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PATHOLOGY AND CONTROL OF *DITYLENCHUS DIPSACI* ASSOCIATED WITH *PHLOX SUBULATA*

By

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ABSTRACT

PATHOLOGY AND CONTROL OF DITYLENCHUS DIPSACI ASSOCIATED WITH PHLOX SUBULATA

By

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Preliminary studies of a die-back condition in Phlox subulata, L., ground phlox or moss-pink, indicated high infestation levels of Ditylenchus dipsaci (Kuhn, 1857) Filipjev, 1936 (bulb and stem nematode). Samples from cultivars of P. subulata in perennial nurseries gave further evidence linking the disease to this nematode and disclosed no resistant cultivars.

Host plant bioassays resulted in plant height and shoot fresh weight reductions in each of six host species grown in infested as compared to steamed soil. Pathogenicity tests were conducted on four cultivars of P. subulata to substantiate the host-parasite relationship. Plants in steamed soil showed significant increases in height and shoot fresh weight compared to plants grown in infested soil. D. dipsaci was extracted from each cultivar.

Aldicarb (Temik® 10G) and oxamyl (Vydate-L) significantly reduced field populations of D. dipsaci and alleviated disease symptoms associated with P. subulata. Field population dynamics indicated four generations of D. dipsaci were produced during 1976.

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INTRODUCTION

Southwestern Michigan is an important production area for ornamental perennial plants in the United States. In the past several years, however, growers have incurred heavy losses of *Phlox subulata*, a popular ground cover, because of a destructive die-back condition. Preliminary investigations of *P. subulata* cultivars revealed large population levels of the bulb and stem nematode, *Ditylenchus dipsaci*, in tissue samples taken from diseased plants growing in normal field culture.

The objectives of this study were (i) to conduct a preliminary survey to search for possible resistant cultivars of *P. subulata*, while assessing the overall extent of infestation by the bulb and stem nematode in four commercial nurseries, (ii) the determination of the physiological race of *D. dipsaci*, (iii) provide proof of pathogenicity of *D. dipsaci* on four cultivars of *P. subulata*, noting changes in plant height and shoot fresh weight between plants growing in infested versus steamed soil and changes in nematode population densities during the investigation, (iv) the establishment of a control program to reduce the economic threat that this nematode poses to perennial growers in southwestern Michigan, and (v) the examination of the population dynamics of *D. dipsaci* associated with *P. subulata* over a one year period in the field. This study was designed to contribute to the understanding of the plant parasitic nematode, *D. dipsaci*, and

to assist in the development of improved nematode control methods for perennial plant growers.

LITERATURE REVIEW

Ground phlox culture

Phlox subulata, L., ground phlox or moss-pink, was described by Bailey as a tufted spring blooming perennial species. The plant grows quickly, forming dense mats, and with its profuse spring bloom it has become a popular and colorful ground cover in the perennial ornamental plant industry (1).

One of the first records of *P. subulata* under cultivation was in 1745, when John Barton sent a plant to Peter Collinson for establishment in the botanical garden of England (35, 45). Collinson catalogued the plant as evergreen false-*Lychnis*, but it was later classified as *P. subulata* by Linneaus. The species received little attention for the next 100 years until it gradually became a popular garden species (45).

Phlox subulata is the type of the genus *Phlox*. Distinguishing morphological characteristics of the species include the mats of dense persistent-leafy sterile shoots which the plant develops, with flowering shoots 5-10 cm in height containing 3-6 nodes. Latent axillary shoots are numerous and conspicuous. The leaves of the plant are linear to subulate, finely ciliated and the upper surface has pilose hairs which are usually glandless. The leaf has a maximum length of 10-20 mm, and their width is 1-2 mm. The inflorescence consists of a 3- to 6-flowered axillary cyme. The pedicelled flowers stand above the foliage, with inflorescences 20 cm or more across in colors ranging from pink to purplish to white. The lobes of the corolla are usually obcordate

and deeply notched; the stamens are slightly exerted (45).

Phlox subulata is often found growing wild on sterile soil or on open, rocky or sandy slopes in various northeastern portions of the United States. The Polemoniaceae family, to which P. subulata belongs, consists of thirteen genera of annual and perennial herbs. The genus Phlox contains a Subulatae section which includes three members of the eastern narrow leafed phlox: P. subulata (ground phlox or moss-pink), P. nivalis Loddiges (trailing phlox), and P. bifida Bick (sand phlox). Of these species, P. subulata is the major commercially propagated perennial narrow leafed phlox.

Bulb and stem nematode

History

Ditylenchus dipsaci (Kuhn, 1857) Filipjev, 1936, the bulb and stem nematode, infects a wide variety of plants and is considered to be a major agricultural problem in several countries throughout the world, particularly in Russia. It was first described by Kuhn on Fuller's teasel as Dipsacus fullonum, L. Bessey observed this species in the United States in 1907, while Allison reported the nematode in North Carolina infecting alfalfa (4). The genus Ditylenchus had 78 species as of 1972 (25).

Taxonomy and morphology

Ditylenchus is a member of the subclass Secerenentea, order Tylenchida, superfamily Tylenchoidea, family Tylenchidae and subfamily Ditylenchinae. Baker's check list of the nematode superfamiles gives a nomenclatural review of D. dipsaci (2). There has been some confusion

between the genus Ditylenchus and its phyletically close relative Anguinia. This confusion has resulted primarily from inexact systematic characters of the genus Ditylenchus. Over the years, these systematic characters have become distinct so that Ditylenchus and Anguina are adequately separated.

Ditylenchus dipsaci is the type species of the genus. A complete morphological description of this nematode has been given by Paramonov (25). In addition, electron micrographs taken of this nematode now enable complete differentiation of the intricate structure of the stomatal region and esophagus area where race differences have been detected (48, 49).

Life cycle

The duration of the life cycle of D. dipsaci was observed to last 19 to 23 days. The nematode undergoes four molts and four larval stages. The egg stage lasts 7 days, the first larval stage in the egg 5 to 5-1/2 days, the second larval stage 2 to 2-1/2 days, the third larval stage 3 to 3-1/2 days, and the fourth larval stage 4 to 5 days. The larval stages are distinguishable by total nematode body length and the nature and location of the genital primordium. Sexes can be differentiated in the third and fourth stage larvae and adults. Four days after the final molt and after mating, the female begins egg deposition. A single male can fertilize more than one female. Egg laying capacity ranges from 207 to 498 per female. The longevity of both males and females is 45 to 73 days in onion (50).

Physiological races

Ditylenchus dipsaci attacks over 400 species of plants, with some species being affected frequently. In 1956, the concept of biological

races of D. dipsaci was introduced (25). Differentiation into races was based on physiological characters, and varied according to the level of physiological and biochemical adaptivity of a particular race to a host plant.

By 1965, approximately twenty races of D. dipsaci were known (25). The biological race concept was further complicated, however, by differentiation not only according to host plant, but also according to countries; thus, a geographical factor was introduced. The potato race in England, for example, is different from the potato races which have been found on the European continent, particularly in the Netherlands (25). Local differentiation of races was also observed.

Ditylenchus dipsaci is a very heterogeneous organism and appears to be widening it physiological adaptibility, leading to an increase in the number of races yearly (25). Different races have been found to infect Hordeum, Beta, Solanum, Allium, Phaseolus, Daucus, Phlox, Hyacinthus, Tulipa, Plantago, Nicotiana, Medicago, Melilotus, Lucerne, and Fragaria (23, 29, 36, 42).

Races of D. dipsaci are not usually differentiated morphologically, although anatomical differences can sometimes be observed. For example, the onion form of D. dipsaci displays variations in the shape of the cardial bulb (25). Races interbreed, and under experimental conditions, they correspond to the concept of hybrids (34).

The origin of new biological races through interbreeding has been shown to result in the genetic determination of race preference for host plants (34). Existing races may be under selection pressure toward speciation. Using compatibility tests to study intraspecific variation within D. dipsaci, a consistent failure in reciprocal crosses between

two races was reported (34). It was suggested that a mechanism for reproductive isolation was developing as evidenced by the reciprocal crosses, and that races and isolating mechanisms approached the sibling species concept. Intraspecific and intrapopulation polymorphism in chromosome numbers of the bulb and stem nematode was reported in the onion, strawberry, phlox, parsley, and dandelion races, adding further support to the speciation hypothesis (34).

The genus had wide adaptive radiation. Some members are mycochilpages and some are phytohelminths, with non-specific or specific pathogenic effects. The evolutionary descent of the genus Ditylenchus created members able to infect both the root and photosynthetic systems. Most species of Ditylenchus show negative reactions to saprobic environments, a further pressure toward foliar habitats for survival. The adult Ditylenchus is environmentally flexible, however, and can survive up to two years outside the plant if necessary (10, 20). The main feature of the destructiveness of Ditylenchus has been described as the variety of hosts it infects coupled with its ability to remain quiescently in the soil for extended periods of time.

Distribution and hosts

The host range of D. dipsaci is distributed throughtout the temperate regions. It has been reported from Europe in Denmark, England, France, Hungary, Sweden, and the USSR. It is reported to infest plants in the United States and Canada, as well as South America in the countries of Argentina, Brazil, and Chile. South Africa, and New South Wales, Australia have also reported this nematode (4, 29, 41, 42).

Ditylenchus dipsaci has been reported on potatoes, sugar beets, lucerne, white clover, rye, many weed species, tobacco, strawberry, and

phlox. Vegetable hosts include celeriac, garlic, leek, onion, lettuce, swedes, and shallots. Several races also infect narcissus, tulip, and hyacinth (4, 9, 23, 25, 29, 36, 41).

Symptomatology

Symptomatology of D. dipsaci infestation generally follows a characteristic pattern. In monocotyledons, the nematode causes excessive tillering together with puffiness of the leaf sheaths. The leaf sheaths remain short, as do the leaf blades, giving rise to symptoms known as onion bloat and tulip root of oats (29). On bulbs, the nematode attacks the scales, and causes light to dark rings, seen in cross section of the bulb, known as brown ring disease of daffodils and hyacinths. Spikkels or raised lesion-like areas which contain nematodes are similarly formed on the leaves. Dicotyledons show stunting, swelling, blistering, and curvature of stems, petioles, and veins.

Ecology

Ditylenchus dipsaci has been determined to enter the stem of the plant through stomates and openings in the epidermis produced by the action of saliva excreted by the nematode. Under stress conditons, this nematode may not restrict itself to stem entry, but may enter root and leaf tissue (27). Ditylenchus dipsaci causes stunting associated with the development of lateral buds; this may be a survival mechanism for the nematode, providing more stems and consequently more food for increasing nematode populations (27). This species occupies an intermediate position between ectoparasitic species which simply puncture cells for feeding, such as D. destructor, D. drepanocerus, or D. myceliphagus, and the endoparasitic Ditylenchus gall-forming

species, such as D. radicola, D. askenasyi, or D. graminophila (27).

All larval stages and adults are capable of invading the plant, but generally the infectious population is considered the fourth-stage larvae. Optimum infection time has been correlated with field temperature, resulting in various infection rates for different seasons and for different geographical areas (13, 41).

Ditylenchus dipsaci displays unique ecological flexibility. The nematodes are capable of migrating to the surface of the soil and surviving a drying out condition in an atmosphere of 50 percent relative humidity for 34 days (39). Efficient osmoregulatory mechanisms are presumed to operate to allow nematode survival in very dry soils in which osmotic pressure is high. The tendency of suction force plus osmotic forces results in nematode desiccation, which aids survival after exposure to temperatures as low as -80 C (19). In this desiccated condition, D. dipsaci has been reported to survive for at least 23 years, longevity increasing with humidity (8, 10, 40).

Disease complexes

Ditylenchus dipsaci can serve as a predisposition agent for several secondary pathogens. In experimental infections in onion, infections of D. dipsaci and Peronospora schleidenii together were 36 percent greater than with the nematodes alone (47). Loss in dry weight accumulation was 28 percent with the nematodes alone, 17 percent with the fungus alone, and 36 percent with a mixed infection.

In the presence of moist conditions. D. dipsaci can predispose onions to 100 percent infection by Botrytis allii, while only 30 percent of the onions became infected in the absence of the nematode (42). This nematode species also plays a significant role in the dissemination

of phytobacteria (42).

Economic losses

Crop losses depend on a combination of factors including host plant susceptibility, infestation level of the soil, soil type, and weather conditions. Most races overwinter better in clay soils than in lighter soils, and seem to prefer cool, moist weather conditions. Spring infection determines the amount of damage that will be done, for the nematodes do not generally invade the tissue during the summer months (39).

Control

Ditylenchus dipsaci is most effectively controlled by regular efforts aimed at exclusion. This is accomplished by routine soil testing, the planting of nematode-free stock plants or seed, and the elimination of weed hosts and volunteer plants which may harbor overwintering populations. In general, sanitation and hygiene must be given high priority in a control program designed to avoid this pest.

Biological control by the use of predatory and fungal parasites of Ditylenchus populations are plausible for population reduction, but are not considered efficient for large scale production programs at this time (42). The fungus Caternaria angullulae is a natural pathogen of D. dipsaci and the tardigrade, Hypsibius myrops, is a predator of this nematode (33, 42). Both species are possible sources of natural innoculum for biological control measures.

A physical method for population reduction has been demonstrated by the use of hot water treatment of infected plant parts. Such treatments have been successful in controlling D. dipsaci on strawberry

and ornamental bulbous species (42).

Crop rotation was previously recommended as the only means of controlling D. dipsaci. However, with the ever increasing numbers of physiological races, chemical control may be the most effective control method. Diethyl O-2-pyrazinyl phosphorothionate, diethyl 4-nitrophenyl phosphorothionate, S-4-chlorophenyl-thiomethyl OO-diethyl phosphorodithionate, and 1,2-dibromo-3-chloropropane have all been reported effective for control of D. dipsaci (11, 46). The State Plant Pathology Institute of Denmark in 1970 reported the use of diethyl O-2-pyrazinyl phosphorothionate and 2-methyl-2-(methylthio)propionaldehyde O-(methylcarbonoxl) oxime for control of D. dipsaci on phlox.

The development of resistant varieties has been considered by some to be the best means of avoiding nematode damage (15). However, resistant varieties may play a lesser role in D. dipsaci control due to the many physiological races of this species (2). Continued use of resistant varieties may also tend to favor the development of more specialized populations of this nematode.

Bulb and stem nematode and phlox

The phlox race of D. dipsaci causes injury described as one of the most pronounced produced by a D. dipsaci race (36). Considerable growth may occur in spite of infestation, and infection may go unnoticed until obvious symptoms appear. Nypels described an infestation in Phlox paniculata in 1898, and in 1899, Ritzema-Bos identified the species involved as D. dipsaci (36). Weiss in 1923, and Steiner and Dodge in 1929, gave accounts of the disease symptoms on phlox produced by nematode infection (33).

Ditylenchus dipsaci has been reported to infect the following species of phlox: P. drummondii, Hook, P. divanicata, L., P. douglasii, Hook, P. paniculata, L., P. subulata, L., (32, 33), P. suffruticosa, Bent, and P. amoena, Sims (9).

Symptoms on phlox were expressed as elongate swellings, in which the nematode congregated, on the terminal portions of the growing stems. On these swollen portions, a group of closely arranged leaves formed a characteristic rosette. The lower leaves of the rosette showed an inward rolling of the leaf margins. The stalk usually was bent slightly at the lower part of the swelling. Taller stems were spindley with elongated nodes; leaves in this area were deformed and contorted with irregular edges. Some leaves developed with little blade area, or on short numerous stems, creating a witch's broom effect. Tissue of both the stem and leaves became brittle, and in advanced stages, wilting and browning of the leaves was followed by death of stems. Many basal buds developing from the crown failed to produce stems. Occasionally, stems split as the invaded tissues began to disintegrate (36).

