DYNAMICS OF PRATYLENCHUS PENETRANS AND ENDOMYCORRHIZAL FUNGUS ASSOCIATED WITH PRUNUS PERSICA (cv. HALFORD AND SIBERIAN C)

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ABSTRACT

DYNAMICS OF PRATYLENCHUS PENETRANS AND ENDOMYCORRHIZAL FUNGI ASSOCIATED WITH PRUNUS PERSICA (cv. HALFORD AND SIBERIAN C).

By

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A two-year study of the population dynamics of an endoparasitic nematode, Pratylenchus penetrans (Cobb) Filipjev and Schuurmans-Stekhoven and an endomycorrhizal fungus, Glomus macrocarpus geosporus (Nicol. and Gerd.) Gerdemann and Trappe was conducted on peach seedling rootstocks (cv. Halford and Siberian C) in 1,2-dibromo-3-chloropropane (DBCP), and 1,3-dichloropropene + methyl isothiocyanate (1,3-D + MIC) treated soils. Population densities of P. penetrans associated with nematode-inoculated trees were significantly greater than those associated with the non-inoculated trees. The population dynamics of this nematode was similar for both cultivars. Significantly greater root and soil population densities of P. penetrans were recovered from Siberian C than Halford. This difference, however, was not evident prior to the end of the second growing season. No statistical mycorrhizal colonization or spore population density differences

were observed between the G. macrocarpus geosporus-inoculated and non-inoculated trees. Endomycorrhizal colonization of Halford roots, however, was significantly greater than that of Siberian C. The increased colonization was not expressed through increased spore production. Soil fumigation had no overall significant influence on root and soil population densities of P. penetrans and mycorrhizal colonization of either cultivar.

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By

Edward J. Kunickis

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To Kevin: the only 'man' in my life, and to his mom; may he always be the 'boy' in her life.

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I wish to express a most sincere appreciation to Mrs. Natalie 'Nic-Nat' Knobloch: a true scientist and friend whose honest devotion and love to the field of taxonomic nematology . . . . . made it all worth while.

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INTRODUCTION

Approximately 10.4 million acres in the world are planted to peach trees, with the United States accounting for 40% of the world production. In Michigan, over 18,000 acres of peach orchards are commercially maintained , with 25% of the total number of peach trees being five years of age or younger. Plant-parasitic nematodes, predominately P. penetrans, are commonly associated with peach, and are reported to cause an estimated 15% annual production loss in the Michigan peach industry. Consequently, increasing peach tree longevity, through use of chemical control measures for plant-parasitic nematodes, has become of great concern to Michigan orchardists.

Symbiotic root associations formed by mycorrhizal fungi are common on most agricultural crops. Mycorrhizal fungi enhance plant growth (through increased absorption of essential nutrients, in particular, phosphorus), increase tolerance to certain soil pathogens, and may be detrimentally influenced by interaction with plant-parasitic nematodes and by the action of non-selective fumigants.

The possibilities of integrating the benefits by these fungi is becoming increasingly attractive, in view of the present high cost of fertilization, ecological

awareness of the public to indiscriminate action of select pesticide chemicals, and the increasingly important role of phosphorus deficient soils as a major limiting factor in crop production in many areas of the world.

Little information is available in the literature today concerning the role of endomycorrhizal fungi on the growth of fruit trees; even less is understood of the probable interactions of plant-parasitic nematodes in this symbiotic association. Therefore, the objectives of this research were: 1) to study the population dynamics of the nematode P. penetrans and the endomycorrhizal fungus G. macrocarpus geosporus associated with Prunus persica (cv. Halford and Siberian C), 2) to investigate the influence of this nematode and endomycorrhizal fungus on the development of each other, and their subsequent effects on peach tree growth, and 3) to study the effect of soil fumigation on the population dynamics of P. penetrans and G. macrocarpus geosporus as related to the growth and development of peach.

LITERATURE REVIEW

Prunus Persica (L.) Batsch

Few fruit species have spread so rapidly and become adapted to so many climatic conditions as the peach; a tree originally native to China (34). The popularity of the peach is closely related to the wide and general appeal of its fresh fruit, its ease of use, and its utility in the production of a variety of preserved products.

Two types of peaches are grown in the temperate zones. Most of the freestone group are sold fresh; clingstone peaches are generally canned, but a small portion of this and the freestone group is frozen.

About 10.4 million acres in the world are devoted to peaches, with the United States producing 40% of the world total (34). In Michigan, a survey by the Michigan Crop Reporting Service (1973) indicated there were over 18,000 acres of peach orchards, with sixty per cent of this acreage located in southwestern Michigan and thirty per cent on West Central Michigan (35).

In 1973, twenty-six per cent of Michigan peach trees were five years of age or younger (35). Consequently, peach tree longevity has been of great concern to Michigan peach growers. Considerable emphasis in peach breeding

is placed on incorporating disease and nematode resistance in order to prolong orchard life (50).

In Michigan, plant-parasitic nematodes are responsible for an estimated fifteen per cent annual production loss in peaches (6).

PRATYLENCHUS PENETRANS

History-

The first recorded observation of a root-lesion (P. penetrans) nematode was by Bastian, in 1865, when he described Tylenchus obtusus. Bastian's description and drawings, however, were inadequate for purposes of scientific identification. De Man, who described Tylenchus pratensis in 1880, is credited with describing the first root-lesion nematode. In 1922, Micoletzky placed the root-lesion nematodes in a new subgenus, Chitinotylenchus. The genus Tylenchus was synonymized with Anguillulina Gervais and van Beneden by Baylis and Daubney in 1926. In 1934, Filipjev's classification of the Tylenchidae, which is the basis of our present concept of the group, defined Chitinotylenchus as a distinct genus excluding the root-lesion nematodes. In this monograph, the root-lesion nematodes were placed in a new genus, Pratylenchus.

The acceptance of the taxon Pratylenchus was not equally shared by all taxonomists (90). This resulted in much confusion concerning the taxonomy of the root-lesion nematodes. The confusion was largely eliminated by the monographs of Sher and Allen (85) and Loof (51) in the revision of the genus Pratylenchus.

Biology -

Pratylenchus penetrans (Cobb, 1917) Filip. and Stek., 1941, is considered the most important species in this genus, causing root destruction to a large number of plants. Members of this genus are commonly referred to as root-lesion nematodes because of the lesions they frequently produce on feeder roots (30). P. penetrans was first reported in 1917 associated with roots of cotton, potatoes, and violets (14).

Today, this nematode is known to have both a world-wide distribution and a wide host range, occurring on more than 350 plants (15). It occurs in most of the world's agricultural areas (51), but is reportedly more numerous in the warmer regions of the temperate zone than in the tropics and subtropics (13).

P. penetrans occurs throughout the United States, southern Canada, and Europe, infecting corn, potatoes (18), onions (11), celery, and other field and garden crops (92, 93). In the U.S.S.R., P. penetrans has been found in the roots of cotton, potatoes, beans, rye, wheat, tomatoes, and strawberries, causing incalculable damage (49). In Holland, Seinhorst reports that P. penetrans is the most important species of Pratylenchus, causing damage to narcissus, strawberry, and lilies. This nematode has been reported in Michigan (46) as the most widespread and economically important plant-parasitic nematode. In addition, its

association on tree fruits is well established and has received considerable attention from various workers (see Orchard Replant Diseases).

P. penetrans reproduces by amphimixis (77, 89), in which eggs are laid singly in soil or in roots. The first-stage juveniles molt within the egg and the second-stage juveniles hatch within 10 days at 23°C (36). Free-living in the soil, the second-stage juveniles orient themselves towards susceptible roots by a heat gradient (23), entering into the zone of cellular maturation (43). Adult males and females are produced following three additional molts. The length of each stage is influenced by soil temperature, 21°C being optimum for activity and reproduction (54). All vermiform stages can infect roots; however, fourth-stage juveniles and adults penetrate more readily than other stages (86).

Overwintering in P. penetrans appears to involve mechanisms of anhydrobiosis. Studies on the survival of this nematode in soil types differing in particle size, moisture retention, aeration and pore size indicate that survival increases with increasing soil dryness (95). Poor survival in wetter soils is attributed to lack of aeration. The rate of survival may further be increased by senescent roots which provide protection for P. penetrans during winter (41); temperatures of 1.0-4.5°C reportedly are optimal for survival (24). All stages are capable of overwintering, but fourth-stage juveniles and adults are most important (22, 41).

Pathology -

P. penetrans is an obligate endoparasite of the root cortex, generally resulting in both primary necrotic symptoms of the root and secondarily induced symptoms of the shoot system. Lesions and discoloration of the root cortex are caused by the mechanical action of the nematodes' stylets rupturing cortical cell walls and by the chemical action of enzyme secretions (65, 96). Stunting, chlorosis, and wilting are the most common symptoms in the shoot system (95).

