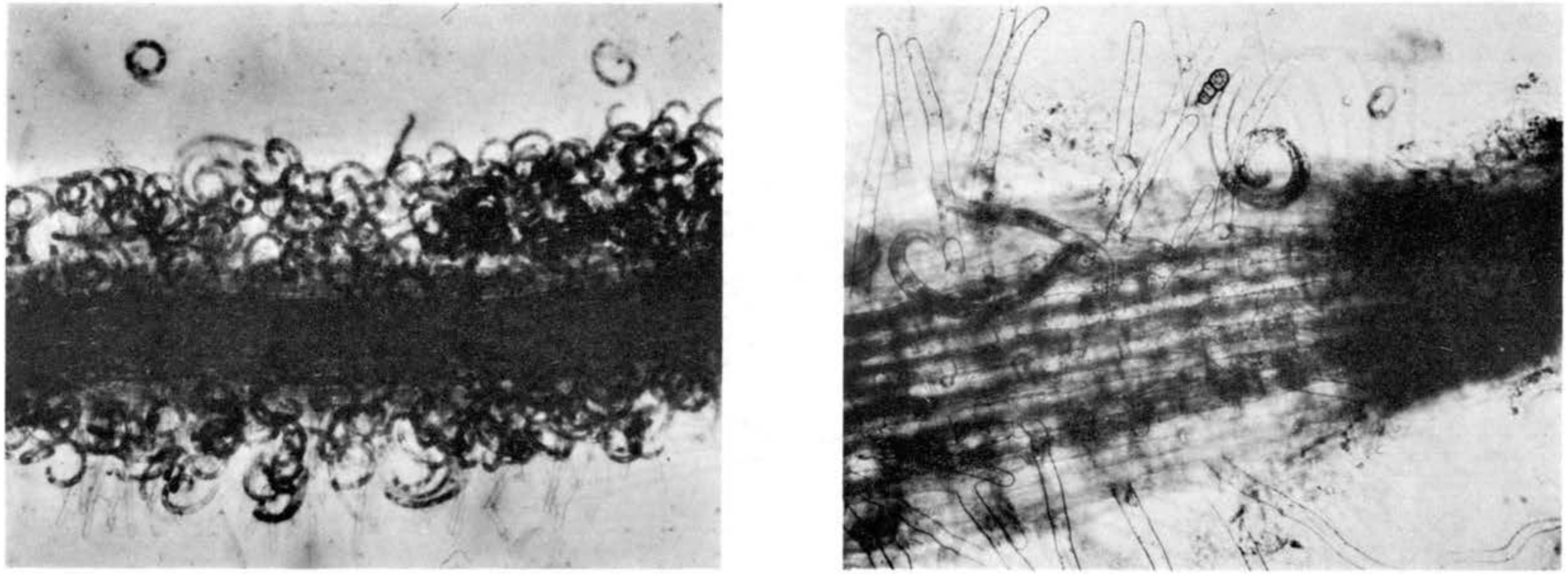
Iris germanica seeds were obtained from a grower in Geary, Oklahoma (22). Seeds were leached in a .05 percent solution of KNO_3 for 24 hours and placed in moist vermiculite prior to storage at 4°C for six weeks. The seeds were then transferred to a beaker of distilled water and oxygenated by bubbling air through the suspension until germination. The germinated seeds were transferred to the petri dishes as discussed in Chapter III. The plants were also inoculated by the same technique. Periodic observations were made as described above.