The host range of the phlox race includes Dianthus, Oenothera, Solidago, Campanula, Gilia, Collomia, and Primula. Morphological studies failed to reveal diagnostic characteristics which would justify the name Ditylenchus phloxidis proposed in 1951 (36). However, hybridization studies involving the phlox race crossed to the onion, strawberry, red clover, narcissus, parsnip, and parsley races contained a relatively high percentage of deviants when phlox females were used, and resulted in almost total hybrid incapacitation when phlox males were used. Parsley and parsnip race females crossed to phlox race males resulted in viability, and in isolated cases, lead to

hybrid populations. It was hypothesized that the phlox race was in an active stage of speciation (36).

A home gardener's handbook written in 1953 lists the major pest of phlox as the "eelworm" (35). The author states that authorities have not elucidated a method for "curing eelworm infestations", and suggested that the only positive control method was to dig and burn infested plants. The stem was thought to be the major point of entry of this nematode, as root cutting propagation of plants did not seem to become infested until after the plant had sprouted 8 to 10 cm.

MATERIALS AND METHODS

Nematode Survey

Sampling procedures

Preliminary sampling of P. subulata, cv Emerald Pink, indicated that high levels of D. dipsaci may cause the die-back condition observed in fields of perennial nurseries in southwestern Michigan. Thus, an extensive survey of all commercial cultivars of P. subulata from selected nursery plantings was conducted.

The first nematode sampling was made August 1, 1975 from two perennial nurseries. Six plants, three healthy in appearance, three with obvious disease symptoms involving chlorotic or necrotic patches of tissue, were randomly selected from each of the fourteen P. subulata cultivars grown. The entire plant, including roots and soil surrounding the root system, was taken during sampling. The plant was labeled and placed in a plastic bag which was closed securely. Plant samples were stored at 5-7 C for one week until nematode extraction. The sampling represented selections from all current commercial varieties of P. subulata grown by the two Michigan nurseries.

A second sampling was taken October 10, 1975 from two other perennial nurseries in southwestern Michigan. Eight plants, four healthy and four with disease symptoms, were selected from each of the three main phlox cultivars grown. Samples were collected and stored as described for the first sampling.

Nematode extraction procedures

Population densities of nematodes in the soil were determined by soil extraction using the centrifugal-floatation technique (16). A 400-mesh screen was used for collection of the nematodes from the 100 cm^3 soil sample. The extracted nematodes were stored in water in 1.5 x 12 cm glass test tubes at 5-7 C until microscopic quantification was made. Shoot and root samples were cleaned of debris and nematodes were extracted from the tissue using the standard shaker technique for 48 hours at 125 rpm (16). A 400-mesh screen was used again for collection of the nematodes which were subsequently stored in water in test tubes at 5-7 C until quantification. Maximum length of storage of nematodes in the water suspension was two weeks.

Physiological Race Identification

Growth conditions

Six plant species known to be hosts for D. dipsaci were selected for greenhouse evaluation for susceptibility to D. dipsaci (15, Table 5). Seeds were germinated on wet filter paper in 9 cm petri dishes at 24-26 C under 2.1 klux cool white 16 hour fluorescent lights. After the radical emerged approximately 3-6 mm, they were transplanted five seeds in four replicate 10 cm clay pots containing either steamed sandy loam soil or infested field soil. All pots were cleaned and steamed at 121 C.

The sandy loam was obtained from a Hudsonville, Michigan, phlox field infested with D. dipsaci. The inital population density of D. dipsaci was 45 nematodes per 100 cm^3 soil as determined by the centrifugal-floatation technique (16). Soil analysis indicated a pH of 6.0, 42 kg per hectare phosphorous, 17 kg per hectare potassium,

220 kg per hectare calcium, and 18 kg per hectare magnesium.

Soil for the steamed treatment was treated for 90 minutes at 121 C. Sandy loam for the field soil treatment was used directly from the field. Plants were maintained at the natural photoperiod conditions of 43° N latitude at East Lansing, Michigan, and watered daily. Greenhouse air temperature ranged from 22 to 30 C with an estimated mean of 26 C. Humidity ranged from 0 to 40 percent with an estimated mean of 20 percent. No fertilizer or pest control chemicals were applied prior to planting or during the 28 days of growth.

Harvesting and evaluation procedures

Plants were collected after 28 days of growth and placed intact, with soil removed, in plastic bags at 5-7 C for 12 hours. Soil from each replicate was also stored in plastic bags at 5-7 C. Each plant was evaluated for plant height (mm) and shoot fresh weight (g). Height measurements were taken using a standard millimeter rule and weight was measured on a Mettler P160 balance, accurate to $\pm$ 1.0 mg. Nematodes were extracted, collected, and stored from shoot and soil samples as stated for the nematode survey above.

Pathogenicity

Growth conditions

Shoot tip cuttings of four cultivars of *P. subulata* were rooted in artifical soil mix under 2.1 klux cool white 16 hour fluorescent lights at 24-26 C for two weeks until 0.5-1.0 cm roots developed on all cuttings. Rooted cuttings ranging from 15 to 40 mm in height were then selected for uniformity and transplanted three per 10 cm clay pot in either steamed sandy loam soil or field soil. All pots were cleaned and steamed at 121 C.

Four replications of three cuttings each were made for four cultivars of P. subulata (White Delight, Emerald Pink, Emerald Blue, and Atropurpurea) for both steamed and field soil. The initial population density of D. dipsaci was 45 nematodes per 100 cm^3 of sandy loam as determined by the centrifugal-floatation technique (16). Soil was steamed following the same procedure as for the physiological race identification. Greenhouse conditions, watering, fertilization, and pest control were the same as outlined for the physiological race identification study.

Harvesting and evaluation procedures

One plant from each replicate was harvested at 0, 11, 29, and 48 days after transplanting into field or steamed soil. Plants were collected and placed intact, with soil removed, in plastic bags and stored at 5–7 C until measurements were made, either immediately or 12 hours after harvesting. At the termination of the investigation, soil from each replicate was stored in plastic bags at 5–7 C for one week until nematodes were extracted. Each plant was measured for plant height (mm) and shoot fresh weight (g) as stated above. Ditylenchus dipsaci was extracted from shoot and soil samples using procedures outlined above.

Control Program Evaluation

Growth conditions

Divisions of stock plants of P. subulata, cv Emerald Pink, were obtained from a commercial nursery in Hudsonville, Michigan, and planted in a 54.9 x 4.9 m plot using a two-row mechanical planter. Soil was previously prepared to seed bed condition. Divisions

containing approximately 15-20 cm top growth plus 5-8 cm of roots were planted April 12, 1976 in sandy loam soil, pH 6.4, containing 27 kg per hectare phosphorous, 7 kg per hectare potassium, 176 kg per hectare calcium, and 16 kg per hectare magnesium. Field plants were not irrigated, fertilized, or unspecified pesticides applied at any time during the two year test period. The plot was hand weeded when necessary. The area was planted to ground phlox in 1973 and 1974, but was fallow in 1975.

Pesticide application

A completely randomized design was used to test the effect of aldicarb (Temik® 10G) and oxamyl (Vydate-L) pesticides for the control of D. dipsaci. Plants were spaced 15 cm in a row, with the rows 0.6 m apart. Each replication consisted of two rows, 9.14 m in length. Six replications of each of four treatments were used. The four treatments consisted of 1) control, 2) aldicarb, 3) oxamyl, and 4) aldicarb/oxamyl combination.

Aldicarb was applied once in May 1976 to treatments 2 and 4. Both treatments were applied via soil application of granular aldicarb at a rate of 6 lbs active ingredient per acre or 0.67 g per square meter. Aldicarb was covered with loose soil using a garden rake. The day aldicarb was applied was very windy, and some contamination of adjacent replications occurred.

Oxamyl was applied as a foliar spray to treatments 3 and 4 as 5 ml per plant at a rate of 4.99 ml per liter or 1.19 ppm, with 2 to 3 ml of the adjuvant Regulaid added to 11.36 liters of oxamyl solution. The oxamyl solution was applied beginning June 2, 1976, and thereafter every two weeks for a total of seven applications to treatment 3, and on June 2, 1976, and thereafter every two weeks for a total of four

applications to treatment 4.

Harvesting and evaluation procedures

Soil, shoot, and root samples were taken randomly at the onset of the experiment from each of the 24 replicates. Nematode extraction procedures were identical to those stated for the nematode survey. When D. dipsaci was determined to be the predominant nematode species involved, only samples from shoot tissue were collected from the replicates to monitor population levels in the foliage.

Samples were collected every two weeks in 1976 and monthly in 1977. Each sample was weighed to include only 1.0 g shoot tissue. In October, 1976, ten plants from each replicate were removed from the field and stored in closed plastic bags at 5-7 C for the winter months from October to May 1977. The entire plant with some soil surrounding the roots was included. Bimonthly samples were taken from shoot tissue of the stored plants and nematodes extracted as stated above. In this way, nematode population density could be monitored over the winter while following commercial handling practices of refrigerating stock plants for the winter. Attempts to plant these stored plants in the field in May 1977 were unsuccessful due to the very hot and dry spring that year.

RESULTS AND DISCUSSION

Nematode Survey

Results

The nematode surveys taken August 1, and October 10, 1975, revealed D. dipsaci present in large numbers in shoot samples (Table 1). Aphelenchoides spp., another foliar parasitic nematode, was present in fewer samples and at lower population densities. Five genera of parasitic root feeding nematodes were extracted from soil samples and of these nematode, Pratylenchus penetrans was found in the highest percentage of samples.

The average numbers of D. dipsaci found in healthy versus diseased plants were categorized according to phlox cultivars (Tables 2, 3, and 4). No complete resistance to D. dipsaci was observed in any of the eleven cultivars of P. subulata sampled, and the degree of infestation symptoms that were expressed varied widely with respect to the number of nematodes extracted.

Soil samples from the ground phlox cultivars surveyed yielded averages of 128.4 nematodes per 100 cm^3 soil for diseased plant samples and 3.8 nematodes per 100 cm^3 soil for healthy appearing plant samples (Table 2). The standard error of the mean was 57.5 and 0.9 for diseased and healthy plant samples, respectively. Soil samples from the cultivar Emerald Pink had the highest nematode population counts of the cultivars, with an average of 616.3 nematodes per 100 cm^3

Table 1. Plant parasitic nematode species detected in eleven *P. subulata* cultivars sampled in 1975.

Nematode Species	Samples with nematodes (%)	Population density
Soil (100 cm^3)		
Ditylenchus dipsaci	69	96.6
Pratylenchus penetrans	16	5.8
Aphelenchoides spp.	19	6.2
Criconemoides spp.	6	5.0
Tylenchus spp.	6	4.5
Tylenchorhynchus spp.	5	8.1
Helicotylenchus spp.	1	1.0
Shoot (g)		
Ditylenchus dipsaci	52	126.5
Aphelenchoides spp.	18	9.1
Root (g)		
Ditylenchus dipsaci	24	4.5
Pratylenchus penetrans	57	29.2
Aphelenchoides spp.	16	15.5
Criconemoides spp.	1	1.0
Tylenchus spp.	1	5.0
Tylenchorhynchus spp.	1	1.0

Table 2. Mean number of Ditylenchus dipsaci recovered from soil associated with diseased and healthy P. subulata.

Cultivar	D. dipsaci per 100 cm^3 soil ($s_{\bar{x}}$; C.V.)	
	Diseased plants	Healthy plants
Atropurpurea	86 (45; 111)	2 (1; 105)
Blue Hills	17 (10; 12)	10 (10; 433)
Crimson Beauty	49 (33; 45)	3 (2; 80)
Emerald Blue	14 (8; 13)	1 (1; 23)
Emerald Pink	616 (307; 792)	6 (2; 168)
Pink Perfection	2 (1; 1)	0 (0; 0)
Red	12 (11; 16)	9 (5; 264)
Red Wing	10 (8; 12)	2 (2; 74)
Rosea	1 (1; 1)	0 (1; 0)
Scarlet Flame	4 (2; 4)	10 (7; 439)
White Delight	25 (12; 34)	1 (1; 39)

Table 3. Mean number of *Ditylenchus dipsaci* recovered from shoot samples of diseased and healthy *P. subulata*.

Cultivar	*D. dipsaci* per gram shoots ($\bar{x}$; C.V.)			
	Diseased plants		Healthy plants	
Atropurpurea	53	(27; 69)	2	(1; 64)
Blue Hills	2	(2; 3)	50	(39; 1333)
Crimson Beauty	4	(4; 5)	1	(1; 34)
Emerald Blue	49	(38; 61)	0	(0; 0)
Emerald Pink	450	(147; 392)	9	(9; 564)
Pink Perfection	0	(0; 0)	1	(1; 22)
Red	207	(64; 102)	0	(0; 0)
Red Wing	123	(91; 126)	0	(0; 0)
Rosea	2	(1; 1)	0	(0; 0)
Scarlet Flame	9	(7; 11)	0	(1; 8)
White Delight	68	(25; 74)	1	(1; 25)

Table 4. Mean number of *Ditylenchus dipsaci* recovered from root samples of diseased and healthy *P. subulata*.

Cultivar	*D. dipsaci* per gram roots ($s_{\bar{x}}$; C.V.)			
	Diseased plants		Healthy plants	
Atropurpurea	2	(1; 166)	0	(0; 48)
Blue Hills	0	(0; 0)	0	(0; 0)
Crimson Beauty	2	(2; 267)	5	(4; 1153)
Emerald Blue	0	(0; 0)	0	(0; 0)
Emerald Pink	5	(2; 508)	1	(1; 284)
Pink Perfection	0	(0; 0)	0	(0; 0)
Red	2	(1; 132)	1	(1; 379)
Red Wing	1	(1; 76)	0	(0; 0)
Rosea	0	(0; 0)	0	(0; 0)
Scarlet Flame	0	(0; 27)	0	(0; 0)
White Delight	1	(1; 124)	1	(1; 560)

soil. Emerald Pink showed much variability, however, with individual diseased plant samples ranging from 3 to 3,463 nematodes per 100 cm^3 soil and a coefficient of variability (C.V.) of 792% (standard deviation per sample expressed as percent of mean.) Other diseased cultivars showed less variability and consisted of, in order of decreasing average number of D. dipsaci extracted per cultivar, Atropurpurea, Crimson Beauty, White Delight, Blue Hills, Emerald Blue, Red, Red Wing, Scarlet Flame, and Pink Perfection. The soil samples from Rosea had the lowest average number of nematodes extracted, compared to the other cultivars, with an average of 0.7 nematode per 100 cm^3 soil and a C.V. of 0.9%.

Soil samples from healthy plants generally had low nematode populations compared to diseased plants. From the cultivar Emerald Pink, individual sample counts ranged from 0 to 21 nematodes, but averaged 6.0 nematodes per 100 cm^3 soil with a C.V. of 167%. The cultivar Scarlet Flame had the highest average for soil samples from healthy plants with 10.3 nematodes per 100 cm^3 and a C.V. of 439%. The lowest nematode counts were from the soil samples of Pink Perfection and Rosea, where no D. dipsaci were extracted. Healthy plants consisted of, in order of decreasing average number of D. dipsaci extracted from 100 cm^3 soil, Scarlet Flame, Blue Hills, Red, Emerald Pink, Crimson Beauty, Atropurpurea, Red Wing, Emerald Blue, White Delight, Rosea and Pink Perfection.