Symptoms induced by the root-lesion nematode are the final physiological reactions of the plant to populations of P. penetrans, and are intimately controlled by environmental factors which affect both the pathogenicity of the nematode and the vigor of the plant. Some of these factors, which directly or indirectly affect the symptom expression of P. penetrans on the plant are discussed below.

Susceptibility of plants to P. penetrans varies considerably according to the species of the plant. Jensen (39) found that all 33 species of cover crop tested were susceptible to this nematode. In similar work by Oostenbrink et al. (71), it was noted that out of 164 cultivated plants tested, all were hosts to the root-lesion nematode. However, significant differences in the relative susceptibility of certain species were observed. Oostenbrink, in 1956, suggested that with P. penetrans, there is a direct relation between susceptibility and the size of the root system. The bigger the root system, the smaller the

amount of damage; the smaller the root system, the more extensive the damage due to similar numbers of nematodes.

Within susceptible species of plants, marked differences occur in the relative amounts of visual symptoms produced. Mountain (61), studying the pathogenicity of P. penetrans found that tolerant plants failed to exhibit necrotic root symptoms, whereas necrotic root symptoms, in the form of lesions, were prevalent on the less tolerant hosts. Hussey and Barker reported that the root-lesion nematode had little effect on nodulation in soybean, peanut, and cowpea, but damaged the nodules of the garden pea (37). In New York, Parker and Mai indicated wide differences in the susceptibility of clonal apple rootstocks to P. penetrans (73).

Above ground symptoms, such as stunting and poor growth, are not always apparent in plants infected with the root-lesion nematode. Studies of cherry trees (72) and apple seedling (52) have shown that the root-lesion nematode can produce poor growth without any obvious symptoms above ground.

The fact that large numbers of P. penetrans can occur in roots without causing any appreciable reduction in growth of the plant, may be due to the fact that these nematodes feed on cortical cells and do not damage the vascular tissues. In certain species of plants, however, water translocation may be impeded by the formation of tyloses, which result in response to P. penetrans (1).

Ecology -

The degree of symptoms expressed by a plant in response to infection is related to time of infection and is clearly influenced by the initial population density present in the soil. Oostenbrink has demonstrated significant linear regressions between the logarithm of initial soil population density or root population density and reduction in growth of susceptible plants (70). Similarly, Seinhorst has shown that measurable damage occurs only when the population density exceeds a certain limit (84). With daffodils, one Pratylenchus penetrans per 500 g of soil is sufficient to induce symptoms; whereas, 500 P. penetrans per 500 g of soil cause potatoes to show symptoms of nematode damage. Ferris (25), working with onions, found that 5 P. penetrans per 500 g of soil resulted in noticeable symptoms.

P. penetrans is generally associated with light, sandy soils (45, 62, 72). Disease symptoms, induced by the presence of P. penetrans are also more prevalent on sandy soils than on clay soils. Whether this is a result of poor plant growth, lack of water, or increased nematode reproduction is largely unknown. Townshend (94) reported that penetration of corn roots by the root-lesion nematode was greatest in soils with a low bulk density because in such soil the size of the pores favored the best conditions for penetration.

Dolliver (20) has shown that the ability of P. penetrans to reproduce in peas was related to the physiological status of the plant. Treatments that reduced plant dry weight slightly, such as less favorable light and temperature, increased root-lesion populations. Treatments that reduced dry weight considerably also reduced nematode numbers. Ferris (26), studying the effects of soil temperature on onion seedling damage by P. penetrans reported that at 7 to 13°C, fewer than 100 P. penetrans per g of root tissue were required to produce damage. In Canada, pot tests conducted on lucerne plants showed that plants infected with P. penetrans were less resistant to cold temperatures than nematode-free plants (88).

These studies indicate the intimate interaction between the host, environment, and root-lesion nematode in determining the degree of the pathogenic relationship.

Nutrients in the soil, whether present naturally in an organic form or amended with inorganic chemical affect the root-lesion numbers directly or indirectly through their influence on microorganisms (97).

Walker has shown that nitrogen added to soils decreases populations of P. penetrans. He suggests that ammonification of the nitrogen compounds produces ammonia, which may be responsible for this population reduction through its nematicidal effects (98). Kirkpatrick et al. (42) found similar results. They showed that populations of

P. penetrans were significantly lower with plants receiving high rates of potassium.

Since phosphorus tends to be residual in most soils when compared to nitrogen or potassium, it is possible that continued use of N-P-K fertilizers may result in a high phosphorus, low potassium condition. This situation could result in increases of root-lesion nematodes.

Few reports exist in the literature on nematode population changes associated with plant growth. Di Edwardo (19), working with strawberries in New Jersey, showed that populations of P. penetrans reached a maximum in the soil in June and in the roots in July. At the end of July, the concentration of nematodes per unit volume of root decreased because of the increased root growth, but increased in September with the infection of new roots. In studies conducted in Canada on rye and tobacco, Olthof (69) showed that populations of P. penetrans were low in the summer and high in the fall. Similarly, Mountain and Boyce noted that the number of root-lesion nematodes per g of peach root declined during the summer and increased again in autumn. They postulated that suberization of roots and decay of tissue suitable for nematode colonization led to this decrease in density.

In 1975, an innovated approach to the study of population dynamics, employing a computer simulated ecosystem model, was conducted by Bird et al. (9). This model assists

the study of population dynamics of P. penetrans as it affects the growth and development of Solanum tuberosum.

ORCHARD REPLANT DISEASES

One of the most important diseases caused by P. penetrans is the replant disease of tree fruit nursery and orchard stock. It is a common practice in orchards that when trees decline (i.e., when trees that have previously produced profitable yields no longer grow or produce satisfactorily), renovation and subsequent plantings of the same or a closely related species is initiated. Under such conditions, if the new plantings grow poorly or fail to become established in comparison to similar plantings in virgin soil or soil never planted to the species concerned, then a situation known as an orchard replant disease or 'replant problem' exists.

"Poor growth of replanted trees is the most obvious symptom of this problem. Above ground, parts of the plant are stunted with short internodes and small leaves. Root systems are small, discolored, and have poorly developed feeder roots. Trees may die after the first or second growing season, or may remain in a severely stunted condition for many years. In some cases, surviving trees may improve with age, but they are not likely to be as large and vigorous as the trees that had the benefit of normal growth during the first few growing seasons (7)."

Replant problems, known also as 'soil sickness', are very economically serious diseases which have been reported in California on apples, cherries, and citrus (56, 57, 76),

and in Canada on peach (48, 62, 63, 64), and in the North Eastern United States (53). It is especially serious in Europe, where it is referred to as 'Bodenmudikeit' (81).

Proebsting and Gilmore (76) first reported that one of the most important causal factors in this disease seems to be the role of phytotoxins produced during the growth of the plant and during microbial decomposition of the residues. It was confirmed by Patrick (74) and later by Ward and Durkee (99), that peach root bark contained a cyanogenic glycoside, amygdalin. Present in living roots, amygdalin is not toxic to peach roots or to peach seedlings, but its degradation products, benzaldehyde and hydrogen cyanide, are highly toxic and inhibit the respiration of peach roots. The breakdown of amygdalin into toxic components is readily accomplished by microorganisms, as well as by enzymes in the peach roots themselves. It was concluded that microbial action on the amygdalin fraction of the roots was primarily responsible for the toxic factor frequently found in orchards.

Once the role of phytotoxins was established in this disease, researchers began studying other soil microorganisms which might prematurely activate the release of these toxic substances into the soil.

Mountain and Boyce (62) isolated 25 genera of nematodes from 167 peach orchards known to have replant problems. They reported that although all were considered as potential

factors, only P. penetrans showed consistent association with the replant disease.

In a later work (63), they showed that the root-lesion nematode occurred in all commercial peach orchards in Ontario, Canada, and that P. penetrans was the first nematode to attack newly developing roots. Further, control of P. penetrans with nematicides resulted in increased growth of peach trees.

In 1971, a nationwide survey of peach orchards was conducted in Japan. Pratylenchus spp. were found to be one of the most common plant-parasitic nematodes in 100 peach orchards at 30 locations (68). Similar surveys of plant-parasitic nematodes conducted in South Africa and South Australia revealed the ubiquity and economic importance of Pratylenchus spp. in peach orchards (59, 87). Knierim estimated that 20-50 per cent of cherry trees planted on old sites in Michigan were 'unthrifty', exhibiting poor vigor and very little terminal growth. These problem orchards were predominately infested with P. penetrans (47).

From laboratory experiments, Mountain and Patrick (64) showed that P. penetrans could invade and kill peach root tissues in the absence of bacteria and fungi; this initiates the breakdown of amygdalin into its toxic components and reduces the growth of the peach seedlings. They suggested that the root-lesion nematode, under natural conditions, plays a primary role in the etiology of this disease.