RESULTS AND DISCUSSION

Within thirty minutes after inoculation the majority of the nematodes were observed to be present around the root. The nematodes apparently experienced difficulty in feeding due to the presence of a small water film around the root surface. The size of the root produced by the iris offshoot was extremely large in comparison to most other plant roots studied in-vitro. The growth of the iris roots was erratic in that they twisted as the agar was penetrated. Due to this characteristic growth of the iris root in-vitro and as well as to its large diameter, the agar was separated causing the formation of the water film around the root. Some of the plants were lifted from the agar due to the twisting and irregular growth of the root. This caused many of the plates to be useless for observation. The nematodes extracted from the soil were largely in the pre-adult stage of development. The preadults did not molt during storage but molted rapidly when in the root proximity. A root diffusate which stimulates molting in P. projectus apparently exists in iris and functions in the same manner as reported for carnations by Rhoades (25). The nematodes migrated rapidly toward the root and became trapped in the water film. Once the preadults larvae were in proximity to the root, molting was initiated but was not completed. This was apparently due to the inability of the nematodes to maintain sufficient contact with any resistant substrate to gain egress from their detached cuticles. These individuals soon became inactive and died. The adult nematodes that were placed on the root could not feed due to their inability to maintain sufficient traction for stylet insertion while suspended in the moisture film. It was observed that the adults would attempt to feed and that with each thrust of the stylet the nematode would be pushed away from the root surface, precluding feeding. The water film occurred around the roots of both offshoots and seedlings. Different agar concentrations (1.5 percent to 0.35 percent) were tried. The 1.5 percent and 0.35 percent agar offered no improvement over the 0.75 percent agar as the water film continued to form. The 0.35 percent agar dried too rapidly and was abandoned. The 1.5 percent agar caused increased lifting of the plants and was not used further in these studies. The 0.75 percent agar was used for the study.

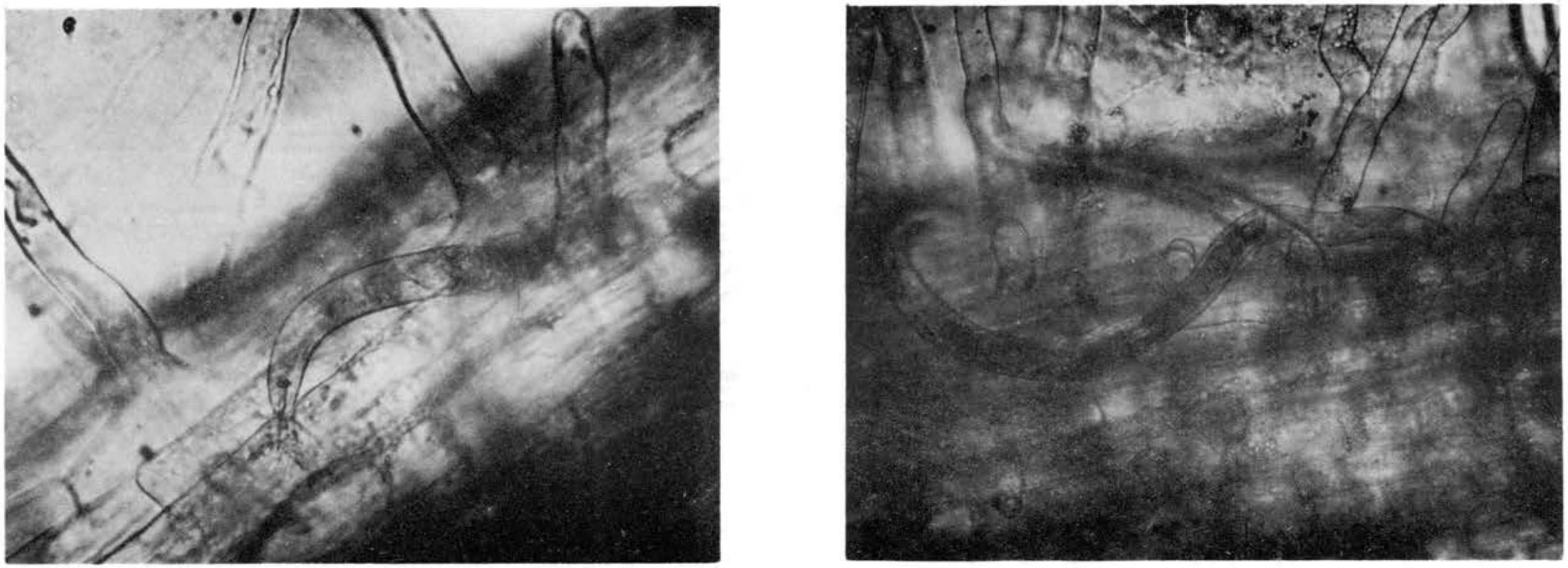
With the elimination of the water film by use of the 0.75 percent agar the nematodes were observed to molt and readily move about. It was observed that in-vitro the nematodes were attracted to the early maturation zone of the root (Figure 12-A). In this region they fed ectoparasitically (Figure 12-B) much as Lindford, et al., reported for P. minutus (17). No males were found in any P. projectus populations from iris and all the observations made were on adult females and larvae. The nematodes characteristically fed by inserting the stylet into epidermal cells (Figure 13, 14) often near the base of root hairs, but they were not observed feeding far out on the root hairs as reported by Rhoades (25). Feeding was most commonly observed near the end of the epidermal cells in the depression between two cells (Figure 13-A). Oviposition was not observed until after feeding began (Figure 14-B). All larval stages produced in-vitro by the feeding females fed in the same manner as the adults. A short period of inactivity was observed during molting. None of the larvae were observed feeding during molting, and the preadult or resistant stage that is commonly found in soil populations was not observed. The preadult stage that was used for inoculum was not observed feeding until after the final molt.