Shoot samples taken from all cultivars had an average of 124.5 nematodes per gram from the diseased plants and 5.1 nematode per gram from healthy plants. The standard errors of the means were 33.4 and 2.7 for diseased and healthy plants, respectively. The cultivar Emerald Pink had the highest nematode counts with an average of 450.4

nematodes per gram. Individual samples yielded from 34 to 584 nematodes, resulting in a high C.V. of 392%. The cultivar Pink Perfection had the lowest nematode count of all cultivars surveyed with no nematodes detected. Foliar samples of diseased plants consisted of, in order of decreasing average number of D. dipsaci extracted per gram of shoots, Emerald Pink, Red, Red Wing, White Delight, Atropurpurea, Emerald Blue, Scarlet Flame, Crimson Beauty, Blue Hills, Rosea, and Pink Perfection.

Foliar samples from healthy plants of the cultivar Blue Hills had the highest average number of nematodes per gram, 50.3. Shoot samples from this cultivar were extremely variable, ranging from 6 to 128 nematodes extracted per gram, leading to a high C.V. of 1,333%. Four cultivars yielded no nematodes upon extraction of healthy plant samples; these included Emerald Blue, Red, Red Wing, and Rosea. The order of decreasing average number of D. dipsaci extracted from healthy plant samples consisted of Blue Hills, Emerald Pink, Atropurpurea, Crimson Beauty, Pink Perfection and White Delight (both with 0.7), Scarlet Flame, and Emerald Blue, Red, Red Wing, and Rosea, all with no nematodes extracted.

Root samples from diseased and healthy plants of the cultivars surveyed revealed averages of 1.5 and 0.6 nematodes per gram of roots, respectively. Standard errors of the means were 0.5 for diseased, and 0.2 for healthy samples. The cultivar Emerald Pink had the highest average nematode count, 3.5 nematodes per gram of roots. Individual samples ranged from 0 to 19 nematodes per gram and resulted in a high C.V. of 508%. Root samples from four cultivars classified as diseased yielded no nematodes. These cultivars were Blue Hills, Emerald Blue, Pink Perfection, and Rosea. The cultivars were, in decreasing order of average number of D. dipsaci extracted from diseased plant samples,

Emerald Pink, Crimson Beauty, Red, Atropurpurea, White Delight, Red Wing, Scarlet Flame, and Blue Hills, Emerald Blue, Pink Perfection, and Rosea.

Healthy plant samples showed Crimson Beauty to be the cultivar with the highest average nematode count from root samples. This cultivar averaged 5.3 D. dipsaci per gram of roots, with individual samples ranging from 0 to 14 nematodes and a C.V. of 1,154%. Six cultivars had no nematodes in root samples of healthy plants; these included Blue Hills, Emerald Blue, Pink Perfection, Red Wing, Rosea, and Scarlet Flame. In order of decreasing average number of nematodes extracted from root samples of healthy plants, the cultivars were Crimson Beauty, White Delight, Emerald Pink and Red, Atropurpurea, Blue Hills, Emerald Blue, Pink Perfection, Red Wing, Rosea, and Scarlet Flame.

Discussion

While no complete nematode resistance was found, some cultivars, notably Pink Perfection, Red Wing, and Rosea, had fewer D. dipsaci per gram of shoots or per 100 cm^3 soil than the others. The cultivars consistently found to posses high population levels of the nematode were Emerald Pink, Atropurpurea, White Delight, Crimson Beauty, and Emerald Blue. The other cultivars expressed differential sensitivities to nematode infection: some displayed a hypersensitive reaction with severe infestation symptoms while not supporting nematodes; others displayed a moderate to sensitive reaction with chlorotic and/or necrotic symptoms.

The high number of nematodes extracted from shoot samples was usually linked to visual disease symptoms of chlorosis, necrosis,

and/or stunted vegetative growth in all cultivars except Blue Hills, Crimson Beauty, Pink Perfection, Rosea, and Scarlet Flame, where low numbers of nematodes were extracted from both diseased and healthy plant samples. These cultivars were either giving hypersensitive reactions to nematode infestation or were unable to support further population increases after low levels of damage had occurred.

Fewer nematodes were extracted from root samples which tends to support the hypothesis that D. dipsaci prefers photosynthetic tissue, when available, for feeding (27). The soil region around the immediate root area supported sufficiently high numbers of nematodes, perhaps reflecting the invasion of soil habitats by D. dipsaci as host plant tissue became damaged and unable to support further nematode increases.

Soil and shoot tissue extractions from healthy plant samples generally had lower average numbers of D. dipsaci than did diseased samples. The exception was the cultivar Blue Hills, which had approximately the same level of nematodes extracted from the soil of diseased and healthy plants, but had a greater number of nematodes extracted from the shoots of healthy than diseased samples, a reverse situation from the other ten cultivars.

Calculation of chi-square values for soil and shoot samples from the ground phlox cultivars surveyed indicated significance when testing the hypothesis that "healthy" or "diseased" condition was independent of the number of nematodes extracted (Table A1 and A2). Classifying the plant as apparently healthy or diseased was significantly related to the number of nematodes extracted from the sample. In most cases, this significance was due to "healthy" plants having very low numbers of nematodes extracted compared to "diseased" plants, where higher

counts generally resulted. In shoot samples, the cultivars Crimson Beauty, Emerald Blue, Emerald Pink, Pink Perfection, Red, Red Wing, and White Delight had nematode extraction averages from healthy plants which were significantly lower than the corresponding diseased samples according to chi-square tests for independence. The same test applied to soil samples indicated that the cultivars Blue Hills, Emerald Blue, Emerald Pink, Red, Red Wing, and Scarlet Flame had significantly lower nematode counts in healthy plants than those from diseased plants. Emerald Blue, Emerald Pink, Red, and Red Wing were the only cultivars with both soil and shoot samples showing significant chi-squares in relation the classification and numbers of nematodes extracted.

Physiological Race Identification

Results

Host plant reaction in field or steamed soil ranged from complete growth suppression (Melilotus indica, field soil) to vigorous growth (Lycopersicon esculentum, cv Rutgers, steamed soil). The survival rates of seeds of all host plants were low, however, once they were transplanted into either soil treatment and placed in the greenhouse environment. Twenty-three percent of the seedlings planted survived in the field soil while 43% survived in the steamed soil. Individual host plant species showed variable survival rates (Table 5). Tomato cv Rutgers had a survival rate of 5/20 for field soil and very high survival, 13/20, in steamed soil as did alfalfa. This relationship of low field soil survival correlated to high steamed soil survival for the same species would be expected if this race of D. dipsaci parasitized these plant species. However, other host species showed equal or reverse reactions from the expected. Tomato cv Stone had the same

survival rate in both field soil and steamed soil. Onion, however, had a reverse situation with the highest survival in field soil, 7/20, while in steamed soil, a lower survival rate resulted, 4/20. One host species, sweet clover, had low survival in both soils: in field soil there was no seedling growth, while in steamed soil 3 of 20 survived.

Table 5. Survival rates of selected host plant seedlings grown in field or steamed soil after 28 days.

Cultivar	Viable plants/Total number of plants	
	Field Soil	Steamed Soil
Allium cepa, cv Sweet Spanish	7/20	4/20
Lycopersicon esculentum, cv Rutgers	5/20	13/20
Lycopersicon esculentum, cv Stone	7/20	7/20
Melilotus indica, cv Yellow	0/20	3/20
Medicago sativa, cv Ranger RCC63	1/20	11/20
Beta vulgaris	5/8	8/8

Survival rates of the various host species were also reflected in average height and shoot fresh weight differences recorded (Figures 1 and 2). In all cases, plant height of the host species was lower in plants grown in infested when compared to those grown in steamed soil. Alfalfa and tomato cv Rutgers were the only two host plants which had significant height increases when grown in steamed as compared to field soil. Sweet clover and alfalfa in field soil conditions were

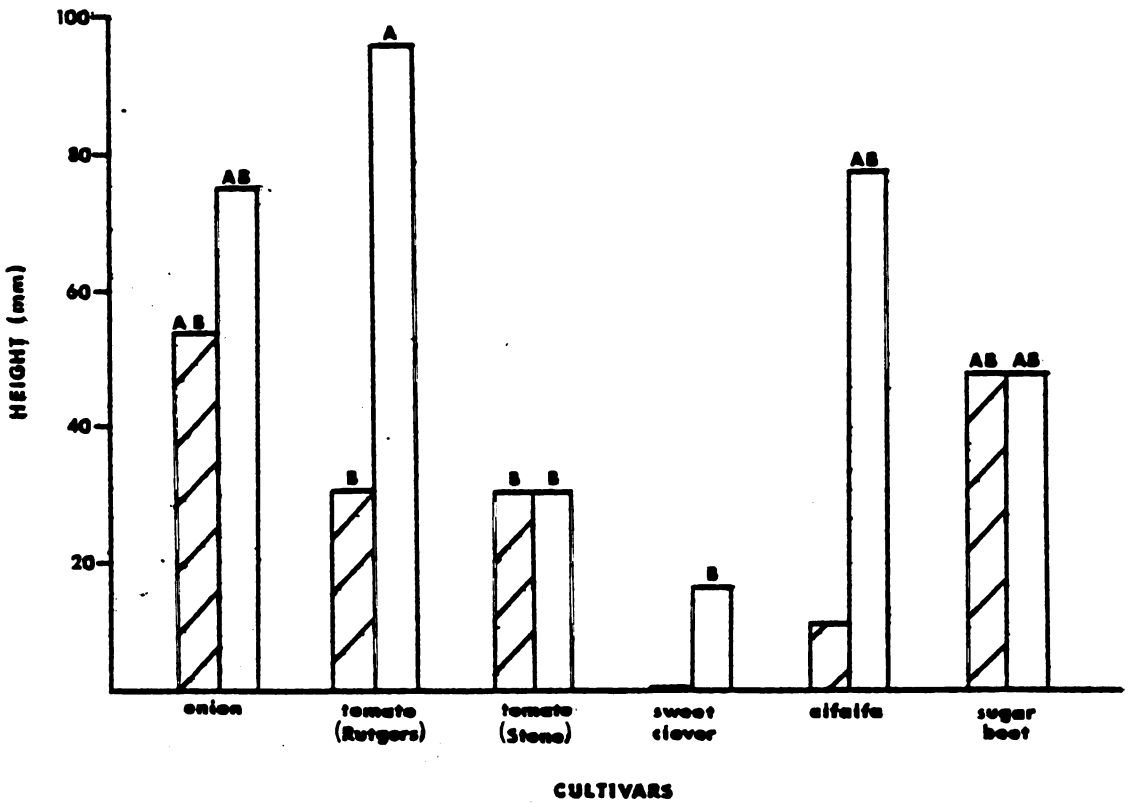


Figure 1. Height of selected host plants of D. dipsaci grown 28 days in field and steamed soil. Field soil ▨, steamed soil □. a, b = means are not significantly different according to Duncan's multiple range test.

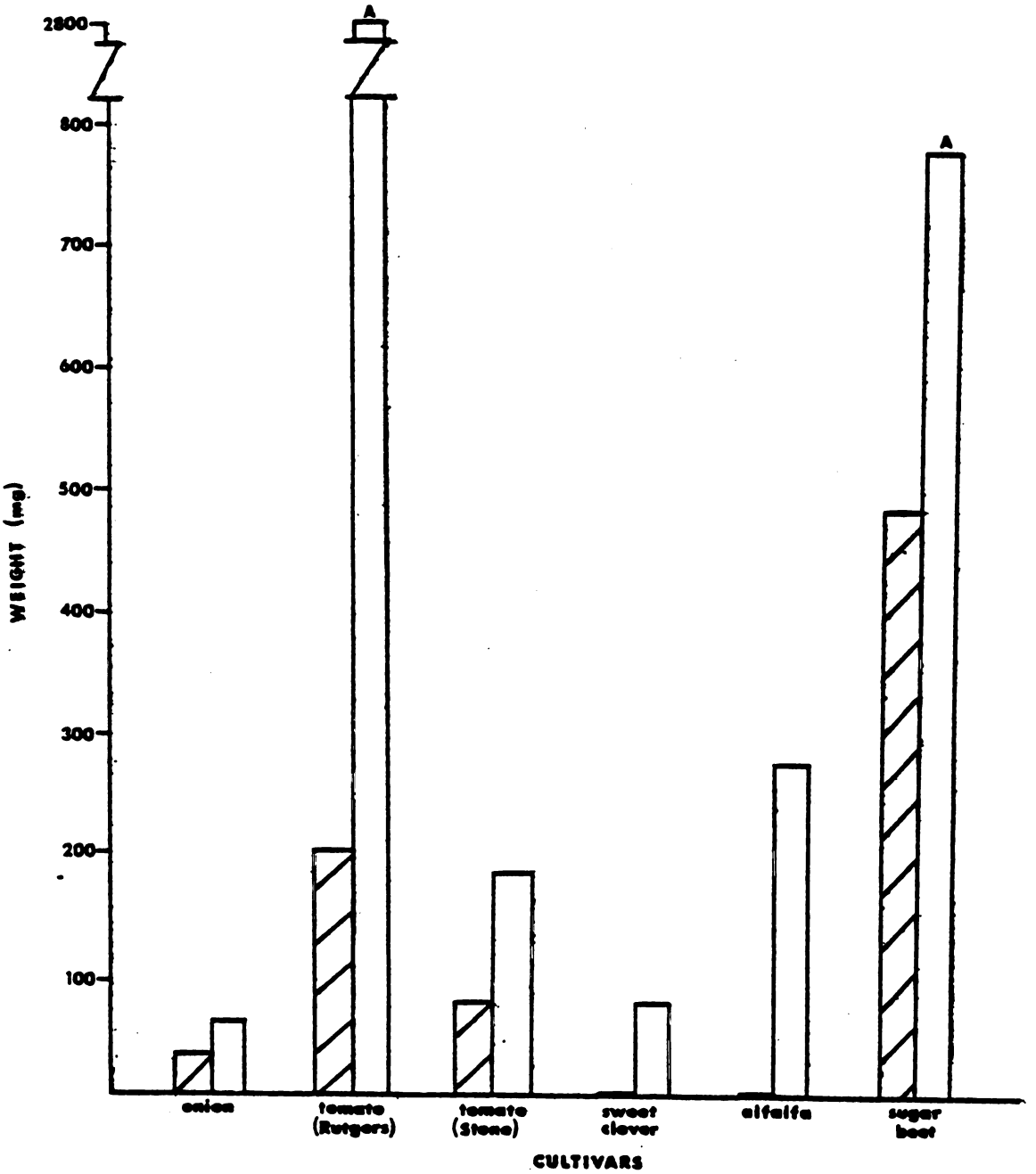


Figure 2. Shoot fresh weight of selected host plants of D. dipsaci grown for 28 days in field and steamed soil. Field soil ▨, steamed soil □. a = means are not significantly different according to Duncan's multiple range test.

significantly different in height from the other host plants as detected by Duncan's multiple range test (Figure 1). While the within species height differences were significant for only alfalfa and tomato cv Rutgers grown in steamed soil, other host plants had similar between species height differences.

All host species had fresh weight differences when grown in field versus steamed soil. Field soil generally resulted in low shoot fresh weight when compared to steamed soil conditions. Shoot fresh weight differences between species were significant, as expected. And, only alfalfa and tomato cv Rutgers grown in steamed soil were significantly higher in shoot fresh weight than the remaining host plants when means were compared by Duncan's multiple range. Low fresh weight values in some cases, notably sweet clover and alfalfa, were the result of poor seedling viability after transplanting to the soil treatment.

Onion was the only host plant species which harbored nematodes in shoot tissue (Table 6). This species averaged 104.6 nematodes per gram of shoots. None of the other host species supported foliar invasion by *D. dipsaci*. Soil populations in all host plants, however, were reduced from the initial level of 45 nematodes per 100 cm^3 soil and ranged from 3.5 nematodes for tomato cv Stone to 12 nematodes per 100 cm^3 soil for tomato cv Rutgers.