P. penetrans can induce root degeneration directly through its own pathogenic properties and indirectly through the formation of extensive infection sites which allow bacteria and fungi to increase the extent of tissue breakdown, which results in further damage through the release of phytotoxic compounds.

Today, factors known to be involved in the replant diseases include plant-parasitic nematodes, fungi, bacteria, weeds, toxic chemicals from senescent roots, soil structure, unbalanced nutrition, and cultural practices (100).

It is apparent that the stages in the etiology of replant disease, in many instances, would be non-specific and difficult to pinpoint. It involves many different factors and organisms, none of which could produce the entire disease complex. An excellent and detailed review of the numerous causal factors implicated in the replant problem is afforded by the 1966 works of Savory (80). Seemingly, however, the role of the root-lesion nematode appears paramount.

VESICULAR-ARBUSCULAR (VA) MYCORRHIZAE

Mycorrhizae are symbiotic associations between fungi and the roots of higher plants in which both members normally benefit from the relationship. They are generally divided into two main groups, ectomycorrhizae and endomycorrhizae (75). The ectomycorrhizae type is restricted almost entirely to trees of the Pinaceae, Fagaceae, and Betulaceae families. Endomycorrhizae occur on Bryophytes, Pteridophytes, Gymnosperms, and Angiosperms throughout the world. Two subgroupings occur within the endomycorrhizae: those produced by septate fungi, which primarily colonize members of the Orchidaceae, Gentianaceae, and Ericales, and those produced by non-septate fungi. Endomycorrhizae produced by non-septate fungi are commonly referred to as vesicular-arbuscular (VA) mycorrhizae and occur on more plant species than any other type of mycorrhizae (27).

VA mycorrhizae are formed by certain species of the Endogonaceae, a family of fungi in the Mucorales (29). These fungi form an extensive network of hyphae on feeder roots. Frequently they form conspicuous thick-walled chlamydospores on the root surface and in the soil adjacent to feeder roots. Hyphae of the fungi penetrate epidermal cells and progress through the root and to cortical cells, but they never invade the endodermis, stele, or root meristem tissues (91). Arbuscules, specialized haustorial structures of the fungus, are formed intracellularly by the

repeated dichotomous branching of the main infection hyphae (16, 17). The arbuscules apparently aid bilateral exchange of metabolites between the host cells and the fungus (101). Thin walled spherical structures known as vesicles may also be produced in cortical tissues intra- or intercellularly by the infecting hyphae and function as temporary storage organs.

VA endomycorrhizae are related to water molds and do not produce large fruiting bodies or airborne spores as do mushroom fungi (2). They produce large globose, subglobose, elliptical to ovoid spores that contain globules of oil. In some species, spores are grouped into sporocarps, and in others, spores form singly (67, 91). These fungi are spread in soil by root contact, water, plant transfer, or by man.

The most common endomycorrhizal fungi belong to the genus Glomus (29) and are universally widespread (28). In the absence of a host they stay viable in a dormant state for many years in the soil. When germinated in the presence of the roots of a host, they form endomycorrhizae.

Endomycorrhizae are more efficient nutrient-absorbing roots than non-mycorrhizal roots, especially in the absorption and utilization of phosphorus (27). The extensive network of external hyphae of this fungus, penetrating a larger volume of soil than non-mycorrhizal roots, is believed to be responsible for increased phosphorus uptake.

The development of a mycorrhizal association is dependent on, related to, and contingent with all physical, chemical, and environmental factors which, directly or indirectly, affect either the growth of the symbiont, the host, or both. Light intensity and nutrient availability in the soil influence the degree of mycorrhizal development (60). High levels of nutrition in soil reduces the degree of mycorrhizal infection, whereas low nutrient supply seems to allow increased development (91). Certain soil factors, such as aeration, temperature, and water influence plant root growth directly. These factors may influence mycorrhizal development indirectly since roots must be present to stimulate the fungi. These same factors can also influence the fungi directly. Wet soils and adverse soil temperatures inhibit the development of many mycorrhizal fungi (79). Fumigation of soils destroys many roots pathogens. However, mycorrhizal fungi are also susceptible to non-selective fumigants (31, 44, 66).

SOIL FUMIGANTS

Incorporations of soil fumigants for the control of plant-parasitic nematodes and avoidance of replant problems on trees has received considerable attention (4, 10, 12, 32, 33, 40, 55). Plant growth is generally improved by soil fumigation, but occasionally plants grow poorly after such treatments (58).

Recently, attention has been directed by several researchers to the effects of non-selective soil fumigants on VA endomycorrhizae (31, 44, 66).

Kleinschmidt and Gerdemann (44) investigated the cause of chlorotic and stunted citrus seedlings grown in fumigated nursery soils and found the problem to be related to inadequate nutrition caused by killing of VA endomycorrhizae. Formerly, the poor growth was attributed to soil toxicity. Similarly, Bird et al. (8) found no endomycorrhizae in cotton roots grown in methyl bromide-treated soils. However, fumigation with nematicidally active rates of 1,2-dibromo-3-chloropropane (DBCP) or 1,3-dichloropropene related hydrocarbons resulted in significant increases in endomycorrhizal infection. Additional research is needed to further define the trends of other widely applied soil or foliar nematicides in the development of endomycorrhizae, particularly in tree fruits.

Mycorrhizae and its relationship with other soil microorganisms has received limited attention in recent

years. Presently, however, research is being conducted to investigate the probable interaction of mycorrhizae and plant-parasitic nematodes (3, 78, 82, 83). Both of these organisms are usually associated with roots of most plant species. Since, independently, they alter the physiology of plant roots, their mutual presence may also have a marked effect on the biology of each other (8).

MATERIALS AND METHODS

A field experiment was initiated in May, 1974, on a Spinks loamy sand soil, at East Lansing, Michigan. The 90 ft x 120 ft experiment site, which had known history of previous tree fruit growth, was subdivided into 12 experimental units of 4 per row. Each unit was a single row plot with 8 seedlings, 4 of each cultivar, spaced 4 ft apart. For each cultivar, the seedlings were germinated and developed in the following soil environment:

1) Non-inoculated control
2) *Glomus macrocarpus geosporus*-inoculated soil
3) *G. macrocarpus geosporus* and *P. penetrans*-inoculated soil
4) *P. penetrans*-inoculated soil

Field preparation for the incorporation of soil fumigants was conducted on May 14, 1974. A randomized complete block design was used with each experimental unit replicated 4 times. The soil fumigants were injected 8-10 inches beneath the soil surface with a broadcast eleven-shanked pump-driven John Blue applicator. The soil treatments consisted of:

1) Nontreated control
2) 1,2-dibromo-3-chloropropane (DBCP), 4.0 gal/A.
3) 1,3-dichloropropene + MIC (Vorlex), 4.0 gal/A.

After soil aeration, the peach seedlings were planted on June 19, 1974. To encourage auxilliary shoot break and to provide uniform growth and initial development, all seedlings were 'headed back' to a height of 12 inches on July 15, 1974.

The peach rootstock cultivars, Halford and Siberian C, were grown from seed germinated three months prior to field planting. The seeds were stratified (moist-chilling) for 90 days in polyethylene containers, at 45°F. Following stratification (March 1, 1974), 96 seeds, 48 of each cultivar, were planted singly in 10 clay pots filled with steam sterilized sandy soil. Concurrently, 24 seedlings of each cultivar were inoculated with a 3.0 ml water suspension of 50 P. penetrans (treatment # 4). The nematodes were extracted from greenhouse maintained culture boxes employing a modified centrifuge-flotation technique (38), and stored at 12.5°C for 3 days prior to seedling inoculation.

Peach seedlings were established under greenhouse conditions (80-90° F day, 60-70° F night), with normal cultural and insect control practices. Soil fertilization was maintained with a 50% Hoaglands solution applied every 7-10 days, with the pH adjusted to 6.7.

Ten days before field planting, isolates of an endomycorrhizal fungus, resembling G. macrocarpus geosporus (29) were extracted from established mycorrhizal culture boxes employing a modified centrifuge-flotation technique. The

spores were individually segregated and stored at 12.5°C for 12 hours prior to seedling inoculation. Ten spores in water suspension were added directly to exposed roots of 12 P. penetrans-inoculated (treatment #3) and to 12 non-treated Halford seedlings (treatment #2). Similar inoculations were conducted with Siberian C seedlings. The remaining 12 pots of each cultivar served as non-treated controls (treatment #1). To ensure that contaminating microorganisms in the inoculum were also added to other treatments, the inoculum was washed, and the resulting wash water passed several times through a 400 mesh screen. Three ml of the wash water was then added to all other treated and non-treated control pots.