The process of puncturing cell walls was observed on several occasions. It was a slow process requiring from five to twenty minutes for those individuals observed. The nematode placed its labial region against the cell wall and initiated a series of five to ten relatively



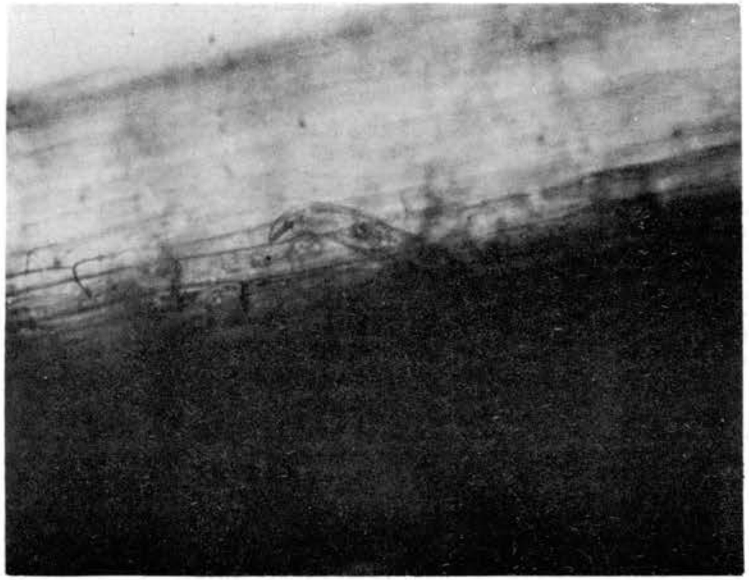
A B

Figure 12. Lateral root segments of Iris germanica cv. 'Snow Goddess' In-Vitro. A. Maturation zone of a lateral root. (Note: number of nematodes attracted to root segment.) B. Paratylenchus projectus feeding ectoparasitically in the early maturation zone of a lateral root. (Note: proximity of root hairs to root apex)



A B

Figure 13. Paratylenchus projectus feeding in-vitro on epidermal cells. A. Feeding site is in the depression between two cells. (Note: granular aggregation around stylet tip). B. An enlarged view of P. projectus feeding on epidermal cells. (See Figure 11-B)



A

B

Figure 14. Paratylenchus projectus feeding in-vitro on epidermal cells of Iris germanica. A. Feeding on mature zone of main root. (Note: granular aggregation around stylet tip). B. Female feeding and oviposition. (Note: stages of egg development)

slow stylet thrusts. At the end of each series of thrusts, the stylet was completely withdrawn and a period of inactivity of three to fifteen seconds ensued followed by another series of stylet thrusts. As penetration of the cell progressed and the stylet became more deeply inserted, both the series of thrusts and the periods of inactivity were of longer duration.

It was observed that stylet insertion varied in the depth to which the stylet penetrated the cell. It varied from approximately fifty percent to as little as ten percent of the total stylet length (Figure 13, 14-A). After the cell wall had been penetrated and the stylet tip situated well into the host protoplast, there was a period of inactivity. This period of inactivity varied among the individuals observed. The periods of inactivity were usually for 30 to 60 minutes prior to the initiation of metacorpal activity.