Table 6. Mean number of D. dipsaci from soil and shoots of host plants grown for 28 days in field or steamed soil.

Cultivar	D. dipsaci per 100 cm^3 soil or per gram			
	Field Soil		Steamed Soil	
	Soil	Shoots	Soil	Shoots
Allium cepa, cv Sweet Spanish	9.5	104.6	0	0
Lycopersicon esculentum, cv Rutgers	12.0	0	0	0
Lycopersicon esculentum, cv Stone	3.5	0	0	0
Melilotus indica, cv Yellow	8.0	0	0	0
Medicago sativa, cv Ranger RCC63	8.2	0	0	0
Beta vulgaris	7.5	0	0	0

Discussion

More than twenty physiological races of D. dipsaci are known (22) and since many plant species are infested by this nematode, it is difficult to carry out a greenhouse host plant assay in a comprehensive way. Therefore, host varieties were chosen that have been reported to be parasitized by D. dipsaci (15). Some of the reported varieties could not be obtained (e.g., Triticum durum, var Walsatch, and Lycopersicon esculentum, cv Stone Improved). The tomato variety Stone Improved was substituted by two others more readily obtainable. Wheat was not used as a host plant. Since plant and parasite genomes are known to closely interact in the expression of pathogenicity (40, 42), the variety substitutes may have limited nematode infestation symptoms. The objective of this investigation was to distinguish, by

host preference and nematode reproductive increases, the physiological race of D. dipsaci that parasitizes selected P. subulata cultivars. Limitations were therefore placed on the method of greenhouse bioassays in the case of the tomato variety substitutions.

Host plants maintained detectable levels of D. dipsaci in the soil. Only Allium cepa, cv Sweet Spanish, supported foliar invasion of D. dipsaci compared to the other host plants. Still, onion seedlings had a higher survival rate in field compared to steamed soil. Soil population levels of D. dipsaci also remained relatively high. This paradox may be the result of cultural problems, and chi-square calculations indicated that field survival was not significantly different from that of steamed soil survival rates (Table A3). Shoot fresh weight and plant height for onion were not different as indicated by Duncan's multipe range test, yet the values for the field treatment were less than steamed soil values in both cases. Thus, onion showed a higher, although not significant, survival rate, lower height and shoot fresh weight in field compared to steamed soil. The significant observation of D. dipsaci infestation on onion was that it was able to survive and reproduce freely.

Tomato cv Rutgers had no significant difference in survival rate for the two soil treatments. Although it maintained the highest soil population of D. dipsaci, it did not exhibit invasion of foliar tissue. Plants in steamed soil had significant height and shoot fresh weight increases over plants in field soil which may have been damaged by nematode feeding attempts.

Tomato cv Stone did not have significant survival rates, shoot fresh weight or height differences in field soil or steamed soil. No nematodes were extracted from shoot tissue and soil populations were

the lowest of the host plants tested. Since L. esculentum, cv Stone Improved, was reported as a host cultivar for D. dipsaci, it is apparent that the substitution of the cultivar Stone for Stone Improved was not justified.

Sweet clover and sugar beet had significant survival differences between the two soil treatments. Only eight plants of sugar beet were given either soil treatment, two per replicate, due to germination difficulties. Height and shoot fresh weight differences were not significant for these two host plants. D. dipsaci was not able to infest the foliage of these species and soil population levels were similar.

The alfalfa variety Ranger had been previously described as a host species of D. dipsaci. In this study, no significant height, shoot fresh weight, or survival differences were detected by Duncan's multiple range and chi-square tests. Survival was very low in both soil treatments, which would tend to support the premise that cultural problems existed with this species.

From the above comparisons of nematode counts, survival rates, height and shoot fresh weight differences, it is apparent that the nematode causes stunting of host plants and fresh weight reductions over control plants in greenhouse bioassays. The unknown race of D. dipsaci infesting P. subulata was able to survive and multiply on the host plant A. cepa, cv Sweet Spanish, and on the four cultivars of P. subulata tested in the pathogenicity study. Since both onion and phlox have distinct D. dipsaci races, either race could be responsible for the observed disease symptoms. While the onion race has not been specifically reported to infest phlox, it is possible that interbreeding between the onion and phlox race of D. dipsaci has

occurred, since both of these economic species are extensively grown in the Hudsonville, Michigan area, and nematode races are known to interbreed freely (29). Without specific controlled hybridization studies between the phlox and onion races, these races are difficult to distinguish. It is presumed that the ranges of the races tend to overlap, although the primary host plant may vary. Thus, three possible physiological races of D. dipsaci parasitizing P. subulata exist: the phlox race, the onion race, or a phlox-onion hybrid race.

Several sources of error exist in determining the identity of the physiological race. Increased replication would have avoided problems associated with host seedling survival in some cases. Cultural problems such as root restriction, no fertilization, and weed competition in field soil treatments influenced the outcome of this experiment. Weed competition in field soil was a severe problem, although replicates were kept hand weeded as much as possible. The most important source of error appears to have been cultural, either in transplanting from petri dishes to greenhouse conditions or while under the greenhouse environment. Perennial seedlings, such as those of alfalfa and sweet clover, were the most difficult to establish.

Pathogenicity

Results

Phlox subulata cultivars growing either in field or steamed soil exhibited differences in plant height and shoot fresh weight. The plant height of the cultivar White Delight grown in field soil averaged 36, 30, and 27.75 mm per plant over the three harvest dates, while the same cultivar grown in steamed soil averaged 43.25, 52.5, and 80 mm (Figure 3). The average height of Emerald Pink grown in field soil fluctuated over

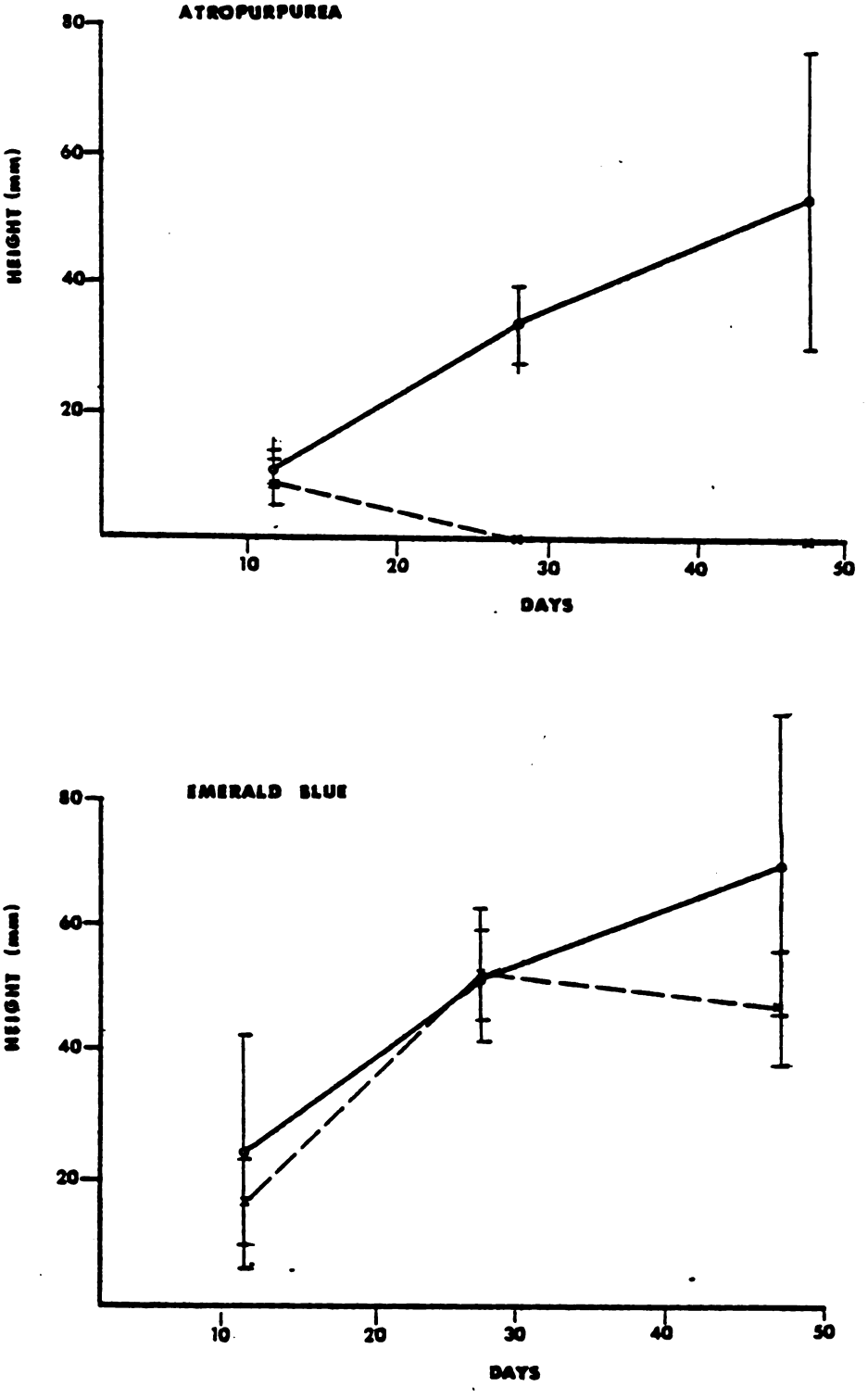
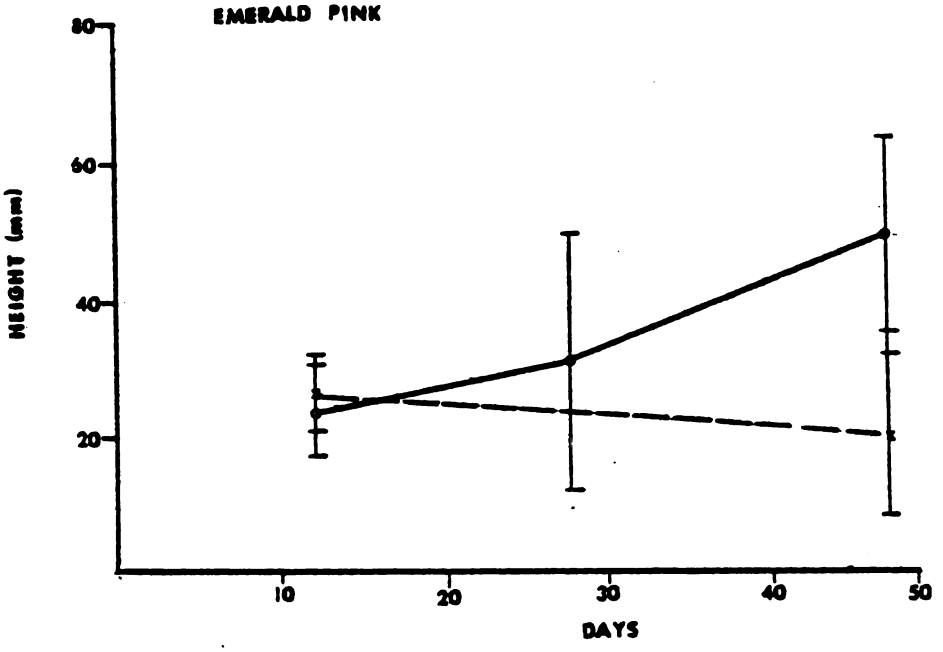
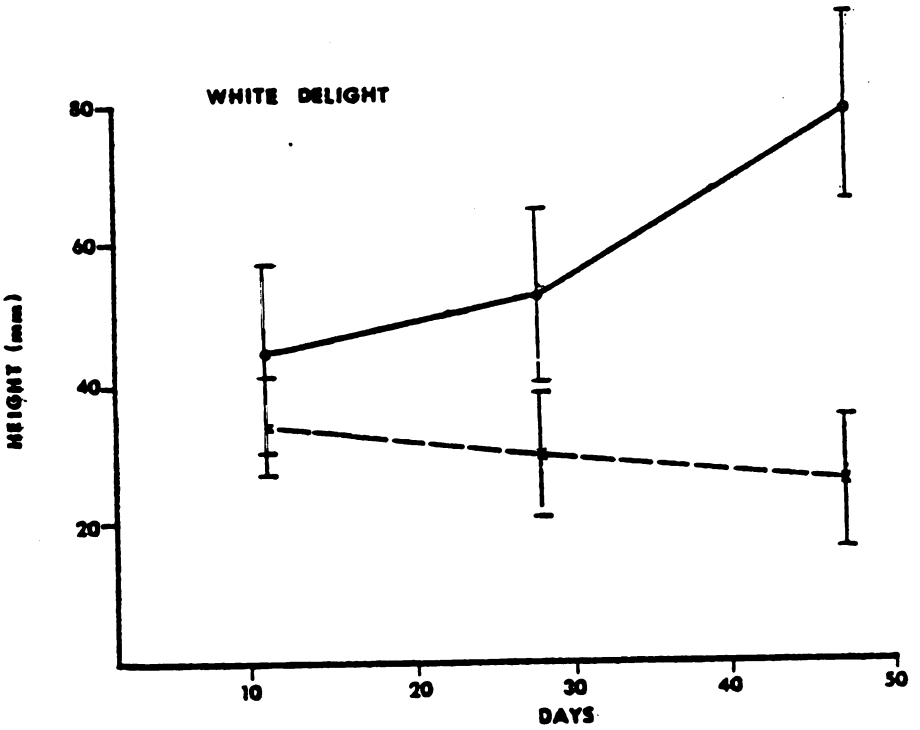


Figure 3. Height (mm) of four cultivars of Phlox subulata grown in field and steamed soil. Plants grown in field soil ×———×, steamed soil •———•.

Figure 3 (cont'd.).





the three harvest dates from 25, 0, to 20.5 mm. Growing in steamed soil, this cultivar showed a steady height increase of 22.25, 31.5, and 47 mm. Emerald Blue grown in field soil did not exhibit the stunted growth as did the other three cultivars, with an average height of 16.75, 54.5, and 47.75, although these averages were less than those for Emerald Blue plants growing in steamed soil, 24.5, 53, and 69.5 mm. Atropurpurea exhibited a very stunted response in field soil, with 9.5, 0, and 0 mm averages over the three harvest dates. In steamed soil, this cultivar showed dramatic height increases of 10.5, 36.5, and 56.25 mm at the three harvest dates, respectively.

Shoot fresh weights of the four cultivars likewise reflected the height differences previously described. White Delight grown in field soil showed progressive decreases in shoot fresh weight, from 0.168, 0.129, to 0.009 gram per plant (Figure 4). Plants growing in steamed soil, however, had shoot fresh weight increases of 0.113, 0.362, and 0.508 gram at the three harvest dates. The cultivar Emerald Pink grown in field soil had variable shoot fresh weight changes of 0.052, 0, and 0.031 gram, tending to decrease over time, while in steamed soil it had fresh weight gains as the plants increased in height, progressing from 0.114, 0.197, to 0.278 gram per plant.