Population densities of P. penetrans were determined from analysis of rhizosphere soil and feeder root samples conducted on May 9, 1974, September 6, 1974, May 28, 1975, September 8, 1975, and April 7, 1976. Each seedling soil sample was the composite of 4 sub-samples collected at 4-8 inch soil depth, and stored at 12.5°C. Extraction of P. penetrans, based on a 100 cm^3 soil sample, employed a modified centrifuge-flotation technique. Identification and quantification of the nematodes were determined at 80X magnification with a compound light microscope.

To quantify root population densities of P. penetrans, feeder root samples were collected from each seedling. On September 8, 1975 and April 7, 1976, feeder roots were

washed, divided into 0.5-1.0 cm sections and mixed thoroughly. One gram root samples were selected at random and placed in 250 ml flasks. Seventy-five ml of a mixture of 10 ppm ethoxyethyl mercuric chloride (EMC) and 50 ppm dihydrostreptomycin sulfate (DSS) incubation solution (5) were added to each flask, and incubated on a gyratory shaker at 150 rpm for 48 hours. Each flask was then removed from the gyratory shaker, and the incubation solution poured through a 500 mesh screen. The nematodes were rinsed from the screen, collected in 10.0 ml water and stored at 12.5°C. On sampling dates May 9, 1974, September 6, 1974, and May 28, 1975, individual root samples varied from 0.5 to 7.6 grams. The roots were incubated on a gyratory shaker at 100 rpm for 72 hours. The incubation solution was poured through a 400 mesh screen and nematode population densities averaged and recorded on a per gram basis.

Following the last three root processing analyses, the efficacy of the gyratory shaker for extraction of P. penetrans was investigated. Processed root samples, selected at random, were placed in a blender with 500 ml of water and macerated for 30 seconds (21). The macerated root tissue was poured through a 60 mesh screen onto a 500 mesh screen. The residue collected on the 500 mesh screen was processed following normal procedural methods for the recovery of plant-parasitic nematodes from soil

samples. The nematodes recovered from macerated tissue were quantitatively compared with, but not added to final nematode counts.

Sampling dates for the quantification of VA mycorrhiza root infection coincide with those for P. penetrans. The roots were processed utilizing a modified root clearing and staining technique similar to one previously outlined by Bird, Rich and Glover (8). Percentages of infection (numbers of vesicles or arbuscules present in each root sample) were estimated through microscopic observation at 40X magnification. Recovery of VA mycorrhizal spores was obtained using a modified centrifuge-flotation technique.

Height and trunk diameter measurements of all seedlings were determined on July 24, 1974 and March 24, 1976. Growth indices were formulated for the evaluation of overall growth characteristics and to allow comparison of relative growth.

RESULTS

Root-Lesion Nematodes-

Soil and root populations of P. penetrans were initially associated only with trees that received nematode inoculum (Table 1.). By the fourth sampling date, considerable P. penetrans contamination was evident and this nematode was associated with all trees. Through the end of 1975, population densities of P. penetrans associated with the inoculated trees were significantly greater than those associated with the non-inoculated trees. Population dynamics of P. penetrans were similar for both cultivars (Table 2). Significantly greater population densities of P. penetrans were recovered from Siberian C than Halford, as indicated by the larger root population densities on the fourth and fifth sampling dates and soil population densities on the third and fourth sampling dates (Table 3).

Soil fumigation had no overall significant influence on the root and soil population densities of P. penetrans (Table 4). The only exceptions were that on the second and last sampling dates, root population densities of P. penetrans in DBCP treated soils were significantly less than those of the other treatments. Similar results were obtained for the soil population densities on the last sampling date.

TABLE 1. *Pratylenchus penetrans* recovered from roots and soil of *P. penetrans*-inoculated and non-inoculated peach trees.

	P. penetrans per g root tissue				
Treatment	5/9/74	9/6/74	5/28/75	9/8/75	4/7/76
Non-inoculated	0.0 x[1]	0.5 x	0.3 x	193.2 x	181.6 x
Inoculated[2]	2.3 y	32.7 y	192.6 y	677.4 y	271.7 x

	P. penetrans per 100 cm^3 soil			
	5/9/74	9/6/74	9/8/75	4/7/76
Non-inoculated	0.0 a	0.2 a	15.7 a	28.7 a
Inoculated	0.8 b	7.5 b	103.5 b	52.9 b

1 Column means followed by the same letter are not significantly different (P=0.05).

2 Each tree inoculated with water suspension of 50 *P. penetrans* (3/1/74 and 6/20/74).

TABLE 2. *Pratylenchus penetrans* recovered from roots and soil of *P. penetrans*-inoculated and non-inoculated Halford and Siberian C peach cultivars.

Variety and treatment	*P. penetrans* per g root tissue				
	5/9/74	9/6/74	5/28/75	9/8/75	4/7/76
Halford					
Non-inoculated	0.0 a[1]	0.4 a	0.3 a	122.2 a	115.5 a
Inoculated[2]	1.5 b	43.4 b	172.5 b	256.3 b	230.9 a
Siberian C					
Non-inoculated	0.0 a	0.5 a	0.2 a	264.1 a	247.6 a
Inoculated	3.0 a	21.9 b	212.7 b	1098.4 b	312.4 a

	P. penetrans per 100 cm^3 soil			
	5/9/74	9/6/74	9/8/75	4/7/76
Halford				
Non-inoculated	0.0 a[3]	0.4 a	14.2 a	24.6 a
Inoculated	0.3 b	8.9 b	60.1 b	28.0 a
Siberian C				
Non-inoculated	0.0 a	0.0 a	17.1 a	32.8 a
Inoculated	1.3 b	6.0 b	146.8 b	77.7 b

1 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.
2 Each tree inoculated with a water suspension of 50 *P. penetrans* (3/1/74 and 6/20/74).
3 Within cultivars, column means followed by the same letter are not significantly different (P=0.05).

TABLE 3. *Pratylenchus penetrans* recovered from roots and soil of Halford and Siberian C peach cultivars.

	P. penetrans per g root tissue				
Variety	5/9/74	9/6/74	5/28/75	9/8/75	4/7/76
Halford	0.8 a[1]	21.9 a	86.4 a	190.5 a	173.2 a
Siberian C	1.5 a	11.1 a	106.5 a	681.2 b	286.6 b

	P. penetrans per 100 cm^3 soil			
	5/9/74	9/6/74	9/8/75	4/7/76
Halford	0.1 x	5.0 x	37.1 x	26.3 x
Siberian C	0.6 y	3.0 x	82.0 y	55.2 y

1 Within extraction techniques, column means followed by the same letter are not significantly different (P=0.05).

TABLE 4. Influence of two soil fumigants on Pratylenchus penetrans recovered from roots and soil of Halford and Siberian C peach trees.

Treatment	*P. penetrans* per g root tissue				
	5/9/74	9/6/74	5/28/75	9/8/75	4/7/76
Nontreated	0.5 a[1]	20.1 a	84.3 a	485.2 a	263.8 a
DBCP[2]	1.1 a	9.5 b	132.5 a	298.2 a	124.9 b
1,3-D + MIC[3]	1.2 a	19.9 a	72.7 a	524.8 a	304.2 a

	P. penetrans per 100 cm^3 soil			
	5/9/74	9/6/74	9/8/75	4/7/76
Nontreated	0.3 a	5.1 a	59.9 a	48.7 a
DBCP	0.3 a	1.9 a	56.7 a	28.4 b
1,3-D + MIC	0.6 a	4.9 a	61.8 a	45.2 a

1 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

2 Applied 35 days before planting at 4.0 gal/A (broadcast).

3 Applied 35 days before planting at 40.0 gal/A (broadcast).

G. macrocarpus geosporus had no overall influence on root and soil population densities of P. penetrans (Table 5 and Table 6). On the third and fourth sampling dates, however, P. penetrans root populations were significantly less in the G. macrocarpus geosporus trees than the trees not inoculated with the fungus. The trends were similar for both cultivars (Table 7 and Table 8).

There were no statistically significant or biologically evident interactions between the tree inoculations and soil fumigation treatments in relation to population densities of P. penetrans.

Mycorrhizae-

There were no statistical mycorrhizal colonization or spore population density differences between the G. macrocarpus geosporus-inoculated and non-inoculated trees for either cultivar (Table 9). Colonization of Halford roots, however, was significantly greater on all sampling dates than that of Siberian C. This increased colonization was not expressed through increased spore production (Table 9).

Tree treatments generally had no significant influence on mycorrhizal colonization or spore production (Tables 10, 11, 12, 13). The exceptions to this for mycorrhizal colonizations were with the P. penetrans-inoculated treatments on Siberian C (first sampling date) and P. penetrans-inoculated treatments on Halford (third

TABLE 5. *Pratylenchus penetrans* recovered from roots of *P. penetrans* and *Glomus macrocarpus* var. geosporus inoculated and non-inoculated peach trees.