After the stylet tip was inserted and before pulsation of the median bulb, an aggregation of granular matter formed over the stylet tip (Figure 13-A, 14-A). The first observation of this process was recorded by Rhoades (25). Before valvular activity of the median bulb, muscular pulsations that altered the shape of the median bulb without the opening of the metacorpal valve were observed in several individuals. The contractions caused the entire body of the nematode to move; however, this did not affect the location of the stylet. Upon initiation of valvular activity of the metacorpus, the body movement stopped. Activity of the median bulb began at a seemingly slower rate and became constant at 190 to 200 pulses per minute. The rate remained constant in each nematode until just prior to stylet removal. The length of time for feeding varied with individual nematodes. The female in Figure 14-B was observed feeding on the small cell continuously for seventeen days without any observed inactivity. Other individuals were observed feeding on a single cell for seven days. Despite prolonged movement of other nematodes around one feeding individual, no interruptions of pulsation were observed. Larvae and adults were observed to move to the early maturation zone of the root as it advanced. Feeding in the more mature regions was not observed except for those individuals which remained for several days at one feeding site as the root growth progressed (Figure 14-A). No two individuals were observed to feed on the same cell or on a cell that had previously been fed upon. Cells that had been fed upon developed a distinct aggregation of granular material around the point of stylet insertion. At times the cell became devoid of these granular materials except in the outer regions of the root hair and around the stylet tip. Cells that had been fed upon for several days occasionally became more than half filled with the accumulation of the granular material. The granule formation in the host cell remained after the nematode terminated feeding. Feeding sites <u>in-vitro</u> could easily be recognized by the accumulations of the granular material.

One female was observed to lay sixteen eggs in seven days. The observation and activity of this female continued but the cluster of eggs became so dense that an accurate count of eggs could not be made.

The rate of elongation of the root tip was reduced following prolonged feeding of many individuals. Figure 12-B shows the development of root hairs just behind the root cap indicating that the region of maturation had advanced to the base of the root cap and elongation ceased.

Movement of nematodes about the roots was not confined to the early maturation zone of the root but feeding was observed predominantly in that zone.

CHAPTER V

TOLERANCES OF IRIS, GLADIOLUS, DAYLILY AND CANNA TO THREE NEMATICIDES

The plant types selected (iris, gladiolus, daylily and canna) were found to be frequently interplanted in gardens. Due to the phytotoxicity of some nematicides at various rates on certain plant genera and with insufficient information relating to the nematicides to be used for this study, screening was essential. The objective of this experiment was to determine the tolerances of four herbaceous plants--iris, gladiolus, daylily and canna, to three nematicides ($\overline{O},\overline{O}$ Diethyl O /p. (methylsulfinyl) Phenyl7 Phosphorothidate (Dasanit), KN_3 (Potassium Azide), and 2, 4-Dimethyl-2-Formyl-1, 3-Dithiolane Oxine Methylcarbamate (Tirpate) (Table II).

METHODS AND MATERIALS

Table 3 presents the information concerning the materials, formulations and rates used in the study. Rates were based on the manufacturer's recommendation (x), and the additional rates used were 2x, 3x and 4x. A check treatment was included. An individual treatment area consisted of four square feet.

Materials at the proper rate were placed in individual non-vented polyethylene bags and brought to an equal volume with sifted builders sand. Materials were applied around the plants by the use of a

TABLE II

LIST OF NEMATICIDES, MANUFACTURER AND FORMULATIONS USED FOR THE CONTROL OF *Paratylenchus projectus* ON *Iris germanica*

Material	Manufacturer	Formulation
Dasanit 0,0 Diethyl 0 /p. (methylsulfinyl) Phenyl/ phosphorothidate	Chemagro Corporation Kansas City, Mo.	15% Granular
Nemacur Ethyl 4-(methylthid)-m tolyl Isopropy Phosphosamidate	Chemagro Corporation Kansas City, Mo.	15% Granular
Tirpate 2,4-Dimethyl-2-Formyl-1, 3-Dithiolane Oxine Methylcarbamate	3M Company Minneapolis, Minn.	10% Granular
Potassium Azide (KN_3)	Pittsburg Plate Glass Company Pittsburg, Pa.	10% Granular

TABLE III

FORMULATIONS AND RATES OF NEMATICIDES USED FOR DETERMINING PHYTOTOXICITY TOLERANCES

Material	Formulation (ai)[1]	Rate ai/acre	Rate of Formulation/4 sq. ft.
Dasanit	15% granular	10 lbs.	2.8 g.
Dasanit	15% granular	20 lbs.	5.6 g.
Dasanit	15% granular	30 lbs.	8.4 g.
Dasanit	15% granular	40 lbs.	11.2 g.
Potassium Azide	15% granular	2 lbs.	0.5 g.
Potassium Azide	15% granular	4 lbs.	1.0 g.
Potassium Azide	15% granular	6 lbs.	1.5 g.
Potassium Azide	15% granular	8 lbs.	2.0 g.
Tirpate	10% granular	10 lbs.	4.2 g.
Tirpate	10% granular	20 lbs.	8.4 g.
Tirpate	10% granular	30 lbs.	12.6 g.
Tirpate	10% granular	40 lbs.	16.8 g.