Emerald Blue, which did not show height differences, had fluctuating shoot fresh weights. Grown in field soil, the values were 0.073, 0.237, and 0.136 gram per plant; in steamed soil, the plants showed steady fresh weight gain over the three harvest dates: 0.171, 0.271, and 0.417. The cultivar Atropurpurea grown in field soil had measurable shoot fresh weight only on the first harvest date; the remaining plants of the replicates did not survive to subsequent harvest dates. These plants averaged 0.059, 0 and 0 gram per plant. In steamed soil,

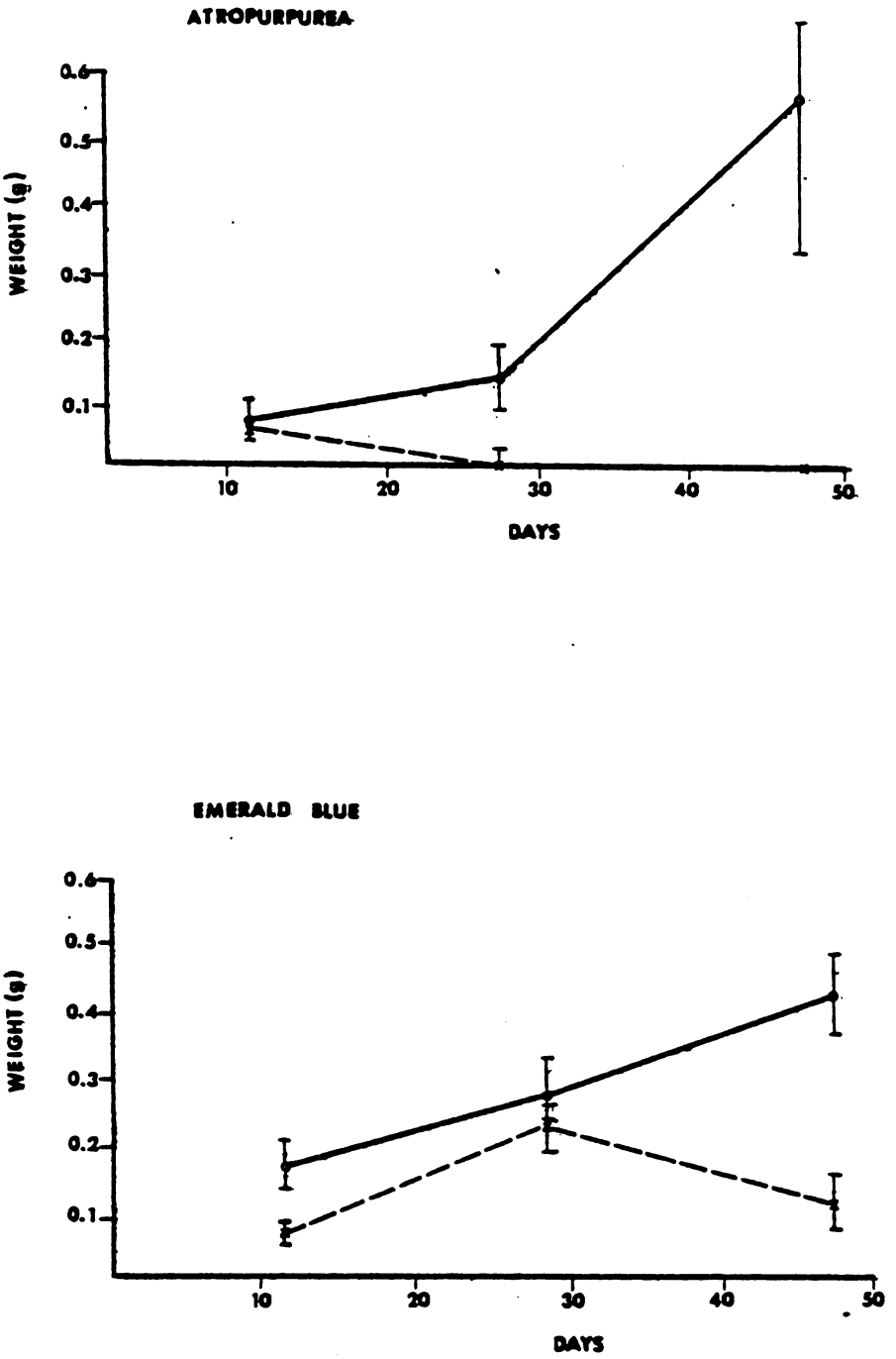
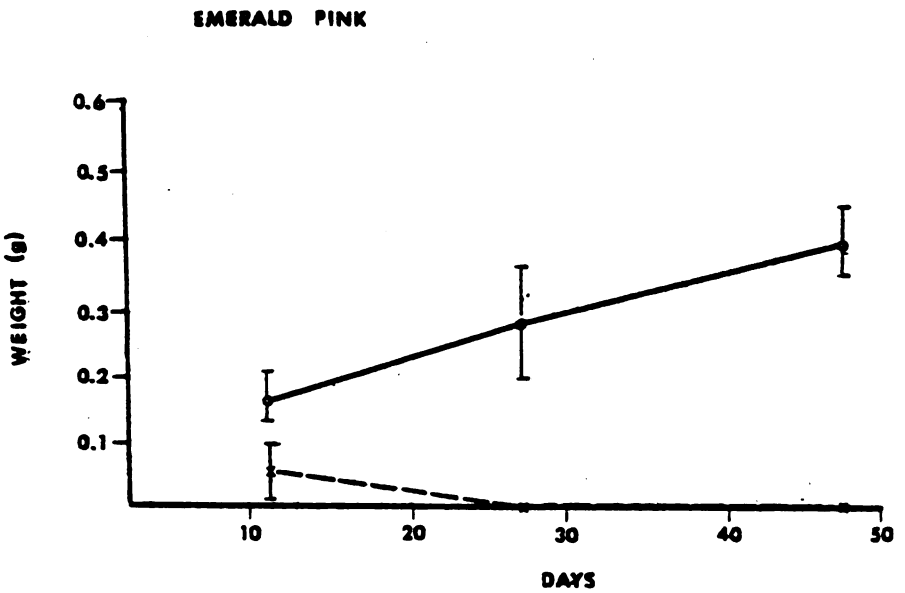
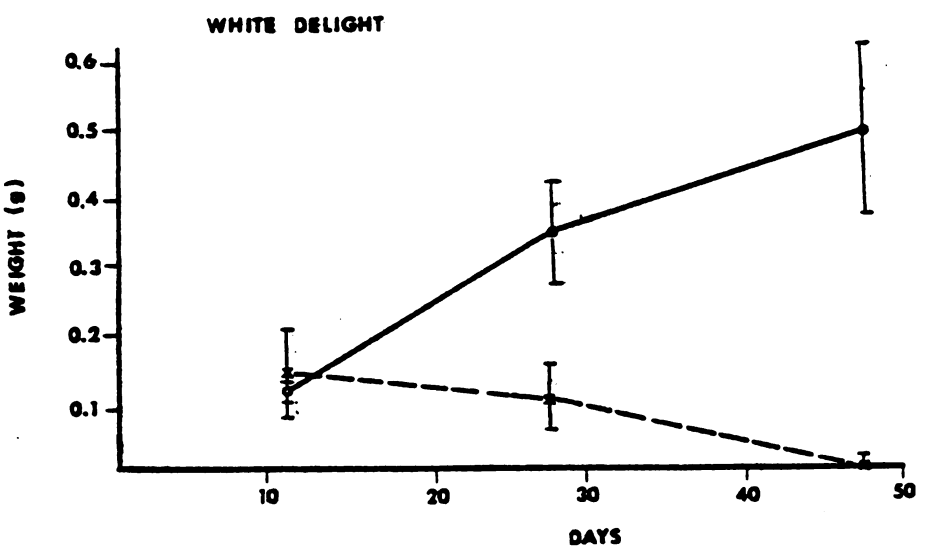


Figure 4. Shoot fresh weight of four cultivars of Phlox subulata grown in field and steamed soil. Plants grown in field soil x———x, steamed soil •———•.

Figure 4 (cont'd.).





steady shoot fresh weight increases of 0.066, 0.137, and 0.571 gram per plant occurred over the three harvest dates.

Survival rates of plants grown in the two soil treatments greatly influenced their average height and shoot fresh weights as noted previously. Under field soil conditions, Emerald Pink and Atropurpurea had high mortality rates with 9 and 7, respectively, of a total of 16 plants surviving until the end of the experiment (Table 7). White Delight had fewer losses with 14 of 16 plants surviving. Only the cultivar Emerald Blue had the entire 16 plants of the field soil treatment survive until the third harvest date. Contrastingly, in steamed soil, White Delight, Emerald Blue, and Atropurpurea all had 100% survival. Emerald Pink was the only cultivar with plant mortality occurring in the steamed soil; 15 of 16 survived.

Table 7. Survival rates of P. subulata cultivars grown in field or steamed soil after 0, 11, 29, and 48 days of growth.

Treatment	Cultivar	Plants viable (4 possible) Days				Total
		0	11	29	48	
Field Soil	White Delight	4	4	3	3	14
	Emerald Pink	4	3	0	2	9
	Emerald Blue	4	4	4	4	16
	Atropurpurea	4	3	0	0	7
Steamed Soil	White Delight	4	4	4	4	16
	Emerald Pink	4	4	3	4	15
	Emerald Blue	4	4	4	4	16
	Atropurpurea	4	4	4	4	16

Ditylenchus dipsaci was extracted from shoot tissue of the four cultivars at increasing levels which corresponded to the observed gradual decline in height and shoot fresh weights of plants grown in field soil (Table 8). The cultivars all supported soil populations of D. dipsaci, although at much reduced levels than initally present (Table 9).

Table 8. Mean number of D. dipsaci recovered from shoot tissue of four P. subulata cultivars grown in field or steamed soil at four harvest dates.

Cultivar	Number of D. dipsaci per gram							
	Field Soil				Steamed Soil			
	Harvest Dates				Harvest Dates			
	0	11	29	48	0	11	29	48
White Delight	0	1.2	2.7	0.7	0	0	0	0
Emerald Pink	0	8.0	---*	0.5	0	0	0	0
Emerald Blue	0	0	1.0	1.0	0	0	0	0
Atropurpurea	0	6.0	---*	---*	0	0	0	0

* All plants dead.

Table 9. Mean number of D. dipsaci recovered from soil samples of four P. subulata cultivars grown in field or steamed soil after 48 days.

Cultivar	Number of D. dipsaci per 100 cm^3 soil	
	Field Soil	Steamed Soil
White Delight	7.5	0
Emerald Pink	2.2	0
Emerald Blue	5.5	0
Atropurpurea	1.5	0

Discussion

Phlox subulata is commercially propagated by division of stock plants. Thus, plant height and shoot fresh weight are important indices of plant quality relative to the annual acreage planted from ground phlox divisions. Any factors which reduce optimal plant growth result in immediate economic loss in terms of total numbers of plants produced and their quality. *Ditylenchus dipsaci*, found in large numbers in *P. subulata*, has been demonstrated to significantly retard plant growth over time (Tables B3, B4, B5, and B6). Analyses of variance comparing shoot fresh weight and plant height to harvest date indicated that significant interaction of plant response at harvest date with respect to increasing plant height or shoot fresh weight depended whether or not the plants were grown in nematode infested or steamed soil. A trend comparison indicated a highly significant linear increase in plant height and shoot fresh weight with progressive harvest dates when planted in steamed soil. No significant trend response was detected when field soil was used. Steamed soil allowed maximum plant growth, thereby resulting in significant height and weight increases over stunted plants grown in field soil.

Emerald Pink and Atropurpurea, which had high plant mortality rates and consequently low average plant height and shoot fresh weight, had the highest numbers of nematodes detected per plant at the first harvest date. The remaining two harvest dates had low nematode counts due to reduced survival rates of replicates of these two cultivars. White Delight had a variable nematode count over the three harvest dates, while Emerald Blue showed the least sign of nematode infection with an average of 1 nematode detected at the second and third harvest dates. This cultivar had only minimal declines in plant height

and shoot fresh weight.

None of the plants growing in steamed soil yielded D. dipsaci from shoot samples. Soil levels of nematodes declined in all cultivars after 48 days from the initial population level of 45 nematodes per 100 cm^3 soil. The field soil treatment harbored only a low nematode level while the steamed soil still remained nematode-free after 48 days. This may have been due to cultural conditions such as root restriction, no fertilization, and weed competition in the field soil treatments which would make the host plant physiologically weak. Plants were hand watered and excess water from the clay pots collected in petri dishes situated underneath each pot so the plants were continually moist, which may have created an unnatural environment for D. dipsaci compared to field situations.

The reductions in plant height and shoot fresh weight of the four cultivars were related to the degree of nematode infestation. Ditylenchus dipsaci feeds on living cells of either stem or leaf tissue, inducing enzymatic changes and sometimes emptying cellular contents of mesophyll or stem cortical cells which eventually leads to cellular destruction. In addition, reductions in height and shoot fresh weight can be directly related to a reduction in photosynthetic capacity, leading to imbalances in nutrient and water absorption rates from restricted shoot and root growth.

Infestations of D. dipsaci on P. subulata can cause severe economic losses for growers, especially through the two parameters investigated in this study, plant height and fresh weight of shoots, which determine the quality and viability of ground phlox divisions. Vegetative increases of stock plants depend on the health and vigor of the plants, a condition seen to be significantly increased for P. subulata plants

grown in steamed soil.

A contributing factor to the increased plant growth of the steamed soil treatment resulted from the high survival rates of the plants. Plants grown in field soil, while competing with nematode infection, had more difficulty in becoming established. Once the plants were established, nematode damage continued to suppress plant growth, particularly laminar growth and expansion, as indicated by lower height and weight of the shoots and the presence of nematodes in the damaged plants. Less than normal surface area would result in more limited light and nutrient absorption capacities, causing further plant growth reductions.

However, conclusions drawn from greenhouse evaluations are limited by several factors and do no necessarily reflect similar field comparisons. Mentioned above were cultural problems such as root restiction, lack of fertilization, and weed competition as factors influencing the pathogenicity results. In addition, the four replicates used displayed variability, especially in the field soil treatment, so that adequate comparisons could not be made in some cases, notably Emerald Pink and Atropurpurea, due to loss of replicates. The three harvest dates limited detection of any significant differences in nematode population growth occurring between the 20 day harvesting interval, although D. dipsaci is reported to have a 19 to 23 day egg and larval development stage and an adult life expectancy of 45 to 73 days (50).

Control Program Evaluation

Results

General Trends

Two systemic nematicides, aldicarb and oxamyl, were used during 1976 and 1977 at Hudsonville, Michigan. Approximately 1,500 plants were given four treatments: 1) control, 2) aldicarb granular application, 3) oxamyl appied as a foliar spray, and 4) aldicarb and oxamyl combination. The field plot was a completely randomized design with six replications of each treatment.

The nematicide treatments induced significantly reduced nematode populations in 1976. Aldicarb, oxamyl and aldicarb/oxamyl treatments were all effective in controlling nematode population growth, and no differences in effectiveness of the two pesticides or their combination were detected.

Since aldicarb and oxamyl alone each controlled the nematode population, the aldicarb/oxamyl combination treatment was discontinued in July 1976. Oxamyl treatments to treatment 3 were discontinued in August 1976 as population levels declined (Figure 5).

Population density in all treatments at the onset of the nematicide trials or at growing degree day accumulation point 0 (Table 10) represented the number of nematodes present in plants transplanted in the field in May 1976. These plants contained a range of nematodes, averaging from 16.2 *D. dipsaci* per gram of shoots for the oxamyl treatment to 40.7 *D. dipsaci* per gram of shoots for the aldicarb/oxamyl combination treatment.

Ten plants from each replication of the four treatments were collected in October 1976 and overwintered at 5–7 C in closed plastic

Table 10. Mean number of D. dipsaci recovered from shoot samples of P. subulata, cultivar Emerald Pink, treated in the field with selected nematicides in 1976.

Growing Degree Days Base 10 (C)	D. dipsaci per gram of shoots			
	Nematicide Treatment			
	Control	Aldicarb*	Oxamyl**	Aldicarb/Oxamyl
0	22.7	19.8	16.2	40.7
93	133.2	25.7	100.7	15.7
151	65.5	0.7	2.5	0.5
297	48.8	0.2	1.3	0.0
428	113.7	0.0	1.7	0.0
574	388.2	4.2	0.8	0.7
748	357.2	1.0	0.7	0.5
868	895.3	7.5	1.7	1.8
1047	644.0	0.0	1.2	0.0
1169	915.7	1.2	2.2	0.5
$\bar{X}$	358.4	5.8	12.8	6.0

* Aldicarb granular application.