Treatment	*P. penetrans* per g root tissue				
	5/9/74	9/6/74	5/28/75	9/8/75	4/7/76
Non-inoculated	0.0 a[1]	0.2 a	0.2 a	183.9 a	174.8 a
G. macrocarpus geosporus[2]	0.0 a	0.5 a	0.3 a	202.4 a	197.4 a
G. macrocarpus geosporus + *P. penetrans*	1.9 b	28.5 b	95.5 a	549.8 a	238.8 a
P. penetrans[3]	3.9 b	37.3 b	292.2 b	804.9 b	304.5 a

1 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

2 Each tree inoculated 10 days before planting with water suspension of 10 spores *G. macrocarpus geosporus*.

3 Each tree inoculated with water suspension of 50 *P. penetrans* (3/1/74 and 6/20/74).

TABLE 6. *Pratylenchus penetrans* recovered from soil of *P. penetrans* and *Glomus macrocarpus* var. *geosporus*-inoculated and non-inoculated peach trees.

Treatment	*P. penetrans* per 100 cm^3 soil			
	5/9/74	9/6/74	9/8/75	4/7/76
Non-inoculated	0.0 a[1]	0.1 a	12.4 a	30.5 a
G. macrocarpus geosporus[2]	0.0 a	0.0 a	19.4 a	26.9 a
G. macrocarpus geosporus + *P. penetrans*	1.0 b	6.8 b	89.5 b	42.9 a
P. penetrans[3]	0.6 b	9.2 b	117.4 b	62.9 a

1 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

2 Each tree inoculated 10 days before planting with water suspension of 10 spores *G. macrocarpus geosporus*.

3 Each tree inoculated with water suspension of 50 *P. penetrans* (3/1/74 and 6/20/74).

TABLE 7. *Pratylenchus penetrans* recovered from roots of *P. penetrans* and *Glomus macrocarpus geosporus*-inoculated and non-inoculated Halford and Siberian C peach cultivars.

Variety and treatment	*P. penetrans* per g root tissue				
	5/9/74	9/6/74	5/28/75	9/8/75	4/7/76
Halford					
Non-inoculated	0.0 a[1]	0.3 a	0.3 a	85.3 a	127.1 a
G. macrocarpus geosporus[2]	0.0 a	0.6 a	0.3 a	159.1 a	103.9 a
G. macrocarpus geosporus + *P. penetrans*	2.0 b	40.2 b	141.1 ab	179.1 a	196.9 a
P. penetrans[3]	1.1 ab	47.5 b	230.9 b	333.5 b	264.8 a
Siberian C					
Non-inoculated	0.0 a	0.0 a	0.1 a	282.5 a	222.4 a
G. macrocarpus geosporus	0.0 a	0.4 a	0.3 a	245.7 a	299.2 a
G. macrocarpus geosporus + *P. penetrans*	1.8 a	16.8 b	76.9 a	920.5 ab	280.7 a
P. penetrans	6.7 b	27.1 b	353.5 b	1276.3 b	344.2 a

1 Within cultivar, column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

2 Each tree inoculated 10 days before planting with water suspension of 10 spores *G. macrocarpus geosporus*.

3 Each tree inoculated with water suspension of 50 *P. penetrans* (3/1/74 and 6/20/74).

TABLE 8. *Pratylenchus penetrans* recovered from soil of *P. penetrans* and *Glomus macrocarpus geosporus*-inoculated and non-inoculated Halford and Siberian C peach cultivars.

Variety and treatment	*P. penetrans* per 100 cm^3 soil			
	5/9/74	9/6/74	9/8/75	4/7/76
Halford				
Non-inoculated	0.0 a[1]	0.1 a	9.7 a	23.9 a
G. macrocarpus geosporus[2]	0.0 a	0.0 a	19.5 a	25.3 a
G. macrocarpus geosporus + *P. penetrans*	0.3 b	9.6 b	53.1 a	24.5 a
P. penetrans	0.3 b	10.3 b	67.0 a	31.5 a
Siberian C				
Non-inoculated	0.0 a	0.0 a	15.0 a	37.0 a
G. macrocarpus geosporus	0.0 a	0.0 a	19.2 a	28.5 a
G. macrocarpus geosporus + *P. penetrans*	1.7 b	4.0 ab	125.9 b	61.2 a
P. penetrans	0.9 b	8.1 b	167.8 b	94.2 a

1 Within cultivar, column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman Keuls Multiple Range Test.

2 Each tree inoculated 10 days before planting with water suspension of 10 spores *G. macrocarpus geosporus*.

3 Each tree inoculated with water suspension of 50 *P. penetrans* (3/1/74 and 6/20/74).

TABLE 9. Mycorrhizal infection and spore population densities of Glomus macrocarpus geosporus-inoculated and non-inoculated Halford and Siberian C peach cultivars.

Variety and treatment	Per cent mycorrhizal infection				Spores per 100 cm^3 soil	
	9/6/74	5/28/75	9/8/75	4/7/76	9/8/75	4/7/76
Halford						
Non-inoculated[3]	18.0 a[1]	23.6 a	41.6 a[2]	50.0 a	34.8 a	97.5 a
G. macrocarpus geosporus	25.0 a	34.7 a	44.0 a	54.1 a	44.1 a	104.5 a
Siberian						
Non-inoculated	1.4 b	6.9 b	22.2 a	26.4 b	28.0 a	45.9 a
G. macrocarpus geosporus	5.5 b	4.2 b	22.2 a	25.0 b	29.5 a	85.4 a

1 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

2 F value 3.893* indicates significant difference (P=0.05); however, Student-Newman-Keuls Multiple Range Test not sensitive enough to detect location of difference.

3 Each tree inoculated 10 days before planting with water suspension of 10 spores G. macrocarpus geosporus.

4 Within cultivar, column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

TABLE 10. Mycorrhizal infection from roots of *Pratylenchus penetrans* and *Glomus macrocarpus geosporus*-inoculated and non-inoculated peach trees.

Treatment	Per cent mycorrhizal infection			
	9/6/74	5/28/75	9/8/75	4/7/76
Non-inoculated	11.8 a[1]	18.0 a	44.4 a	41.6 a
G. macrocarpus geosporus[2]	12.5 a	19.4 a	31.9 a	45.8 a
G. macrocarpus geosporus + *P. penetrans*	16.6 a	8.3 a	34.7 a	33.3 a
P. penetrans[3]	14.6 a	6.2 a	19.4 a	34.7 a

1 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

2 Each tree inoculated 10 days before planting with water suspension fo 10 spores *G. macrocarpus geosporus*.

3 Each tree inoculated with water suspension of 50 *P. penetrans* (3/1/74 and 6/20/74).

TABLE 11. Mycorrhizal infection from roots of *Pratylenchus penetrans* and *Glomus macrocarpus geosporus*-inoculated and non-inoculated Halford and Siberian C peach cultivars.

Variety and treatment	Per cent mycorrhizal infection			
	9/6/74	5/28/75	9/8/75	4/7/76
Halford				
Non-inoculated	16.6 a[1]	27.8 a	55.5 b	55.5 a
G. macrocarpus geosporus[2]	24.9 a	30.5 a	44.4 ab	58.3 a
G. macrocarpus geosporus + *P. penetrans*	24.9 a	38.9 a	44.4 ab	49.9 a
P. penetrans[3]	29.1 a	19.4 a	27.8 a	44.4 a
Siberian C				
Non-inoculated	2.8 b	8.3 a	33.3 a	27.8 a
G. macrocarpus geosporus	2.8 b	8.2 a	19.4 a	33.3 a
G. macrocarpus geosporus + *P. penetrans*	8.3 b	8.3 a	24.9 a	16.6 a
P. penetrans	0.0 a	5.5 a	11.1 a	24.9 a

1 Within cultivar, column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

2 Each tree inoculated 10 days before planting with water suspension of 10 spores *G. macrocarpus geosporus*.

3 Each tree inoculated with water suspension of 50 *P. penetrans* (3/1/74 and 6/20/74).

TABLE 12. *Glomus macrocarpus geosporus* spores recovered from soil of *Pratylenchus penetrans* and *G. macrocarpus geosporus*-inoculated and non-inoculated peach trees.