[1]ai - active ingredient

specimen jar 14.5 cm. x 5 cm. with holes drilled in the lid. Each treatment was applied in a trench which encircled the plant approximately 6 in. from the base. Treatments were watered-in by filling the trench with water and allowing the water to soak in at which time the trenches were filled with soil.

The experimental design was a randomized block using: (a) four plant types; (b) two plants per type per treatment; (c) fourteen treatments consisting of three different chemicals at four rates including a check treatment. All treatments were replicated four times.

Plants were established June 3, 1970, at the Oklahoma State University Horticulture Department Nursery on sandy clay loam soil. The experimental area was kept free of weeds and irrigated regularly. Treatments were applied July 7, 1970, after all plants were in active growth. Differences in plant growth and appearances were recorded.

RESULTS AND DISCUSSION

No differences were observed in the plant due to treatment within six weeks following application of the nematicides. Natural senesence occurred among all treatments of the same type of plants prior to observation of any symptoms of phytotoxicity. This indicates that there were no apparent phytotoxic effects from the nematicides. The results of the experiment indicate that Dasanit, Potassium Azide and Tirpate may be used for plant parasitic nematode control at the rates used for the four plant types.

CHAPTER VI

THE EFFICACY OF FOUR NEMATICIDES ON *Paratylenchus projectus* IN AN *Iris germanica* PLANTING

The objective of this study was to determine the efficacy of four nematicides (Ō,Ō Diethyl 0 /p. (methylsulfinyl) Phenyl/ phosphorothidate (Dasanit), KN_3 (Potassium Azide, 2,4-Dimethyl-2-Formyl-1, 3-Dithiolane Oxine Methylcarbamate (Tirpate) and Ethyl 4-(methylthid)-m tolyl isopropy Phosphosamidate (Nemacur) (Table 2) on *Paratylenchus projectus* populations in a planting of *Iris germanica*.

METHODS AND MATERIALS

Twenty plantings of *I. germanica* were surveyed in the Stillwater, Oklahoma, area to find a population level of *P. projectus* sufficient for a control study.

The planting selected yielded an average of approximately 300 *P. projectus* per 50 ml. of soil (11). The planting also contained relatively low numbers of the genera *Meloidogyne*, *Tylenchorhynchus* and *Xiphinema*. The area was established in 1922 as a commercial iris nursery. From the time of establishment to the present, the planting has received a low level of maintenance. The soil in the planting was compact and contained an abundance of weeds. The plants in the rows had been allowed to spread and as a result the area had become a complete cover of iris.

Only granular formulations were used in this study due to the higher mammalian toxicity of the concentrated liquid forms. The granular materials will probably be used by the hobbiest who is inexperienced in handling nematicides or materials of equivalent toxicity. Nematicides and rates used are presented in Table IV. Rates were calculated on the basis of formulation rather than active ingredient. Rates used were taken from the manufacturer's median recommended rate (x) and were 1/2x, 1x and 2x.

The design was a randomized block utilizing four replications of each treatment which was comprised of a nematicide at a given rate. The treatment area was two feet by six feet and an adjacent check of the same dimensions was used. The adjacent checks were alternated to completely encompass the treatment area. A two-foot buffer zone was provided between each replication to prevent movement of materials from plot to plot. The treatment areas and adjacent checks were marked by the use of package string, and pre-treatment samples were collected June 24, 1970. The treatments were applied June 24, 1970, by the use of a specimen jar (14.5 cm. x 5 cm.) with holes drilled in the lid. The correct quantity of nematicide for each treatment was placed in a non-vented polyethylene bag and brought to an equal volume to insure uniformity of application by the use of sifted builders sand. Each nematicide was applied evenly over the area without soil preparation.

A standard type soil auger was used in taking samples. Five samples four inches deep were taken from the corners and middle of each treatment area and its adjacent check. Post treatment samples were taken July 14, August 4, and August 28, 1970. Each sample was placed in a non-vented polyethylene bag and thoroughly mixed before processing.

