

AN ABSTRACT OF THE THESIS OF

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Title: Response to Fenamiphos, Extraction Techniques and Population Dynamics of *Pratylenchus penetrans* on Western Oregon Red Raspberry.

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The effects of fenamiphos on soil and root populations of *Pratylenchus penetrans* were evaluated in four red raspberry cv. Willamette fields in Northwestern Oregon. Field 1 was a silty clay loam with 5.3% organic matter (OM). Field 2 and 3 were silty loam soils with 3.25 and 2.55% OM, respectively and field 4 was a silty clay with 7.1% OM. The nematicide, fenamiphos (10 kg a.i./ha) was applied in broadcast or band treatments on November 15, 1989. Additional plots in field 3, received a band-nematicide treatment on December 28, 1989 to evaluate the effect of application date on the control of *P. penetrans* in red raspberry. Field 4 had plots in sites with and without grass and weed ground cover in the aisles between raspberry rows to examine effects of ground cover on nematicide efficacy. Nematodes from soil and roots were sampled monthly from all plots in each field from October 1989 to October 1990.

Soil populations of P. penetrans sampled within rows decreased between the October and December sampling dates in all four fields. Soil populations in 3 fields increased in density during mid-summer and reached their highest peak in the middle of September. A similar pattern occurred in P. penetrans soil populations from plots with or without ground cover in aisles between rows of raspberry in field 4. However, in this field, numbers increased in July and reached their peak density in August. Root populations of P. penetrans from red raspberry reached their highest number during spring and summer at all fields.

No significant ($P>0.05$) differences in effectiveness of fenamiphos were detected between band and broadcast method of application and, also between the 2 application dates. Seasonal mean densities of soil populations from band application was only significantly lower than in nontreated controls in areas with ground cover in field 4, respectively. High variability in the numbers of P. penetrans in soil and roots of raspberry was observed throughout the year. Therefore, conclusions about the effectiveness of fenamiphos were difficult to assess.

The efficiency of Baermann funnels was 43.9%, when a known number of P. penetrans was added to soil. Total yields of P. penetrans extracted from raspberry roots by mist chamber root extraction (MCRE) were higher ($P=0.05$) than yields extracted by polyethylene plastic bag root incubation (PBRI). Approximately 90% of the total P. penetrans recovered was achieved after three and seven days of extraction for PBRI and MCRE, respectively. However, the extraction efficiency of MCRE was 30% higher than PBRI and the daily recovery lasted 28 and 18 days, respectively.

Response to Fenamiphos, Extraction Techniques and Population Dynamics of
Pratylenchus penetrans on Western Oregon Red

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Typed by Mauricio Lolas for Mauricio Lolas

To my parents, Jorge and Elisa

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**RESPONSE TO FENAMIPHOS, EXTRACTION TECHNIQUES AND
POPULATION DYNAMICS OF PRATYLENCHUS PENETRANS ON WESTERN
OREGON RED RASPBERRY**

INTRODUCTION

Red raspberry (Rubus idaeus L.) is in the Rosacea (Rose) family. Plants are perennial with roots living for many years. Cane growth occurs the first season, flowers and fruits are produced the second year and then canes die. New canes are produced each year from underground roots or basal cane buds. In the Pacific Northwest, red raspberry behaves as a deciduous plant, dropping its leaves in cool seasons.

Berries are sold directly to consumers or for processing and fresh marketing. In Oregon, the total harvested area of red raspberry was 4,200 acres during 1990, almost 5% more than the previous year. During 1990, Oregon growers processed 21 million pounds, and sold 0.5 million pounds to fresh marketing. Red raspberry had a total production value of \$7 million in 1990, occupying an important place among the Oregon berry crops.