Treatment	Spores per 100 cm^3 soil	
	9/8/75	4/7/76
Non-inoculated	45.6 a[1]	76.0
G. macrocarpus geosporus[2]	41.9 a	110.1 a
G. macrocarpus geosporus + *P. penetrans*	31.7 a	76.4 a
P. penetrans[3]	17.2 a	67.4 a

1 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

2 Each tree inoculated 10 days before planting with water suspension of 10 spore *G. macrocarpus geosporus*.

3 Each tree inoculated with water suspension of 50 *P. penetrans* (3/1/74 and 6/20/74).

TABLE 13. *Glomus macrocarpus geosporus* spores recovered from soil of *Pratylenchus penetrans* and *G. macrocarpus geosporus*-inoculated and non-inoculated Halford and Siberian C peach cultivar

Treatment	Spores per 100 cm^3 soil	
	9/8/75	4/7/76
Non-inoculated	45.6 a[1]	76.0 a
G. macrocarpus geosporus[2]	41.9 a	110.1 a
G. macrocarpus geosporus + *P. penetrans*	31.7 a	76.4 a
P. penetrans	17.2 a	67.4 a

1 Column means followed by the same letter are not significantly different(P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

2 Each tree inoculated 10 days before planting with water suspension of 10 spores *G. macrocarpus geosporus*.

3 Each tree inoculated with water suspension of 50 *P. penetrans* (3/1/74 and 6/20/74).

sampling date). In both cases inoculations with P. penetrans in the absence of inoculation with G. macrocarpus geosporus may have inhibited mycorrhizal colonization. The exceptions for spore production were with Siberian C, and may indicate a detrimental influence of the initial P. penetrans population densities.

Soil fumigation had no significant overall influence on mycorrhizal colonization of either cultivar (Table 14). In most cases, however, soil fumigation with 1,3-D + MIC or DBCP enhanced population densities of mycorrhizal spores present in the rhizosphere (Table 15).

TABLE 14. Influence of two soil fumigants on *Glomus macrocarpus geosporus* mycorrhizal infection in Halford and Siberian C peach cultivars.

Variety and treatment	Per cent mycorrhizal infection			
	9/6/74	5/28/75	9/8/75	4/7/76
Halford and Siberian C				
Nontreated	17.7 a[1]	18.7 a	31.2 a	43.4 a
DBCP[2]	11.8 a	17.7 a	35.4 a	41.6 a
1,3-D + MIC[3]	10.4 a	18.7 a	31.2 a	35.4 a
Halford				
Nontreated	33.3 a[4]	31.2 a	41.6 a	56.2 a
DBCP	19.4 a	24.9 a	41.6 a	45.8 a
1,3-D + MIC	16.6 a	31.2 a	45.8 a	54.1 a
Siberian C				
Nontreated	2.1 a	6.2 a	20.8 a	30.5 a
DBCP	4.1 a	10.4 a	29.1 a	37.5 a
1,3-D + MIC	4.2 a	6.2 a	16.6 a	16.7 a

1 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls multiple Range Test.

2 Applied 35 days before planting at 4.0 gal/A (broadcast).

3 Applied 35 days before planting at 40.0 gal/A (broadcast).

4 Within cultivar, column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

TABLE 15. Influence of two soil fumigants on Glomus macrocarpus geosporus spores recovered from soil of Halford and Siberian C peach cultivars.

Variety and treatment	Spores per 100 cm^3 soil 9/8/75	4/7/76
Halford and Siberian C		
Nontreated	13.5 a[1]	34.9 a
DBCP[2]	46.8 b	87.0 ab
1,3-D + MIC[3]	41.9 b	128.1 b
Halford		
Nontreated	16.6 a[4]	45.3 a
DBCP	50.9 b	90.6 a
1,3-D + MIC	50.8 b	167.1 b
Siberian C		
Nontreated	10.4 a	24.4 a
DBCP	42.6 a	83.4 b
1,3-D + MIC	33.1 a	89.1 b

1 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

2 Applied 35 days before planting at 4.0 gal/A (broadcast).

3 Applied 35 days before planting at 40.0 gal/A (broadcast).

4 Within cultivar, column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

Ectoparasitic Nematodes

Three ectoparasitic nematodes, Criconemoides curvatum Raski, 1952, Trichodorus atlanticus Allen, 1957 and Xiphinema americanum Cobb, 1913 were recovered from the experimental plots. They were associated with both cultivars (Table 16). On the last sampling date there were significantly more C. curvatum associated with Siberian C than with Halford. In general, soil treatments had no influence on population densities of these nematodes (Table 17). C. curvatum, however, appeared to be enhanced by 1,3-D + MIC (first sampling date) and significantly greater population densities of T. atlanticus were recovered in DBCP treated soils (second sampling date). With the possible exception of C. curvatum, initial seedling inoculation had no influence on the population densities of these three nematode species. C. curvatum may have been introduced into the P. penetrans or G. macrocarpus geosporus inoculum (Table 18).

TABLE 16. *Criconemoides curvatum*, *Trichodorus atlanticus*, and *Xiphinema americanum* associated with Halford and Siberian C peach cultivars.

Nematode and variety	Nematodes per 100 cm^3 soil		
	9/6/74	9/8/75	4/7/76
C. curvatum			
Halford	0.8 a[1]	1.1 a	1.2 a
Siberian C	0.6 a	2.9 a	6.9 a
T. atlanticus			
Halford	0.0 a	0.8 a	4.9 a
Siberian C	0.0 a	1.2 a	5.9 a
X. americanum			
Halford	1.0 a	3.0 a	4.3 a
Siberian C	1.1 a	1.1 a	5.6 a

1 Within species, column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

TABLE 17. Influence of two soil fumigants on Criconemoides curvatum, Trichodorus atlanticus, and Xiphinema americanum recovered from soil of Halford and Siberian C peach trees.

	Nematodes per 100 cm^3 soil							
	C. curvatum			T. atlanticus		X. americanum		
Treatment	9/6/74	9/8/75	4/7/76	9/8/75	4/7/76	9/6/74	9/8/75	4/7/76
Nontreated	0.5 ab[1]	2.5 a	3.1 a	0.8 1	2.9 a	0.7 a	3.4 a	4.7 a
DBCP[2]	0.4 a	1.4 a	1.5 a	1.3 a	10.9 b	1.0 a	1.8 a	4.9 a
1,3-D + MIC	1.1 b	2.0 a	1.4 a	1.7 a	2.6 a	1.3 a	0.9 a	5.4 a

1 Column means followed by the same letter are not significantly different(P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

2 Applied 35 days before planting at 4.0 gal/A (broadcast).

3 Applied 35 days before planting at 40.0 gal/A (broadcast).

TABLE 18. *Criconemoides curvatum*, *Trichodorus atlanticus*, and *Xiphinema americanum* recovered from soil of *Pratylenchus penetrans* and *Glomus macrocarpus geosporus*-inoculated and non-inoculated peach trees.

	Nematodes per 100 cm^3 soil							
	C. curvatum			*T. atlanticus*		*X. americanum*		
Treatment	9/6/74	9/8/75	4/7/76	9/8/75	4/7/76	9/6/74	9/8/75	4/7/76
Non-inoculated	0.4 a[1]	3.2 a	3.7 a	0.9 a	4.2 a	0.5 a	4.3 a	5.6 a
G. macrocarpus geosporus[2]	0.5 ab	1.6 a	1.9 a	0.9 a	4.1 a	0.9 a	1.4 a	2.7 a
G. macrocarpus geosporus + *P. penetrans*	0.5 ab	1.7 a	1.0 a	1.0 a	5.8 a	1.1 a	1.5 a	5.3 a
P. penetrans[3]	1.3 b	1.5 a	2.7 a	0.9 a	7.7 a	1.4 a	1.1 a	6.4 a

1 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

2 Each tree inoculated 10 days before planting with water suspension of 10 spores *G. macrocarpus geosporus*.

3 Each tree inoculated with water suspension of 50 *P. penetrans* (3/1/74 and 6/20/74).

Peach Growth and Development -

Siberian C trees had significantly smaller heights and trunk diameters than Halford trees (Table 19). None of the factors investigated, P. penetrans, G. macrocarpus geosporus, or soil fumigation had any influence on the growth and development of either cultivar during the experimental period (Tables 20, 21, 22, 23).

Both cultivars grew in a seemingly normal manner during the two year experiment with a total mortality of 11% (2% for Halford, 20% for Siberian C).

TABLE 19. Comparison of trunk diameter and height of Halford and Siberian C peach cultivars.

Variety	Trunk diameter (cm) 3/24/76	Height (cm) 3/24/76
Siberian C	2.78 a[1]	110.92 a
Halford	4.35 b	159.46 b

1 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

TABLE 20. Influence of *Pratylenchus penetrans* and *Glomus macrocarpus geosporus* inoculation on the growth of peach trees.

Treatment	Growth index[1] 7/9/74	Growth index 7/24/74	Number laterals 7/24/74	Height (cm) 7/24/74	Height (cm) 3/24/76	Trunk diameter (cm) 3/24/76
Non-inoculated	2.9 a[2]	2.5 a	6.1 a	28.9 a	137.9 a	3.7 a
G. macrocarpus geosporus[3]	3.2 a	2.8 a	7.0 a	29.7 a	134.9 a	3.5 a
G. macrocarpus geosporus + *P. penetrans*[4]	2.5 a	2.4 a	5.9 a	28.2 a	130.8 a	3.4 a
P. penetrans	2.6 a	2.4 a	6.6 a	29.7 a	136.9 a	3.6 a

1 calculated on a 1-4 visual estimation of overall growth characteristics (4= Excellent).