TABLE IV

FORMULATION AND RATES OF NEMATICIDES

Treatment	Formulation	Rate/1,000 sq. ft. Formulation	Rate/12 sq. ft.
1. Dasanit	15% G	1.3 lbs.	7.0 g.
2. Dasanit	15% G	2.6 lbs.	14.0 g.
3. Dasanit	15% G	5.2 lbs.	28.0 g.
4. Nemacur	15% G	1.0 lbs.	5.0 g.
5. Nemacur	15% G	1.6 lbs.	9.0 g.
6. Nemacur	15% G	3.2 lbs.	18.0 g.
7. Potassium Azide	10% G	0.2 lbs.	1.0 g.
8. Potassium Azide	10% G	0.4 lbs.	2.0 g.
9. Potassium Azide	10% G	0.8 lbs.	4.0 g.
10. Tirpate	10% G	0.8 lbs.	4.0 g.
11. Tirpate	10% G	1.6 lbs.	9.0 g.
12. Tirpate	10% G	3.2 lbs.	18.0 g.

A 50 ml. aliquot was taken from the composite of the five samples and processed by a modification of the Christie-Perry technique (5) (20). Samples were read by the author twenty hours after processing.

RESULTS AND DISCUSSION

Immediately after the treatments were applied a rain of one and one-half inches fell during a thirty-minute period. Due to the high degree of soil compaction and the excessive rainfall, run-off undoubtedly caused dilution and dispersal of some materials into adjacent plot areas despite the buffer areas provided.

Table V shows the results of the various treatment with respect to the number of P. projectus present. Due to variation in the degree of soil compaction and the dispersal of the nematicide within the treatment areas, additional variation was created which possibly would not have occurred under more favorable conditions. In a previous study (27) emulsifiable concentrate formulations of two of the nematicides, Nemacur and Dasanit, proved to be highly effective in reducing population of P. projectus on I. germanica. In this study the three Nemacur treatments ranked fourth, sixth and tenth; while Dasanit treatments ranked seventh, ninth and twelfth out of twelve entries. The formulation of material used and the differences in soil conditions could have limited the effectiveness of Dasanit and Nemacur. It has been shown in another study that twice as much chemical is required for control of nematodes in compacted soil (10). Tirpate treatments ranked first, third and eleventh; while Potassium Azide treatments ranked second, fifth and ninth out of twelve entries. The greater effectiveness of Tirpate and Potassium Azide may be due in part to their smaller granule

TABLE V

THE EFFICACY OF FOUR NEMATICIDES ON THE REDUCTION OF *Paratylenchus projectus* POPULATIONS IN A TALL-BEARDED IRIS PLANTING

Nematicide and Formulation	Rate/1,000 sq. ft. Formulation	Means[1]	Rating[2]
1. Dasanit, G 15%	1.3 lbs.	247.50	b c d
2. Dasanit, G 15%	2.6 lbs.	831.00	d
3. Dasanit, G 15%	5.2 lbs.	-262.25	b c d
4. Nemacur, G 15%	1.0 lbs.	-469.00	b
5. Nemacur, G 15%	1.6 lbs.	345.00	b c d
6. Nemacur, G 15%	3.3 lbs.	-331.00	b c d
7. Potassium Azide, G 15%	0.2 lb.	-1062.00	a
8. Potassium Azide, G 15%	0.4 lb.	-382.00	b c
9. Potassium Azide, G 15%	0.8 lb.	-143.00	b c d
10. Tirpate, G 10%	0.8 lb.	699.00	c d
11. Tirpate, G 10%	1.6 lbs.	-798.00	b
12. Tirpate, G 10%	3.2 lbs.	-1277.00	a

1. Means based on reductions of nematodes as compared with adjacent checks over all sample dates.
2. Those values not followed by the same letter are significantly different at the 0.05 level according to Duncan's multiple range test.

size and would permit a better penetration of these materials into the soil surface. The penetration of Nemacur and Dasanit into the soil could possibly have been reduced greatly by larger granule size resulting in a lower rating of effectiveness. In essence, the amount that penetrated into the soil is not determinable and does not precisely represent the efficacy of the nematicide. Potassium Azide is a fumigant and the shallow depth of penetration may have resulted in a significant vapor loss.