The root-lesion nematode (Pratylenchus penetrans (Cobb) Phillip and Stek.) was considered to be a major limiting factor in red raspberry production in the Pacific Northwest (McElroy, 1977). Six species of Pratylenchus have been associated with raspberry, but P. penetrans is the most pathogenic and causes stunting and sometimes cane death (McElroy, 1977; Trudgill, 1983). Pratylenchus crenatus

appears to be of minor importance. Pratylenchus spp. are migratory endoparasites, generally with wide host ranges. These nematodes invade the root cortex, penetrating the root by puncturing the wall of an epidermal cell with their stylet and killing cells during feeding. The feeding areas develop into brownish, elongate lesions that spread along the root axis to finally girdle and kill this part of the root. As a result, plant tops become yellow, stunted and wilt (Dropkin, 1989).

Lesions in the cortex are also sites for other pathogens (Phytophthora, Pythium, Fusarium and Verticillium) that aggravate the damage. Interactions between Pratylenchus spp. and other pathogens have been documented for several crops (Pitcher, 1978; Sikora and Carter, 1987).

Eggs are deposited by gravid females within the roots or in the soil. The first molt occurs within the egg and the second stage juvenile (J2) emerges into the soil or root. As the nematode grows, it molts to become third (J3) and fourth (J4) stage juvenile, and adult. Adult males are common in some species and rare or absent in other species. All migrating stages (J2, J3, J4 and adult) are infective and may move between soil and roots throughout their life. Second-stage juveniles which hatch in the root may extend the lesion or they may leave the root and invade other roots to establish new infestation sites. On a worldwide basis, Pratylenchus is the second most damaging genus of plant parasitic nematodes (Sasser and Freckman, 1987). Recently planted raspberries are extremely vulnerable to this nematode and the damage often results in poor establishment (McElroy, 1977).

Above-ground symptoms resulting from damage produced in the root system

by Pratylenchus spp. often cannot be distinguished from symptoms of other soil borne diseases or environmental stresses. Therefore, the suspicion that plants are affected by nematodes can only be determined by submitting soil and/or root samples to an approved nematology laboratory. These results are necessary to diagnose if nematodes are involved in the current growing problem. Nematode identification and density estimates may also be useful to predict if nematodes presently in the samples could be a problem in the future. Although no formal nematode survey has been conducted, approximately 88% of the red raspberry samples received by the Oregon State University Plant Clinic in 1990 were infested by Pratylenchus spp.

Population estimates are used to plan and evaluate nematode management strategies and to predict potential crop losses. Population estimates should be based on counts from both soil and root environments due the migration and endoparasitic feeding characters. Variation in populations at different times of the year may affect the interpretation of sample estimates. Seasonal fluctuations in numbers of P. penetrans within red raspberry plantings have not been examined in Oregon, however.

Fenamiphos, a systemic organophosphate nematicide, can be applied to raspberry fields from October 1 to December 31 when adequate rainfall can be expected. According to the label, this nematicide may not be applied to raspberries more than once per year or within six months of harvest. The performance of fenamiphos for the control of P. penetrans on red raspberry has not been reported in literature, however.

Raspberry are usually rototilled between the rows and herbicides are applied in the row for weed control. Because of soil compaction resulting from the increased use of mechanical harvesters and poor traction during winter rains, growers may plant a permanent cover crop between the rows (Scheer and Garren, 1987). Cover crops have been shown to influence the population dynamics of plant parasitic nematodes (Dunn, 1972; Robbins *et al.*, 1981; Koenning *et al.*, 1985).

The objective of the present study was to 1) provide basic data on seasonal changes in population levels of *P. penetrans* from red raspberry in Oregon; 2) to assess the effect of fenamiphos, delivered as band or broadcast treatments, on nematode population density, and 3) to examine the efficacy of extraction of this nematode from soil and roots by different methods to provide evaluation of the population measurement.

LITERATURE REVIEW

PRATYLENCHUS SPP. POPULATION DYNAMICS

Population Dynamics of Pratylenchus spp.

Annual crops. Populations of Pratylenchus spp. in soybean field soils tended to decline during the growing season and reached a low point near mid-season when the roots contained very high numbers (Ferris and Bernard, 1961).

Soil and root population densities of P. penetrans in Ontario tobacco fields were generally low in the summer and high in the fall, but the seasonal changes were not consistent from year to year (Olthof, 1971).