2 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

3 Each tree inoculated 10 days before planting with water suspension of 10 spores *G. macrocarpus geosporus*.

4 Each tree inoculated with water suspension of 50 *P. penetrans* (3/1/74 and 6/20/74).

TABLE 21. Influence of *Pratylenchus penetrans* and *Glomus macrocarpus geosporus* inoculation on the growth of Halford and Siberian C peach cultivars.

Variety and treatment	Growth index[1] 7/9/74	Growth index 7/24/74	Number laterals 7/24/74	Height (cm) 7/24/74	Height (cm) 3/24/76	Trunk diameter (cm) 3/24/76
Halford						
Non-inoculated	3.5 a[2]	3.1 a	9.3 a	37.3 a	159.3 a	4.5 a
G. macrocarpus geosporus[3]	3.4 a	3.3 a	9.5 a	35.6 a	154.9 a	4.0 a
G. macrocarpus geosporus + *P. penetrans*	3.2 a	3.2 a	9.1 a	36.8 a	157.7 a	4.4 a
P. penetrans[4]	3.5 a	3.2 a	9.6 a	38.9 a	165.9 a	4.6 a
Siberian						
Non-inoculated	2.3 a	1.9 a	2.8 a	20.8 a	116.3 a	2.9 a
G. macrocarpus geosporus	2.9 a	2.4 a	4.6 a	23.9 a	115.1 a	3.1 a
G. macrocarpus geosporus + *P. penetrans*	1.9 a	1.7 a	2.8 a	19.8 a	103.6 a	2.5 a
P. penetrans	1.8 a	1.6 a	3.7 a	20.3 a	108.2	2.6 a

TABLE 21 (cont'd).

1 Calculated on a 1-4 visual estimation of overall growth characteristics (4=Excellent).

2 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

3 Each tree inoculated 10 days before planting with water suspension of 10 spores G. macrocarpus geosporus.

4 Each tree inoculated with water suspension of 50 P. penetrans (3/1/74 and 6/20/74).

TABLE 22. Influence of two soil fumigants on growth of peach trees.

Treatment	Growth index[1] 7/9/74	Growth index 7/24/74	Number laterals 7/24/74	Height (cm) 7/24/74	Height (cm) 3/24/76	Trunk diameter (cm) 3/24/76
Nontreated	2.95 a[1]	2.25 a	5.53 a	27.2 a	133.3 a	3.49 a
DBCP[3]	2.41 a	2.95 a	7.72 a	33.1 a	132.5 a	3.78 a
1,3-D + MIC	3.32 a	2.44 a	5.97 a	27.2 a	126.6 a	3.37 a

1 Calculated on a 1-4 visual estimation of overall growth characteristics (4=Excellent).

2 Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

3 Applied 35 days before planting at 4.0 gal/A (broadcast).

4 Applied 35 days before planting at 40.0 gal/A (broadcast).

TABLE 23. Influence of two soil fumigants on growth of Halford and Siberian C peach cultivars.

Variety and treatment	Growth index[1] 7/9/74	Growth index 7/24/74	Number laterals 7/24/74	Height (cm) 7/24/74	Height (cm) 3/24/76	Trunk diameter (cm) 3/24/76
Halford						
Nontreated	3.6 a[2]	3.0 a	8.4 a	34.4 a	159.4 a	4.3 a
DBCP[3]	3.5 a	3.5 a	10.8 a	42.2 a	151.5 a	4.6 a
1,3-D + MIC[4]	3.1 a	3.0 a	8.9 a	34.8 a	148.4 a	4.2 a
Siberian C						
Nontreated	2.3 a	1.5 a	2.7 a	19.8 a	107.2 a	2.7 a
DBCP	2.4 a	2.4 a	4.7 a	24.1 a	113.5 a	3.0 a
1,3-D + MIC	1.9 a	1.8 a	3.0 a	19.6 a	104.9 a	2.5 a

1 Calculated on a 1-4 visual estimation of overall growth characteristic (4=Excellent).

2 Within cultivar, column means followed by the same letter are not significantly different(P=0.05) according to the Student-Newman-Keuls Multiple Range Test.

3 Applied 35 days before planting at 4.0 gal/A (broadcast).
4 Applied 35 days before planting at 40.0 gal/A (broadcast).

DISCUSSION

Root-Lesion Nematode -

Soil and root population densities of P. penetrans recovered on all sampling dates appear to have been derived from the original inoculations. If an indigenous field population of this species had been present, it would have been differentially altered by the soil fumigation treatments and recovered from the non-inoculated seedlings. As in most studies of endoparasitic nematodes, root population densities were several times greater than soil population densities and were a more reliable indicator in estimating populations than soil population density data.

Significantly greater population densities of P. penetrans were recovered from the roots of Siberian C, than from the roots of Halford. This difference was not evident prior to the end of the second growing season.

Growth retardation of the cultivars attributable to P. penetrans was not observed. This lack of above ground symptoms (ie., stunting, chlorosis, poor growth) of Halford and Siberian C would seem to indicate a host tolerant relationship to parasitism at these population levels of P. penetrans.

Siberian C supported higher population densities of P. penetrans than Halford; however, this should not be

misconstrued to indicate that Siberian C is a more tolerant host cultivar. The reasoning behind this seemingly paradoxical statement is based on the inherent differences in growth charasteristics between the two cultivars. Siberian C usually grows 80% as large as Halford. This relationship maintained itself through nine growing seasons (R. Layne, personal communication). It is this characteristic that supports its use as a dwarfing rootstock. The exact mechanism for the dwarfing characteristics of Siberian C is not well understood. It has been postulated to be the direct result of a smaller or less efficient root system for absorption and utilization of soil nutrients compared to standard rootstocks such as Halford. In 1956, Oostenbrink suggested that with P. penetrans there is a direct relationship between the size of the root system and the susceptibility of the host to nematode damage. The larger the root system, the less the degree of damage; whereas, the smaller the root system, the more extensive the damage caused by a similar number of nematodes. In line with this reasoning, Siberian C had a smaller root-stock system than Halford as determined directly by the considerable difficulty in attaining sufficient root samples for both nematode and mycorrhizal analysis. Siberian C exhibited 32% less growth and development (ie., height, trunk diameter) than Halford, and had a more concentrated area for colonization by similar numbers of

P. penetrans. This resulted in a greater population density of this nematode per gram of root tissue due to its slower growing rootstock. The more vigorous Halford would allow similar numbers of P. penetrans to distribute themselves more uniformly throughout the root system, resulting in fewer P. penetrans per g of root tissue. Support of this can be drawn from a comparison of mean population densities of Halford and Siberian C roots to specific ranges of population densities from the Sept. 8, 1975 root samples: For Halford, the mean population density of P. penetrans, per gram, was 190.5. Twenty-nine trees exhibited population densities of 100 or more. From this total tree number, 89% fell into a range of 100-500. Two trees had a population density of 500-1000, and only one tree exceeded 1142. For Siberian C, the mean population density per gram was 681. Eighteen trees exhibited population densities of 500-1000. Sixty-seven per cent ranged from 100-4080. Considerable variability of this nematode was also evident for this cultivar at the 0-500 range. It is evident that the extremes in population densities of this nematode on Halford trees was smaller than on Siberian and was closely related to the mean population density. Further, the mean population density of P. penetrans on Halford was more indicative of the actual numbers recovered per individual tree.

One of the logical conclusions that can be made concerning the response of Halford and Siberian C to the presence of P. penetrans is that neither cultivar had reached its tolerance level and both cultivars exhibited host tolerance at the populations levels recorded at the end of the research. Had the initial population density been greater, populations would have increased more rapidly and perhaps differences in cultivar response would have become apparent and expressed earlier in the experiment. Siberian C would, with time, rapidly become more susceptible to increasing population densities of P. penetrans as the tolerance level of this cultivar was reached and exceeded. This indication of susceptibility through symptom expression would be the direct result of the intensive root damage caused by the concentrated feeding and parasitism of P. penetrans on the slow-growing rootstock; and Halford, because of its more vigorous rootstock, would continue to be tolerant of the presence of P. penetrans for a longer duration than Siberian C. In this regard, Halford would prove to be the better host for P. penetrans, supporting a greater total number of nematodes over a longer time. Physiological aging, environmental or cultural factors that would unduly stress the normal growth of the plant would naturally influence the longevity of tolerance exhibited by both cultivars to this nematode.