Root systems in the planting were not very extensive. The general depth of root penetration was four inches. This shallow root system could be more easily damaged by the use of high rates of materials. Although no phytotoxicity was observed in the Dasanit and Nemacur plots, the rates used could have placed a stress on the plants causing initiation of new root development from the rhizome. During the months of July and August, P. projectus populations were observed to increase at a rapid rate. This increase was due to the seasonal development of new roots in iris during July and August, which would provide additional feeding sites for the nematode population. This would allow a nematode population increase and indicate the lack of residual activity of the nematicides in the soil. This residual effect was observed four weeks after treatment in Dasanit and Nemacur plots. During this four week period the nematode population was supressed but thereafter it increased through the termination of the study. From the data collected it is almost impossible to correlate the variable effects of soil condition, condition of the rhizosphere, plant response, and chemical reaction to the population of P. projectus.

The normal growth characteristics of iris create a very favorable environment for the nematode. *Iris germanica* develops underground stems; thus frequently produces new rhizomes and roots. The residual effects of most nematicides remain for approximately six weeks. The combination of the short residual effect of modern nematicides and the continued growth of the iris dictates sequential applications of effective materials for control of *P. projectus* in iris plantings.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The pathogenicity of *Paratylenchus projectus* on *Iris germanica* was evidenced by the significant reduction of lateral root development. The reduction of epidermal surface area affects the absorptive efficiency of the root. The health and vigor of offshoots produced by a parent rhizome are dependent upon stored nutrients and energy in the parent rhizome. The deleterious effect of parasitism should be increasingly or progressively evident after several growing seasons.

The greatest concentration of *P. projectus* in the iris rhizosphere seems to be at a depth where the soil does not dry out readily and contains a stable supply of soil moisture adequate for their activity. The depth at which this occurs would depend upon the particular planting site. At the planting in which this study was conducted, the greatest concentration occurred at the 2 - 4 in. depth and decreased at lower depths. *Paratylenchus projectus* are usually found feeding on lateral roots. Numbers recovered from the lateral roots were ten fold those extracted from the main roots. Occasionally *P. projectus* was found inside the root feeding endoparasitically. Entrance was gained through the exit points of lateral roots.

The roots of *I. germanica* seem to produce a root diffusate which stimulates molting of the preadult stage of *P. projectus*. *In-vitro* the *P. projectus* are attracted to the early maturation zone of lateral roots. Feeding by nematodes was most commonly observed near the end of the epidermal cells in the depression between two cells. Oviposition did not occur until after active feeding had been initiated. The process of puncturing cell walls is much the same as reported by Rhoades (25) for *P. projectus*. Feeding of two or more nematodes on the same cell was not observed. The duration of feeding varied from three to seventeen days depending on the individual nematode. Feeding caused the formation of a distinct granular aggregation near the point of stylet insertion. Prolonged feeding caused a decrease in the rate of elongation of lateral roots.

The normal growth characteristics of iris provide a very favorable environment for *P. projects*. The effective granular nematicides used in this study for the control of *P. projectus* in *I. germanica* plantings have a short residual activity. Due to the growth characteristics of the *I. germanica* and the short residual effect of these modern nematicides, sequential applications at the rates indicated would appear to be necessary for effective control.

Gladiolus, daylily, iris and canna were tolerant to the granular nematicide rates used in this study for the control of *P. projectus* in *I. germanica* plantings.

The results of this study indicate:

(1) *Paratylenchus projectus* are pathogenic to *I. germanica* cv. 'Snow Goddess'.

(2) The feeding of *P. projectus* is predominately on the early maturation zone of lateral roots causing a decreased rate of elongation.

(3) *Iris germanica* apparently produces a root diffusate which stimulates the molting of preadult larvae of *P. projectus*

(4) Tirpate and Potassium Azide can provide effective control of *P. projectus* in *I. germanica* plantings.

(5) Gladiolus, daylily, iris and canna are tolerant to the rates used of the granular formulations of Potassium Azide, Tirpate, Dasanit and Nemacur.

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Personal Data: Born in Enid, Oklahoma, September 11, 1944, the son of Mr. and Mrs. Ollie William Eck.

Education: Attended Fairview High School, Fairview, Oklahoma, from September, 1958, to May, 1962; attended Oklahoma State University from September, 1965, to January, 1970, receiving the Bachelor of Science degree with a major in Forestry; completed requirements for the Master of Science degree in Horticulture in May, 1972.

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