The first three weeks after planting, numbers of P. penetrans in potato roots increased rapidly and then decreased the fourth week (Bird, 1977). This pattern was followed by a second increase in weeks five to six or seven and a second decrease in the following weeks, depending on the potato cultivar. Pratylenchus penetrans populations in a potato field increased in roots, tubers, and soil as the growing season progressed. During fall and winter, the total population decreased and the percentage of adults increased (Dickerson et al., 1964).

Pratylenchus scribneri numbers from corn and potato roots, in Wisconsin, increased four months after planting and then decreased in September (MacGuidwin, 1989). Nematodes in soil increased four to five months weeks after planting in both crops. In North Carolina, P. zaeae numbers were highest in October and in January through May in fields planted to various crops (Barker, 1968). The highest numbers

of P. zeae in corn and tomato, in Trinidad, West Indies, occurred in March (spring) and the lowest in January (summer) (Singh, 1976). Most nematode populations increased during the early growing period and reached a peak two to three months after planting. This rise in nematode activity coincided with an increase in the rate of root growth. Pratylenchus brachyurus populations in and around the roots of corn grown in Ibadan, Nigeria, reached their peak in soil in June and in roots in July. Both soil and root populations had a second peak in November. In soil around weeds, populations peaked only in October (Egunjobi, 1974).

Perennial crops. Less is known about the fluctuation of Pratylenchus spp. population in the soil and roots of perennial crop plants.

In red clover, P. penetrans began root invasion three weeks after seeding, and four generations were reported per year. Populations increased sharply in early August, mid-September and early November. Soil temperatures ranging from 0.5 to 6.1 C in early November indicated that P. penetrans can reproduce at relatively low soil temperatures.

Pratylenchus penetrans densities in strawberry roots increased steadily from early March and peaked in late July (Di Edwardo, 1961). At this point, strawberry root growth started to increase and the nematode population level decreased. During this period of increased plant growth activity, the root systems were almost completely renewed, greatly increasing the total volume of roots per plant and consequently reducing the relative number of nematodes per unit of volume of root. During September, the P. penetrans number per unit volume of root increased again

because of the migration of the nematodes to the new roots and the decrease in root growth rate. Soil populations increased during April and June and reached a peak in the early part of July. Pratylenchus coffeae populations peaked on strawberry in April or May and declined in mid-summer. Winter populations were low (Riggs et al., 1956).

Pratylenchus penetrans soil populations peaked in May and mid-September in Western Oregon peppermint (Pinkerton and Jensen, 1983). Root populations peaked in late May and decreased in August. Another root population increase was observed in early October and was followed by a decrease during November and December. Merrifield (1990) found that P. penetrans populations in peppermint fields peaked in early May, decreased through the summer, peaked again in August, and decreased through the fall to a low winter level.

Pratylenchus vulnus on peach was found in high numbers throughout the year. A rapid increase occurred between August and December, especially in the roots of trees showing poor vigor (Fliegel, 1969). Populations of P. zaeae on peach were low from winter through summer, but there was an increase during September and October, followed by a gradual decline toward the end of winter.

The maximum population of P. vulnus in roots of different varieties of blackberry occurred in early June, coinciding with the highest root growth rate (Goheen and Williams, 1955). The population rapidly declined during summer and remained at a low level during fall and winter.

Effect of no-tillage and tillage soil regimes on Pratylenchus spp. and other plant-parasitic nematode populations.

In Nigeria, numbers of Helicotylenchus pseudorobustus and Meloidogyne incognita juveniles were greater in no-till plantings than in conventionally tilled planting of maize in rotation with several crops (Cavennes, 1974). Thomas (1978) compared seven tillage regimes and also found nematode densities to generally be higher in no-tillage plots than in any other tillage practices. In Indiana, P. scribneri Steiner was more evenly distributed in no-till plots than in conventional plots (Alby *et al.*, 1983). However, populations of plant-parasitic nematodes were higher in conventionally tilled field plots than in no-till plots of grain sorghum followed by winter rye in Georgia (Stinner and Crossley, 1982). Similarly, Pratylenchus spp. density on maize in Nigeria, was greater in conventional tillage than in no-till systems (Cavennes, 1974).