The fact that Siberian C may be more susceptible to P. penetrans than other rootstocks should be seriously taken into consideration in commercial selection of rootstocks and experimental peach breeding programs. While the dwarfing characteristics of Siberian C continue to be commercially appreciable, caution in prevention and control of populations of plant-parasitic nematodes is essential to prevent significant production losses.

Unlike most studies of P. penetrans in relation to the growth and development of peach, this nematode did not appear to have a detrimental influence on the health of the trees during the course of the experiment. As previously indicated, this may be the result of the initial, low inoculum levels of P. penetrans and the consequentially slow buildup of population levels. In addition, consideration should be made of the fact that the experimental plants were not subjected to many environmental stresses common to commercial nursery trees (ie., bare-root transplanting from nursery to final orchard site). These cultural factors could stress the plant and render them more susceptible to damage by P. penetrans. Conceivably, the experimental conditions could have been too artificial to allow any significant conclusion concerning the influence of P. penetrans on commercial production of peach trees. If these environmental stresses had been introduced into the experiment, a direct cause and effect relationship

would have been difficult to discern. Consequently, deleterious effects of growth may have been misinterpreted as resulting from nematode pathogenicity, without regard to damage caused by mechanical factors.

Mycorrhizal Effects -

In the present investigation the presence of an indigenous mycorrhizal population, as indicated by early mycorrhizal development in both the fumigated and non-fumigated experimental plots and in the Glomus macrocarpus geosporus-inoculated and non-inoculated Halford and Siberian C cultivars, precluded any attempts to correlate anticipated differential responses to this Glomus species with other experimental variables.

Further, critical evaluation of the significant differences in the per cent mycorrhizal colonization between Halford and Siberian C peach cultivars could not be reasonably compared due to their inherently different growth characteristics and their lack of growth response to inoculated soil or tree treatments.

It may be postulated that the greater mycorrhizal development on Halford was attributable to either 1) greater susceptibility to indigenous soil mycorrhizae, or 2) less specificity of indigenous mycorrhizae to this cultivar. Seemingly, the converse of this statement would also be applicable to mycorrhizal development in Siberian C.

Ectoparasitic Nematodes -

The indigenous populations of Criconemoides curvatum, Trichodorus atlanticus, and Xiphinema americanum had no detectable influence on the experimental peach cultivars. If the initial population densities of these nematodes had been above the tolerance limits for peach, they would have been differentially influenced by the soil fumigation treatments and detected in the data analysis.

The recovery of T. atlanticus is the first report of this species in Michigan and possibly in the North Central region (G.W. Bird, personal communication). Although a detrimental population density of this nematode was not in evidence, several mycorrhizal root samples showed the 'stubby' appearance characteristic of the pathogenicity of this genus. The population dynamics of this species may require careful monitoring to determine its precise relationship to the growing peach.

RESEARCH NOTE ON MYCORRHIZAL QUANTIFICATION

Colorimetric Quantification of Vesicle Colonization

One of the major problems in working with VA mycorrhizal fungi is accurate quantification of their association with roots of host plants. While several techniques have been developed, they all depend on an individually variable visual interpretation (an estimation based on comparisons of different levels of association as indicated by the numbers of vesicles, arbuscules, or hyphae in the stained root tissue).

Preliminary experimentation was conducted on an alternate quantification technique involving a colorimetric analysis of the amount of acid-fuchsin stain taken up by mycorrhizal infected roots.

Soybean seeds were planted in flats containing a population of unknown mycorrhizal spores. This culture, previously maintained on sorghum, formed spores that resembled those of a Glomus spp., but no positive identification was determined. After 14 days, the seedlings were removed from the flats. Each soybean root system was thoroughly rinsed to remove adhering rhizosphere soil. Individual seedlings were planted in 18 4-inch clay pots containing steam-sterilized sandy soil and maintained under summer greenhouse conditions (80-95° daytime; 70-80° night).

After 66 days, the senescent plants were removed for analysis of mycorrhizal infection. Individual root systems were divided into proximal, mediam and distal sections and a representative sample selected for staining. Roots were stained using a modified root clearing and staining technique outlined by Bird, Rich and Glover (8).

Visual inspection of the stained roots was made to note the distribution and location of infection and general characteristics of mycorrhizae. Root infection was then visually quantified, as determined through several 'practice' samples in which stained, one cm roots segments of approximately equal diameter were rated for estimated percentages of vesicular infection (ie., presence of vesicles in root tissues) (Table 24). Preceeding each estimation of vesicular infection, the 'practice' samples were broken open and the actual numbers of vesicle counted to compare accuracy with visual estimation. Actual numbers of vesicles per unit root length were then segregated into infection classes (Table 25).

'Practice' sampling continued until estimated percentages of vesicular infection per gram of root tissue closely resembled those achieved through actual counting.

Twelve one cm stained root segments, visually quantified and representative of each infection class rating, were immersed into test tubes and destained for 24 hours in 1% HCl. The roots segments were removed and the

TABLE 24. Index for quantification of vesicular infection of a VA endomycorrhizal fungus.

Index value	Per cent vesicular infection
0	0
1	1-10
2	11-30
3	31-70
4	71-90
5	91-100

TABLE 25. Index values and visual quantification of vesicles of a VA endomycorrhizal fungus.

Infection class	Vesicles per cm root
0 (0)	0
1 (1-10)	1-85
2 (11-30)	86-255
3 (31-70)	256-595
4 (71-90)	596-765
5 (91-100)	766-850

resulting stained solutions colorimetrically analyzed on a Bausch-Lomb DB-G Spectrophotometer, operating at a wavelength of 549nm. Per cent transmission values were compared to estimated vesicular infection (Table 26).

The results of colorimetric analysis of vesicular infection revealed 1) decreasing per cent transmission as percentages of vesicular infection increased, 2) visual determination of vesicular infection, on a small scale, (ie., per gram of root tissue) was reasonably accurate, and 3) probable future use of colorimetric analysis in accurately assessing and reporting mycorrhizal infection through utilization of standard infection curves for particular VA endomycorrhizal-host relationships. While a graph of per cent transmission versus per cent vesicular infection would present a normal curvilinear relationship, numerous limitations in the technique must be realized before appreciation of its practical value can be considered. The lack of a mycorrhizal specific stain is of particular importance as it was necessary to make the questionable assumption that all plant root tissue takes up stain in a similar manner, regardless of physiological condition. This obviously would not be the case in comparing a mycorrhizal root with a non-mycorrhizal root, considering differences in area due to the presence of vesicles and hyphae. A colorimetric characterization would also include hyphal and arbuscular staining contributions in determining

TABLE 26. Index values, visual quantification of vesicles and per cent transmission at 549 nm of a VA endomycorrhizal fungus.

Infection class rating	Vesicular infection (%)	Transmission (%)
0 (0)	0.0	100.0
1 (1-10)	8.5	99.5
2 (11-30)	17.0	98.2
3 (31-70)	53.3	96.3
4 (71-90)	74.5	92.5
5 (91-100)	97.0	75.0

per cent infection, while the visual technique depends only on the vesicle numbers.

Even with a mycorrhiza-specific stain, one must consider two additional obstacles: 1) changes in plant tissue permeability due to natural factors or staining processes which may affect the amount of stain available for uptake by the fungus, and 2) staining variation caused by differences between mycorrhizal species or within a species dependent on host, stage of development, and density.

In addition, there are the physical requirements for absolute consistency of the concentration and quality of reagents, time, and temperature factors.

Respective of its apparent anomalies, this approach demonstrates the vital need for accurate, reliable, and reproducible quantification of VA endomycorrhizae to both parallel and augment its definitive relationship in plant growth processes.

Root Clearing and Staining Technique

The degree of accuracy and replicability of any colorimetric analysis of VA mycorrhizae is strongly related to consistency of stain concentration, and as such, a detailed outline of the presently employed root-clearing and staining technique follows:

1) Rinse roots thoroughly. Autoclave roots for 5 minuted in 10% KOH at 121°C.

2) Immerse roots in 3% hydrogen peroxide (H_2O_2) until bleached. Darkly pigmented root systems of woody plants (eg. peach) may require 1 to 1½ hours to achieve bleaching. With lightly pigmented herbaceous root systems (eg. corn, tomato, onion) a 10-15 minute immersion in H_2O_2 has proven adequate.

3) Immerse roots in 1% HCl for 5 minutes.

4) Stain for 10 minutes in acid-fuchsin - chloryl hydrate (900 ml H_2O_2, 100 ml chloryl hydrate + 0.5g acid fuchsin) at 121°C.

5) Before roots cool, destain in lactophenol (500 ml phenol, 500 ml lactic acid, 1000ml glycerol, and 500 ml distilled water) for 5 minutes at 121°C.

6) Remove roots and place in fresh lactophenol. (Prolonged exposure to light apparently breaks down acid-fuchsin stain, consequently, processed roots should be stored in a dark location.)

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