** Oxamyl spray applications.

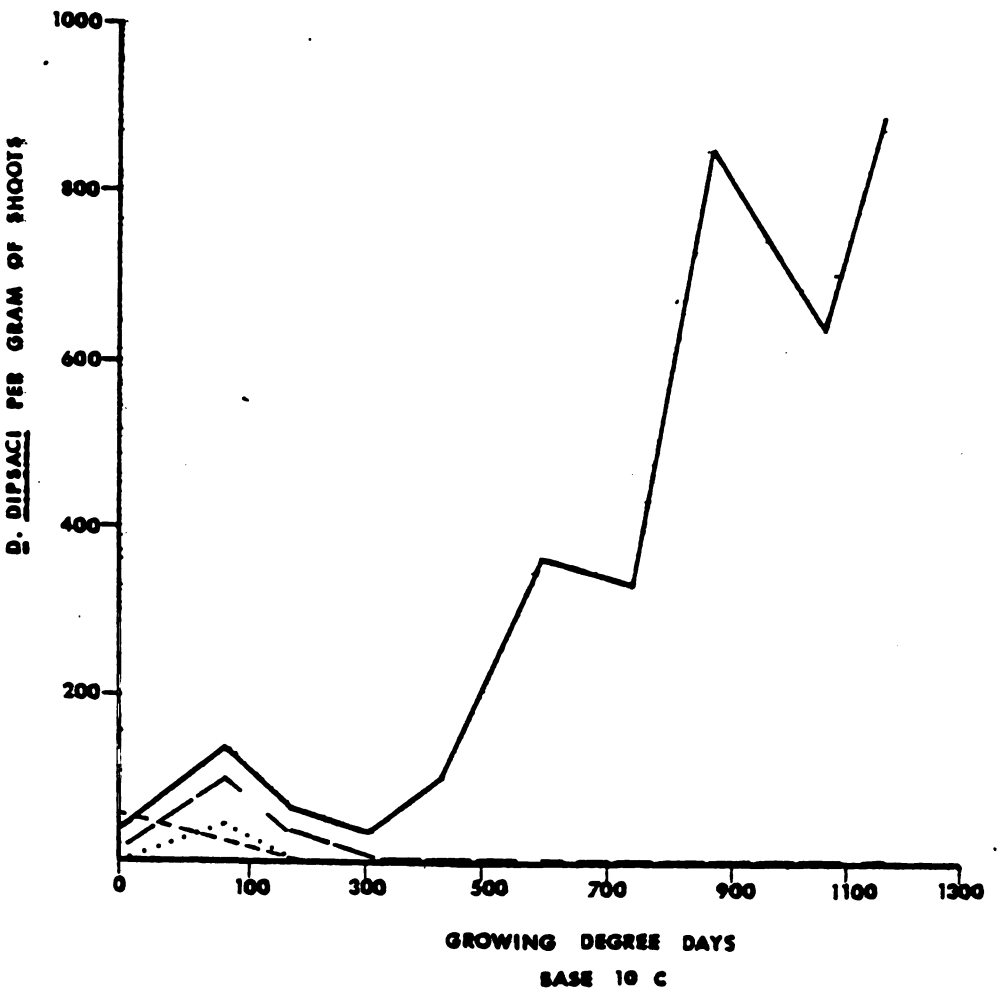


Figure 5. Mean number of D. dipsaci recovered from shoot samples of P. subulata, cultivar Emerald Pink, treated with no nematicide ———, aldicarb ·····, oxamyl — — —, or their combination— — —, in 1976.

bags. These plants were planted out in May 1977. The previous scheme was an attempt to simulate grower handling practices of refrigerated winter storage of stock plants, however, due to an excessively hot and dry Spring in 1977, none of the refrigerated plants survived transplanting. Consequently, all data collected from field plots in 1977 were from plants which overwintered in the field.

In 1977, the same treatment plan as the previous season was followed with slight modifications. The four treatments, each with six replicates, were monitored monthly for nematode population changes and applications of nematicides were given only when it was determined necessary to slow the spread of nematode infestation.

The nematode population, in all treatments, exhibited an increase early in 1977, although quite reduced in number when compared to 1976 (Table 11, Figure 6). After an early peak in June 1977, the nematode population gradually declined so that by August, the numbers were very low. Control plants gradually declined and became unsuitable for maintaining continued nematode population growth. Nematode numbers in these control replicates began to decline gradually. Oxamyl was applied one time in 1977 at the same rate as 1976 to control nematodes which may have been spreading from diseased control replicates to those containing healthy plants treated with oxamyl. The aldicarb and aldicarb/oxamyl combination treatments had low numbers of nematodes throughout the 1977 season and further nematicide treatments were not given to these plots in 1977.

Field Population Density

Data from the 1976 control plot afforded the opportunity to observe the natural population dynamics of *D. dipsaci* infesting *P. subulata*.

Table 11. Mean number of D. dipsaci recovered from shoot samples of P. subulata, cultivar Emerald Pink, treated in the field with selected nematicides in 1977.

Growing Degree Days Base 10 (C)	D. dipsaci per gram of shoots			
	Nematicide Treatment			
	Control	Aldicarb	Oxamyl*	Aldicarb/Oxamyl
0**(January)	305.8	3.4	3.5	0.5
0 (February)	18.8	11.7	0.0	0.2
0 (April)	80.2	14.7	3.0	3.7
202***	3.6	0.5	4.8	0.0
580	247.0	36.3	15.7	0.2
1040	26.7	8.0	19.0	1.2
1260	8.0	6.0	1.2	2.7
1477	19.8	8.8	0.5	11.0
1534	5.0	3.0	5.7	3.8
$\bar{X}$	82.4	10.3	5.9	2.6

* Oxamyl spray application.

** Plants overwintered in refrigerated storage at 5-7 C.

*** Plants overwintered in the field.

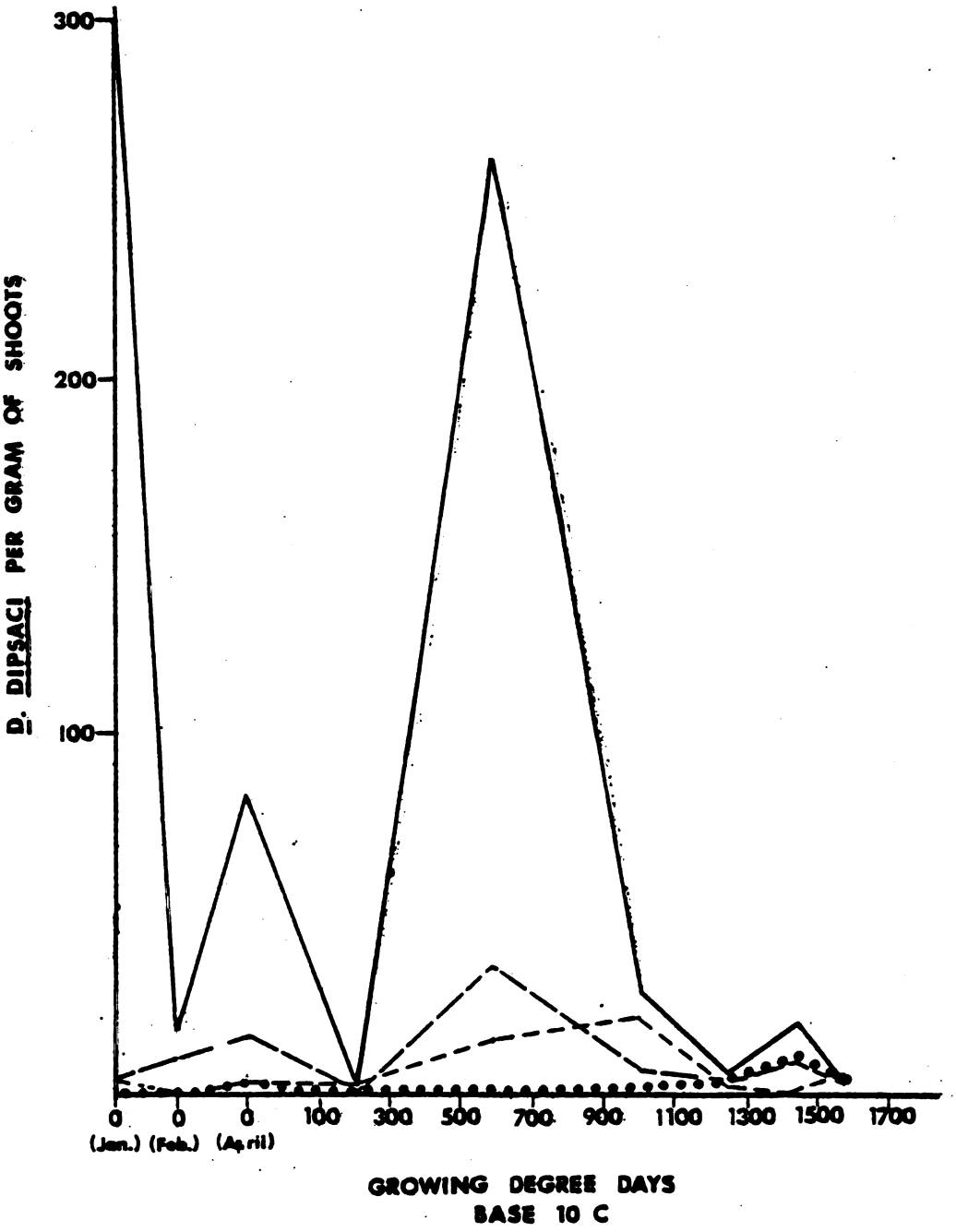


Figure 6. Mean number of D. dipsaci recovered from shoot samples of P. subulata, cultivar Emerald Pink, treated with no nematicide ———, aldicarb – – – –, oxamyl ——– –, or their combination •••••, in 1977.

The initial population of D. dipsaci present in ground phlox divisions transplanted into the field in Arpil 1976 averaged 22.7 nematodes per gram of shoots. After two weeks, the population increased to 133.2 nematodes. This can be considered the first population peak of the year, resulting from the maturation of eggs laid in stored ground phlox divisions. During the next four weeks, there was a gradual decline in the population so that the seventh week averaged 48.8 nematodes per gram of shoots. The second population peak occurred around the tenth week of the experiment with 388.2 nematodes recovered from a gram of shoots. This increase may represent a second generation of nematodes, whose number increased in response to increased Spring growth of the phlox plants in the field. The third population peak occurred approximately two weeks later, the fourteenth week into the experiment, with an average 895.3 nematodes per gram of shoots. Over the following two week interval, the population declined but was followed by a fourth population peak the eighteenth week of the experiment. Further population behavior was not monitored, as the eighteen week sampling date was completed in September 1976.

In September, the plants in the control treatment were severely stunted in growth compared to the treated plants, and had conspicuous patches of necrotic tissue as well as occasional plant death in the row.

By January 1977, the plants overwintering in plastic bags at 5-7 C from the control treatments had begun to deteriorate and nematode population levels declined to an average 305.8 nematodes per gram of shoots (Table 11). By March 1977, the population was 18.8 nematodes followed by a surge to 80.2 nematodes per gram of shoots in April 1977. By this time, plants stored were severely deteriorated and consequently,

the data taken during the 1977 growing season were from samples taken from plants overwintered in control treatments in the field.

In May 1977, these field plants had very low nematode levels, 3.6 nematodes per gram of shoots. As Spring progressed, the nematode population reached its seasonal maximum in June 1977 with 247.0 nematodes per gram of shoots. During the following months, there was an oscillating decline so that by October 1977, the population was at a low level comparable to the Spring, 5.0 nematodes per gram of shoots.

Regression analysis of the control treatment data gave a coefficient of correlation of 0.89 for the 1976 data, supporting the linear relationship of nematode population growth over time (Figure 7). In contrast, regression analysis of the 1977 control treatment data did not give a significant correlation coefficient of population density over time.

The control treatment in 1976 had significantly greater numbers of nematodes when compared to any of the nematicide treatments (Tables B7 and B8). However, this difference was not significant in 1977 (Table B9).

Aldicarb Treatment

Plants receiving a granular application of aldicarb 10G in April 1976 exhibited a decrease in nematodes the fourth week after application (Table 10). An average 19.8 nematodes per gram of shoots were recovered from plants of this treatment when transplanted to the field. Two weeks after aldicarb had been applied, the nematode population had increased slightly to 25.7 nematodes per gram of shoots. By the fourth week, the aldicarb was fully effective and nematode levels dropped to negligible numbers over the next six weeks from 0.7, 0.2,. to 0 nematodes per gram of shoots. The eleventh week showed an increase to 4

nematodes, but the population oscillated between 0 and 7.5 for the remaining eight weeks of the experiment (Figure 5). Treated plants were in excellent condition, with much vegetative growth by the end of the growing season.

The plants from the aldicarb treatment overwintered in refrigerated storage in good condition. The nematodes present in these plants also overwintered at 5-7 C satisfactorily (Table 11). A slight increase in number, from 3.4 to 14.6 nematodes per gram of shoots was detected from January 1977 to April 1977. In contrast, nematodes in plants overwintering in the field were at low levels upon testing in May 1977, with an average of 0.5 nematode per gram of shoots.

During 1977, the nematode population growth peaked for the aldicarb treatment in June 1977 with 36.3 nematodes per gram of shoots, following a pattern similar to the control treatment. The population then declined to 8.0 nematodes per gram of shoots and oscillated between 8.8 and 3 nematodes per gram of shoots for the remainder of the season (Figure 6). No aldicarb treatments were applied in 1977.

Oxamyl Treatments

The oxamyl treatment, initially contained plants with 16.2 nematodes per gram of shoots (Table 10). As with the aldicarb treatment, the nematode population in this treatment had an increase to 100.7 nematodes per gram of shoots by the third week of the experiment when oxamyl sprays were started. The nematode population immediately declined to 2.5 nematodes by the fifth week and remained at a steady low level, ranging between a high of 2.2 nematodes in September 1976 and a low of 0.7 nematodes in August 1976. The average number of nematodes recovered per gram of shoots from the oxamyl treatment over

1976 growing season was 12.8 nematodes (Table 10).

Plants from the oxamyl treatment overwintered satisfactorily in plastic bags held at 5-7 C. Nematode population levels in these plants appeared to have been maintained in storage (Table 11), with approximately the same level present at the beginning as at the end of winter storage. Plants overwintering in the field had population levels similar to those of stored plants, 4.8 and 3.0 nematodes per gram of shoots, respectively.

Levels of nematodes in the oxamyl treatment progressively increased in 1977 and reached a high in early August of 19.0 nematodes per gram of shoots. This is in contrast with the aldicarb treatment which had its population peak in June 1977. Oxamyl was then applied, using the same rate as in 1976. The nematode population dropped by the next sampling to 0.5 nematodes per gram of shoots. By October 1977, the population had recovered to 5.7 nematodes (Figure 6).

Aldicarb/Oxamyl Combination Treatment

Plants in the aldicarb/oxamyl combination treatment, had the highest population level, 40.7 nematodes per gram of shoots, of all treatments when transplanted in the field in April 1976. Aldicarb was applied in May and two weeks later, the population declined to 15.7 nematodes per gram of shoots. At this time, oxamyl spray was applied. The fifth week through the end of the experiment showed little population recovery, even when oxamyl spray was discontinued after the seventh week. This treatment had a mean of 6.0 nematodes per gram of shoots during the course of the experiment.