The presence or absence of a small-grain crop prior to tillage treatment may have an influence on the nematode community (Baird and Bernard, 1984). Heterodera glycines J2 were most numerous in July in the conventional tillage treatments not preceded by wheat. By October, this difference was no longer evident, however. Multiple cropping systems affected nematode population densities, whereas tillage treatments, conventional or no-tillage, had little effect (Gallaher *et al.*, 1988). The mean population densities of M. incognita J2 and P. brachyurus were not affected by tillage but increased under vetch-corn double cropping.

Subsoiling increased corn yield as well as root and nematode distribution in

the soil profile with little increase in either root length or nematode numbers (Rich *et al.*, 1986). Meloidogyne incognita and Criconemella spp. population levels increased on corn following subsoiling, but numbers of P. zeae Graham were not affected. No influence of tillage or subsoiling was apparent on Hoplolaimus columbus and Criconemella spp. populations under rye-corn double cropping (All *et al.*, 1984).

Tillage treatment in Georgia affected vertical distribution of nematodes. Population densities of Hoplolaimus columbus were greater in the 20-33 cm and 33-46 cm soil layers in conventionally tilled soybean plots that received in-row subsoiling than in conventionally tilled plots that had not been subsoiled, but total numbers of nematodes in the two tillage treatments did not differ (Barker *et al.*, 1975). Bare ground plots had more C. xenoplax than plots without winter annuals (suppression of growth of winter annuals over the entire floor) and weedy plots in large peach orchard blocks in North Carolina (Meagher and Meyer, 1990).

The effective use of nematicides for nematode management in no-till systems will require information on soil characteristics affecting the movement and behavior of the pesticides (Schmitt and Nelson, 1987). Densities of Belonolaimus spp. were significantly reduced by fenamiphos in cultivated plots and in plots which were not cultivated. However, there were no significant differences in densities of Meloidogyne spp. J2 among tillage or nematicide treatments (Elliot *et al.*, 1982).

**BEHAVIOR, MODE OF ACTION AND USES OF FENAMIPHOS IN THE
CONTROL OF PRATYLENCHUS SPP. AND OTHER PLANT PARASITIC
NEMATODES.**

Fenamiphos (ethyl 3-methyl-4-(methylthio) phenyl (1-methylethyl) phosphoramidate) is one of the most important nonfumigant organophosphate nematicides available for use in the control of plant-parasitic nematodes. It is marketed under the tradename NEMACUR (Loser and Kimmerle, 1971; Mobay Chemical Corporation, 1985).

Fenamiphos has been found effective against various endo- and ectoparasitic nematodes attacking numerous field, vegetable and fruit crops (Mobay Chemical Corporation, 1985). NEMACUR is registered for a wide variety of crops and ornamentals (Mobay Chemical Corporation, 1985; Peterson and Winterlin, 1986).

Fenamiphos is highly nematicidal, easily absorbed by the foliage and roots of plants, and translocated throughout the sieve elements of the phloem, without causing injury (Zeck, 1971). However, this systemic behavior was not effective in the control of P. vulnus in beans when fenamiphos was applied as foliar treatments (Marban-Mendoza and Viglierchio, 1980c). Therefore, more studies are needed to assess the efficacy of this systemic function. Plant tissues have not been damaged by several applications of fenamiphos at effective nematicidal rates, indicating high plant tolerance to this compound (Mobay Chemical Corporation, 1985).

Behavior of fenamiphos in soil and plants.

Fenamiphos is normally applied by broadcasting granules or by spraying an

emulsifiable concentrate. Physical or water incorporation is necessary for maximum efficacy by either formulation. Nematicides must often move downwards to reach and kill nematodes located below the application depth (Homeyer and Wagner, 1981; Mobay Chemical Corporation, 1985). Fenamiphos moved slowly through the soil and was effective 25 to 30 cm below the soil surface. Furthermore, fenamiphos was present for up to 12 weeks after application, indicating good persistent activity (Homeyer and Wagner, 1981). This slow dispersion provides prolonged contact between nematodes and the active ingredient (Homeyer, 1971). The low water solubility of fenamiphos (0.04-0.07%) precludes the rapid loss of the product from sandy soils in the absence of large addition of water during and after incorporation. This allows enough time for roots to absorb the product (Johnson *et al.*, 1981).