Nematode population levels increased slightly for this treatment during storage at 5-7 C, from 0.5 to 3.6 nematodes per gram of shoots. In plants overwintered in the field, no nematodes were detected in any

of the six replicates of this treatment in May 1977.

The population of D. dipsaci progressively increased during the 1977 growing season, from 0 to 0.2, 1.2, 2.7, to a seasonal high of 11.0 nematodes per gram of shoots during the fifth through the eighth month of 1977. By October 1977, the population had decreased to 3.8 nematodes per gram of shoots. The mean of the aldicarb/oxamyl treatment in 1977, with no nematicides applied, was 2.6 nematodes.

Discussion

Field Population Density

Although the data from the 1976 control treatment resulted in a significant coefficient of linear correlation of population growth over time, a closer look at the data shows negative deviations from the regression line from the fifth through the thirteenth weeks as compared to an oscillatory population behavior as the season progressed beyond the thirteenth week (Figure 7). Perhaps a more correct view of the graph of the 1976 control treatment data would be a consideration that the nematode population reproduces continuously, giving rise to a nonlinear relationship of population to time. If the assumption is made, as with linear regression analysis, that the rate of change of population density depends only on conditions at some specific point in time and not on the past history of the population, an artificial situation develops, for it ignores, among other reasons, the age structure of the population (28). If, for example, a food supply to a population was increased suddenly, the population response may be the rapid rate at which adult females in the population lay eggs, but there would be a natural delay before those eggs hatched and matured into feeding and breeding adults. Thus, an adequate description of

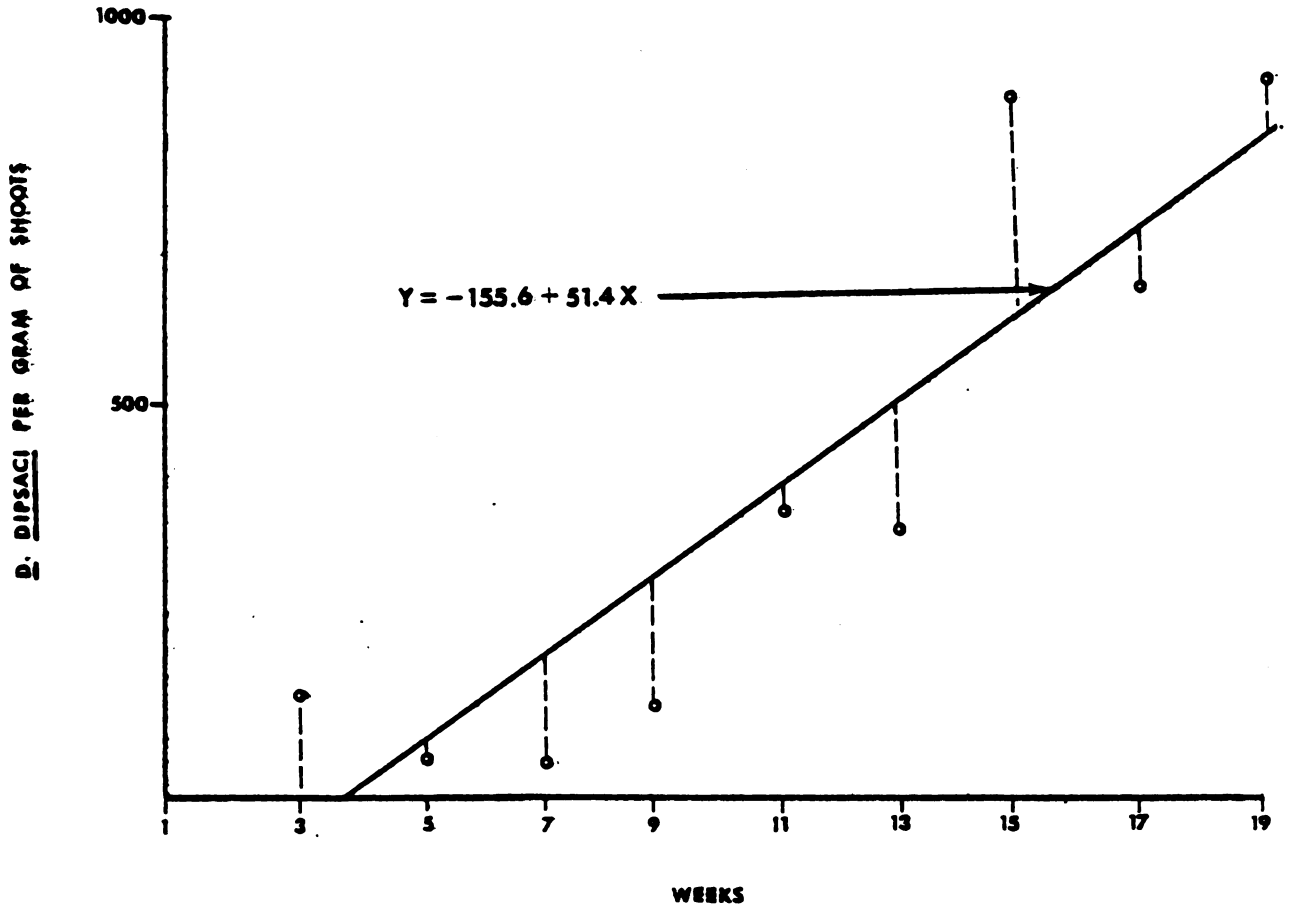


Figure 7. Regression of mean number of D. dipsaci recovered from shoot samples of P. sublata, cultivar Emerald Pink, over time for the 1976 control replicates.

the population requires a knowledge of not only total numbers present, but members of each age group. This study did not record such information, but certain inferences about the way the population should vary naturally under the effects of age structure can be derived mathmatically (28).

A complete analysis of the behavior of a population with overlapping generations would allow for variation in fertility and mortality rates as they interact with age. A simplified case would be one in which adults and juvenile stages are not assumed to compete with one another for food or resources. Since D. dipsaci spends 65% of its juvenile stage in the egg (50), this assumption generally holds for this situation. A further assumption that the population is regulated by limiting factors acting on adults, with food and resources for the young nematodes being present in excess is also necessary. When assumptions and data are supplied to the probability of adult individuals dying in a given time interval, the number of eggs laid by each adult (i.e., half the number laid by each female if there is a 1:1 sex ratio) in the interval, the probability that the egg survives to the adult stage, and the time taken from egg to adult, then the rate of decrease or increase over time and the equilibrium density of the population can be hypothesized (28). Computations of these values lead to an oscillating population (Figure 8). This corresponds to the graph of the control data for 1976. The oscillatory behavior of the population can be divided into seven categories:

Period (i) Week 1 to Week 3, an increase in the population as eggs mature from phlox plants overwintered in refrigerated storage.

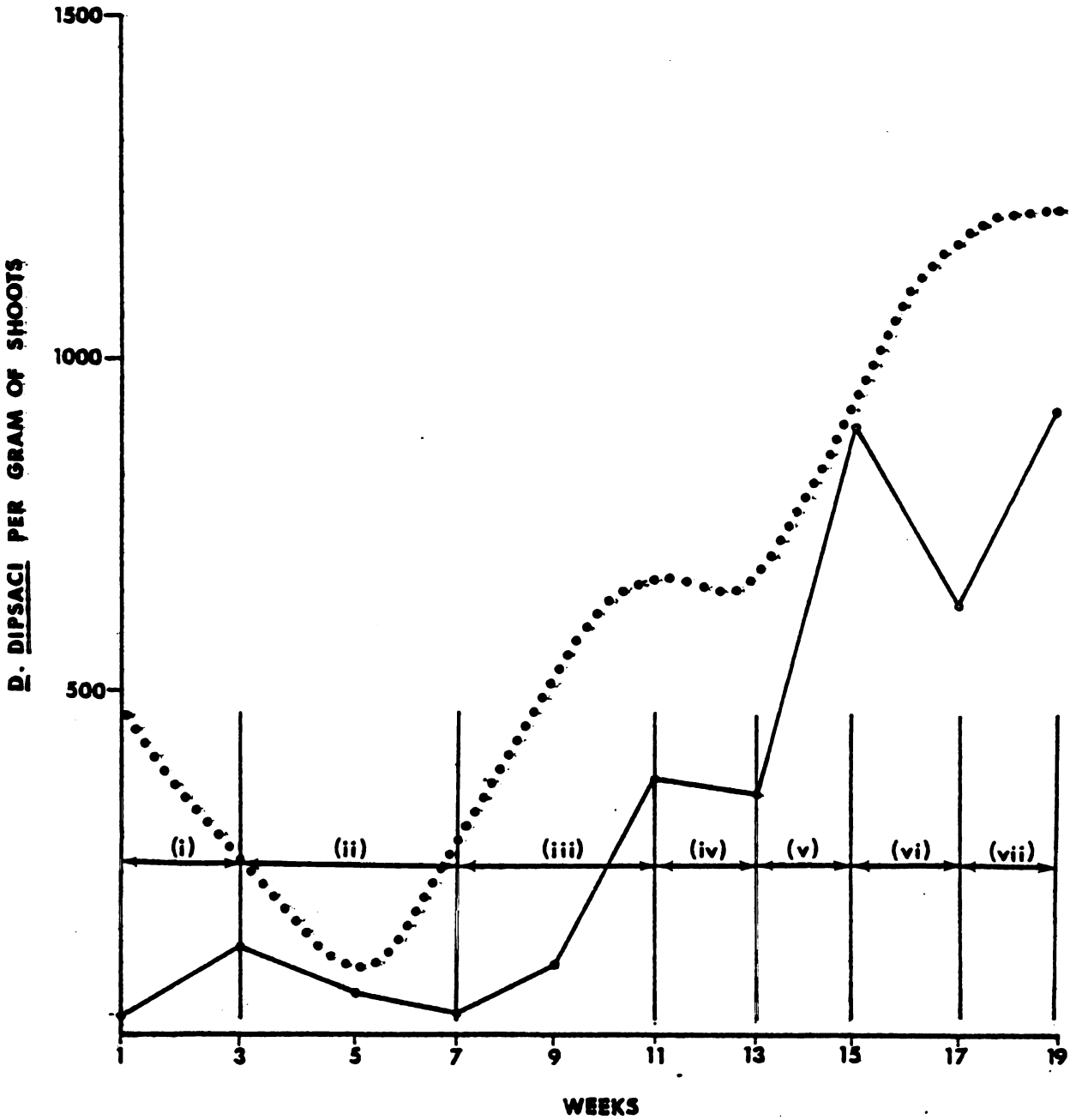


Figure 8. Field population dynamics of D. dipsaci recovered from shoot samples of P. subulata, cultivar Emerald Pink. 1976 control treatment data points ●——●, theoretical population data points ●●●●●●●.

Period (ii) Week 3 to Week 7, deaths but no population restoration.

Period (iii) Week 7 to Week 11, increases in the population as the eggs laid during period (ii) become adults.

Period (iv) Week 11 to Week 13, slight decrease, because adult population from Week 5 to Week 7 was very small and therefore few eggs were laid which would hatch during this period.

Period (v) Week 13 to Week 15, renewed increases as numbers of new adults increase.

Period (vi) Week 15 to Week 17, slight decrease due to small adult population during period (iv).

Period (vii) Week 17 to Week 19, renewed increases as numbers of new adults increase.

During subsequent time intervals, a decrease in the population may have been noted if sampling continued. New adults would fail to emerge during this interval because the adult population may have been above its equilibrium level during period (v) or (vii); therefore, few eggs would have been laid. The oscillations are clearly due to the delay of approximately two intervals or 28 days, the period necessary for eggs to develop into adults.

Although it is a rather uncomplicated model, the application of the data from this experiment does display the population behavior predicted when age structure is taken into account. In general, both fertility and mortaility (not fertility alone as assumed by the model) of an individual are likely to be a function of age and of the population density at that time, and of many other factors (28).

To predict behavior of such a population, the effects of age, density, and other relevant factors on fertility and mortality must be known. This requires data be taken on numbers of nematodes in different age classes alive at a given time. In this simplified model, an approximate picture of the effects of various factors on the behavior of a population is given, and even though age classes were not recorded, the model closely follows the natural population dynamics of D. dipsaci.

Aldicarb Treatment

How quickly a pesticide becomes effective depends on many interrelated variables such as dosage, placement of the granules, type of soil, plants and pests, soil temperature, and particularly, in the case of aldicarb, on the availability of soil moisture (37). There was a dry period of several weeks after aldicarb application in 1976 and this may have delayed systemic activity for several weeks, as the first indication of pesticide effectiveness was detected the fourth week after aldicarb application. Residual action of pesticidal activity also depends on several interacting factors, but the primary factor is pest susceptibility. D. dipsaci has been reported to be controlled by aldicarb in Denmark in 1970, and its sensitivity to this nematicide was determined to be high from this experiment. Residual activity of aldicarb in this treatment lasted the entire growing season of 1976, as no population peaks were recorded.

Aldicarb or 2-methyl-2-(methylthio)propionaldehyde O-(methylcarbonoxl) oxime is a carbamoyloxime cholinesterase inhibitor. It is structurally similar to acetylcholine, and it controls nematodes presumably through the inhibition of cholinesterase, although exact data has not been reported (37). Once aldicarb is released by soil

moisture acting on the granule, it is absorbed by the root system of the plant and moved mainly through the xylem to other portions of the plant. Immediately after cellular penetration, it is attacked by enzymes and transformed into other products (37). Nematodes are killed by direct contact in the soil or by systemic action when feeding on tissue containing the toxicant. Although there are differences in susceptibility among species, nematodes can be killed by exposure for two days at concentrations of 5 ppm or less. As with all nematicides, aldicarb is most effective when nematodes are free in the soil and not protected by either plant tissues or egg membranes, but in the case of this foliar nematode, it appeared to be very effective.

By the fifth week, the nematode population in 1976 dropped to a very low level, and remained thus for six weeks. By the eleventh week, residual activity of the pesticide was slowly becoming dissipated, but plants still had vigorous vegetative growth. The nematode population began to recover slightly at this time, although the following sampling indicated that the population had again risen, only to drop after the next two week interval. When plotted, these values result in a divergent oscillation pattern, extending over a period of ten weeks. Assuming an egg stage of approximately 28 days for D. dipsaci (50), these oscillation peaks follow more or less a 28 day time interval. Aldicarb application resulted in the perturbation of the stable nematode population at the beginning of the growing season. As residual activity of the pesticide declined, nematode reproduction resumed in an attempt to reach a constant level or point of equilibrium, for reproduction rate is a function of nematode density. When the population level fell below the equilibrium point, more eggs were laid to increase density, thereby resulting in increases in the number of

adults, perhaps exceeding the equilibrium point in some instances when all the eggs matured. Various factors then enter in to bring the population back down to its minimal level, perhaps by residual action of aldicarb. As oscillations of increasing amplitude about an equilibrium point increased, other physical restraints developed so that the population did not increase indefinitely.

Plants taken from the field and overwintered at 5-7 C showed gradual increases in population level over a period of four months. It is assumed that the aldicarb had been completely broken down at that time and allowed for the gradual restoration of the population. In contrast, plants overwintering in the field had very low numbers of nematodes in May 1977. These plants were in excellent condition and flowered profusely in the Spring of 1977. Harsh winter conditions may have played a role in supressing nematodes in these field plants, as D. dipsaci does not overwinter well in light, sandy soil such as that found in this experimental plot (40).

By June 1977, numbers of nematodes had increased in aldicarb replicates primarily because two of the six replicates had larger increases than the other replicates. These two replicates were positioned adjacent to control replicates and may have become contaminated from the diseased plants.

Oxamyl Treatment

The pesticidal effects of oxamyl were noticeable by the following two week sampling after application of the spray. Oxamyl or oxamy-(methyl)-N,N-diethyl-[methyl carbamoy)oxy]-1-thiooxamidate is applied to the foliage of the plant with a surfactant. The pesticide enters the surface of the leaf through openings in the epidermis. It is

translocated through the plant as a sugar conjugate and is dispelled by the roots, where control of ectoparasites results. Nematodes are killed by direct contact with the toxicant in the soil or in treated tissue. D. dipsaci appeared to be quite susceptible to oxamyl, as adequate population control was exercised over the growing season with regular applications.

Nematode population density fluctuated slightly in 1976, but was never allowed to recover sufficiently before more oxamyl was applied. The population dynamics of this treatment indicated that the nematodes were very strongly perturbed as with the aldicarb treatment, as no divergent oscillations were recorded. A very low, stable level of nematodes resulted, perhaps indicating possible pesticide resistance build up in nematodes from this treatment. At the end of the 1976 growing season, plants were growing vigorously and appeared to be in excellent condition.

In refrigerated storage, the nematode population fluctuated slightly so that by the end of storage, refrigerated plants and field plants yielded approximately the same number of nematodes. Of all the treatments, the oxamyl treatment averaged the highest nematode survival in plants overwintering in the field. This may have resulted from low residual activity of oxamyl or from some physiological superiority of plants in this treatment enabling the nematode to better survive the harsh winter conditions. An alternative explanation to these increased numbers may be that the low residual effect of oxamyl allowed nematodes from adjacent treatments to infest these plant in the fall after the last sampling or early in the season before sampling was resumed.

In the 1977 growing season, most replicates of this treatment in the field did not have high levels of nematodes. However, it was noticed that some oxamyl treatments, particularly those replicates directly adjacent to control replicates, averaged high numbers of nematodes whereas distant replicates did not. One replicate of oxamyl in June 1977 yielded 24 nematodes per gram of shoots while the remaining five replicates of this treatment yielded none. This replicate was situated next to a control replicate. By August of that year, the replicate, along with a second similarly positioned oxamyl replicate, had increases of 73 and 91 nematodes per gram of shoots, respectively. Nematode increases in these replicates may have resulted from contamination of nematodes from diseased plants of the control replicates, or may have been due to natural nematode population recovery.

Aldicarb/Oxamyl Treatment

The aldicarb/oxamyl treatment showed very little population fluctuation over the 1976 growing season due presumably to the combined effects of the aldicarb and oxamyl pesticides. The population showed a somewhat variable pattern during the ninth through the nineteenth weeks, rising and falling between 1.8 and 0 nematodes per gram of shoots. The double dose of nematicides restricted the magnitude of oscillation as compared to the aldicarb treatment alone, but still reflected a similar reaction to the perturbation of the population equilibrium.

However, the aldicarb/oxamyl treatment did not follow the same population flucutations in storage as the aldicarb treatment. The nematode level remained low so that by May 1977, 3.8 nematodes were recovered from the tissue of plants receiving this treatment.

Plants overwintering in the field had no detectable nematode population in May 1977. The population gradually increased, without oscillations, to a peak in September. This slow recovery may have been due to the concentrated application of the two pesticides or from some increased residual capacity resulting from the pesticide combination, limiting the amount of contamination resulting from diseased plants in control replicates. The delayed population peak in 1977 of this treatment may have resulted from several factors. The pesticide combination may have effected the physiology of the ground phlox in a way that was adverse to the survival of the nematode. Or, the nematode may have been adversely affected by the pesticide combination in such a way as to cause problems with fertility in adults maturing from eggs laid while residual activity of the pesticides was still strong. Such interferences with the life cycle of D. dipsaci may have caused the late population maximum seen in this treatment in 1977.

The outcome of the nematicide control program cannot be indiscriminately applied to commercial propagation practices without some limitations. Plants given the nematicide treatments differed from those in commercial production mainly in the cultural conditions provided. Commercial plantings are not usually left to overwinter in the field every year as were the nematicide treated plants. Attempts to keep plants in refrigerated storage over the winter months were not successful when hot, dry Spring conditions did not allow for survival of transplanted, refrigerated plants. In addition, commercial plantings are irrigated on a regular basis throughout the growing season. Plants in the nematicide trial plot were given no irrigation. Weeds were a minor problem the second year of the experiment, which may account for the wide fluctuations in nematode numbers in all treatments, as many

weeds are known to be hosts of D. dipsaci. Efforts were made to contain the spread of weeds throughout the plot, but over the course of the second season, the phlox planting did not approach commercial management conditions.

Various sources of error resulted from technique problems in this experiment. Considerable variation among the six replicates of the control treatment may have been due to contamination by aldicarb from adjacent replicates in April 1976. Although application was made with a hand operated spreader and immediately raked into the soil, control replicates lying on the east side of aldicarb replicates did not, at any time, approach the population densities seen in other control replicates over the two year period. Even these minimal amounts of drift aldicarb were sufficient to control D. dipsaci.

Since the nematode displayed such extreme sensitivity to the aldicarb, perhaps a better experiment would have been a factorial experiment with the two nematicides to distinguish lower and upper limits of control, allowing for increased cost savings when these pesticides are used in production as well as providing information on phytotoxic effects on P. subulata. In addition, the overall plot dimensions should have been increased with more guard rows established, to provide buffering and thus avoid nematode contamination problems with the treatments. Increased guard row buffering may have avoided the necessity of an oxamyl spray in August of 1977. Any residual effects that the oxamyl may have had the second year of the experiment were obscured by the nematode contamination that appeared to have occurred.

Certain limitations on the nematode extraction procedure also posed problems in the experiment. Modifications of the shaker technique were required for this experiment. Instead of the recommended 48 to 72

hour extraction on the gyratory shaker, the time period in this study was decreased to 24 hours, due to severe deterioration of the phlox tissue that resulted over extended periods in the ethoxyethylmercuric chloride–dihydrostreptomycin sulfate extraction solution. Forty-eight hours in the solution resulted in tissue fragmentation and accumulation which interferred with accurate nematode quantification. Also, the very high infestation levels present in control replicates at the end of the 1976 growing season resulted in population estimation, by quantifying 1 ml samples of the extraction solution. Extrapolation to the total number of nematodes in the 15 ml sample allowed for error due to differential sedimentation of the nematodes in the water solution, although attempts were made to keep sedimentation at a minimum when the 1 ml samples were taken.

The nematicide eventually selected for use in controlling D. dipsaci infestations on P. subulata will be determined by the individual grower after consideration of a number of economical factors. Once the degree of infestation of the nematode on the crop has been determined, losses can be translated into economic terms such as revenue losses or cost increases or both. All direct costs of the control method chosen must be determined as well as indirect costs, including major changes in crop rotation and management practices. The ratio of costs involved to benefits received can then be derived to determine if indeed these new control methods are profitable. The above cost analysis compared with the alternative of eliminating the crop entirely and releasing resources for other purposes will give each grower an individualized recommendation as to which nematicide to select (37). Both nematicides tested herein must be handled with extreme care as both are

potentially lethal. In addition, aldicarb has an added adverse effect on the area bird populations, which often feed on the granules after soil incorporation. Thus, the convenience of aldicarb one time application is offset by adverse environmental impacts.

SUMMARY

1. A survey of eleven cultivars of P. subulata revealed high population densities of D. dipsaci in shoot samples from the plants. All cultivars were found to be infested to a certain degree, with several cultivars showing severe infestation levels which resulted in hypersensitivity symptoms of necrosis and chlorosis of the leaf tissue. Other cultivars with high infestation levels did not display hypersensitive symptoms. Complete cultivar resistance was not found, although several cultivars harbored very low numbers of nematodes with minimal visual evidence of nematode damage.

2. In a study identifying the physiological race of D. dipsaci present, significant height and fresh weight increases were observed in three of the six host plants grown in steamed soil as compared to field soil. Nematodes were extracted from the soil of host plants grown in field soil, however, only the onion species supported foliar invasion and reproduction of D. dipsaci. This data, in addition to data from the pathogenicity study, indicated either the onion race, the phlox race, or a hybrid of these two races was present.

3. Pathogenicity studies involving four cultivars of P. subulata revealed significant increases in height growth and shoot fresh weight of plants grown in steamed soil as opposed to those grown in field soil. Nematodes were extracted from all cultivars growing in field soil, although the number recovered varied between cultivars.

4. Implementation of a field control program involved concentrated applications of two test pesticides via continual application and population checks every two weeks during the first growing season. The following year, population levels were monitored monthly and applications of oxamyl were given when determined necessary to adequately control nematode population growth. While pesticide applications brought existing population levels under control and improved the physiological condition of the nematicide treated P. subulata plants, further biological control was exercised in the form of a severe winter, causing population levels to drop lower.

5. Examination of the field population density of D. dipsaci on P. subulata revealed four generations of nematodes over the 1976 growing season. The number of nematodes recovered from diseased shoot tissue was determined to be a function of the fertility, mortality, and age structure of the nematode population the first year of the study, while the poor condition of the plants determined nematode population maximums the second growing season.

In summary, the strategy for control of D. dipsaci involved an initial concentrated effort to reduce population levels in stock plants using nematicides, so that natural overwintering stresses in the field further suppressed population recovery the next Spring. The population growth of the nematode was monitored throughout the second growing season. If the plants are overwintered in refrigerated storage instead of in the field, then early nematode checks and nematicide applications must be made to bring the population in check early in the season to attain maximum plant growth.

APPENDIX A

APPENDIX A

TABLES OF CHI-SQUARE VALUES

Table A1. Chi-square values testing the significance of the relationship of numbers of nematodes extracted from soil associated with P. subulata cultivars classified as "diseased" or "healthy".

Cultivar	Chi-square values		
	Healthy	Diseased	Total
Atropurpurea	0.06	0.00	0.06
Blue Hills	425.73**	12.95**	438.68**
Crimson Beauty	2.55	0.07	2.62
Emerald Blue	6.63*	0.20	6.83*
Emerald Pink	85.27**	2.60	87.87**
Pink Perfection	0.24	0.01	0.25
Red	461.25**	14.04**	475.29**
Red Wing	5.36*	0.16	5.52*
Rosea	0.05	0.00	0.05
Scarlet Flame	1380.89**	42.01**	1422.90**
White Delight	0.37	0.01	0.38
TOTAL CHI-SQUARE: 2440.47**, 4 d.f.			

* Probability of a larger value of chi-square 0.05.

** Probability of a larger value of chi-square 0.01.

Table A2. Chi-square values testing the significance of the relationship of numbers of nematodes extracted from shoot samples of P. subulata cultivars classified as "diseased" or "healthy".

Cultivar	Chi-square values		
	Healthy	Diseased	Total
Atropurpurea	0.02	0.00	0.02
Blue Hills	3594.81**	139.38**	3734.19**
Crimson Beauty	11.83*	0.47	12.30*
Emerald Blue	57.12**	2.22	59.34**
Emerald Pink	44.86**	1.74	46.60**
Pink Perfection	53.21**	1.93	55.14**
Red	31.29**	1.22	32.51**
Red Wing	13.76*	0.53	14.29*
Rosea	0.19	0.00	0.19
Scarlet Flame	0.09	0.00	0.09
White Delight	20.07**	0.78	20.85**
TOTAL CHI-SQUARE: 3975.51**, 4 d.f.			

* Probability of a larger value of chi-square 0.05.

** Probability of a larger value of chi-square 0.01.

Table A3. Chi-square values testing the relationship between number of plants alive or dead compared to treatments of field or steamed soil for the identification of the physiological race of *D. dipsaci*.

Cultivar	Chi-square values				
	Field Soil		Steamed Soil		Total
	Alive	Dead	Alive	Dead	
Onion, cv Sweet Spanish	1.25	0.37	2.38	1.76	5.77
Tomato, cv Rutgers	0.03	0.01	2.38	1.76	4.19
Tomato, cv Stone	1.25	0.38	0.26	0.20	2.09
Sweet Clover, cv Yellow	4.60	1.37	3.56	2.63	12.16*
Alfalfa, cv Ranger RCC63	2.82	0.84	0.73	0.54	4.94
Sugar Beet	5.69	1.65	6.22	4.60	18.16*
TOTAL CHI-SQUARE: 47.31**, 15 d.f.					

* Probability of a larger value of chi-square 0.05.

** Probability of a larger value of chi-square 0.01.

APPENDIX B

APPENDIX B

ANALYSIS OF VARIANCE TABLES

Table B1. AOV: Shoot Fresh Weight, Host Plant Bioassay.

Source	df	SS	MS	F	$F_{.05}$
Total	83	126.54			
Blocks	5	77.92	15.58	39.95	2.33
Soil	1	18.79	18.79	48.18	3.96
Error	77	29.83	0.39		

Table B2. AOV: Plant Height, Host Plant Bioassay.

Source	df	SS	MS	F	$F_{.05}$
Total	83	135317.70			
Blocks	5	78697.46	15739.49	37.11	2.33
Soil	1	23960.02	23960.02	56.49	3.96
Error	77	32660.22	424.16		

Table B3. AOV: Plant Height, Pathogenicity Study.

Source	df	SS	MS	F	$F_{.05}$
Total	23	10900.36			
Blocks	3	3280.05			
Treatments	5	5712.21	1142.44	8.98	4.30
Soil	1	2795.04	2795.04	21.97	4.54
Harvest Date	2	1628.82	814.41	6.40	3.68
Soil x Harvest Date	2	1288.35	644.17	5.06	3.68
Error	15	1908.10	127.21		

Table B4. Mean squares and F values for testing the significance of plant height at three harvest dates to the treatments field or steamed soil for the pathogenicity study.

Source	df	SS	MS	F	$F_{.05}$
Harvest Date Linear:					
Field Soil	1	9.57	9.57	0.07	4.54
Harvest Date Residual:					
Field Soil	1	8.46	8.46	0.07	4.54
Harvest Date Linear:					
Steamed Soil	1	2897.51	2897.51	22.77	4.54
Harvest Date Residual:					
Steamed Soil	1	7.87	7.87	0.60	4.54
Error	15	1908.10	127.21		

Table B5. AOV: Shoot Fresh Weight, Pathogenicity Study.

Source	df	SS	MS	F	$F_{.05}$
Total	23	0.5885			
Blocks	3	0.0516			
Treatments	5	0.4463	0.0892	14.86	4.30
Soil	1	0.2223	0.2223	37.05	4.54
Harvest Dates	2	0.0733	0.0366	6.11	3.68
Soil x Harvest Dates	2	0.1507	0.0753	12.55	3.68
Error	15	0.0906	0.0060		

Table B6. Mean squares and F values for testing the significance of shoot fresh weight at three harvest dates to the treatments field or steamed soil for the pathogenicity study.

Source	df	SS	MS	F	$F_{.05}$
Harvest Date Linear:					
Field Soil	1	9.57	9.57	0.07	4.54
Harvest Date Residual:					
Field Soil	1	8.46	8.46	0.07	4.54
Harvest Date Linear:					
Steamed Soil	1	2897.51	2897.51	22.77	4.54
Harvest Date Residual:					
Steamed Soil	1	7.87	7.87	0.60	4.54
Error	15	1908.10	127.21		

Table B7. AOV: 1976 Control Program.

Source	df	SS	MS	F	$F_{.05}$
Total	239	207.71			
Treatments	39	84.71	2.17	3.53	1.62
Error	200	122.99	0.61		

Table B8. AOV: 1976 Control Program, Control Excluded.

Source	df	SS	MS	F	$F_{.05}$
Total	179	53.89			
Treatments	29	0.78	0.03	0.07	1.62
Error	159	53.11	0.35		

Table B9. AOV: 1977 Control Program.

Source	df	SS	MS	F	$F_{.05}$
Total	215	1639403.43			
Treatments	35	225970.28	6456.29	0.82	1.64
Error	180	1413433.14	7852.41		

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