Fenamiphos is converted into many metabolites within the soil and plants, of which the sulfoxide and sulfone compounds are the most abundant. Both compounds have also nematicidal properties (Waggoner, 1972; Mobay Chemical Corporation, 1985; Krause *et al.*, 1986). In plants, sulfoxide and sulfone are hydrolyzed to phenolic compounds that are then converted to glucosides (Mobay Chemical Corporation, 1985). In soil, the major route of degradation of fenamiphos is thiooxidation, producing sulfoxide as the major metabolite. Fenamiphos is oxidized and disappears completely in about three weeks. The maximum level of sulfoxide is reached at this time.

Sulfone accumulated slowly in soil over a 12-week period but remained in much lower concentration than sulfoxide (Waggoner and Khasawinah, 1974). Of the

three compounds, sulfoxide disperses most readily in soil followed by sulfone. Fenamiphos disperses the least readily. Waggoner and Khasawinah (1974) suggested that the effect of fenamiphos, sulfoxide and sulfone on soil populations of ectoparasitic nematodes is related to the persistence of the compound in the soil. Thus, fenamiphos is the most effective and has the highest persistence, whereas sulfone is the least effective and is the least persistent. Sulfoxide apparently was more active than sulfone against ectoparasitic nematodes but was less active than sulfone against endoparasitic nematodes (Waggoner, 1972; Waggoner and Khasawinah, 1974).

Fenamiphos granules at 20 kg a.i./ha have been evaluated either broadcast or applied in 20-cm bands on either side of the rows of grape vines (Krause *et al.*, 1986). Twenty-eight days after application, fenamiphos was still present in the 30-60 cm layer soil samples, indicating prolonged persistence in soil. Concentrations of fenamiphos in grapes at harvest were below tolerance limits, however. Band or broadcast application methods did not affect the dispersion and persistence of this chemical in the soil. Another study indicated that fenamiphos, due to its low mobility, is the least likely among the contact or systemic nematicides to reach groundwater (Mobay Chemical Corporation, 1985), suggesting that this chemical would be less likely to contribute to environmental contamination.

Mode of Action.

The mode of action of organophosphate nematicides is not clearly understood, but it is generally accepted that their activity is based on the inhibition of

acetylcholinesterase (Marban-Mendoza and Viglierchio, 1980b; Pree et al., 1990). The disruption of the activity of this neuroenzyme causes abnormal transmission of impulses through the nervous system, causing both physiological and behavioral changes (Homeyer and Wagner, 1981; Pree et al., 1990). Acetylcholinesterase activity was suppressed by 97-98% in nematodes treated with 0.5 mM fenamiphos for 48 h and allowed to recover in distilled water for 48 h more (Pree et al., 1990). Unlike other organophosphate and carbamate nematicides tested at the same time, neuroenzyme inhibition was not reversible.

Motility and dispersion of P. vulnus depended upon nematicide concentration and exposure period. More than 90% of the nematodes which were immobilized with 0.005 mM fenamiphos were immobilized after 6 days of exposure. Complete immobilization was detectable with 0.05 mM fenamiphos after 96 h (Marban-Mendoza and Viglierchio, 1980a). Motility of fenamiphos-treated root-lesion nematodes decreased with longer treatment and increased concentration. Pratylenchus vulnus pretreated with 0.01 mM fenamiphos for 12 h and then allowed to disperse on horizontal sand discs at 20 C for 12 h, exhibited a complete suppression of the coordination function involved in dispersion. Since motility and dispersion play an important role in nematode survival, the disturbance of these behavioral patterns, rather than direct mortality, may be an important mechanism in nematode control by fenamiphos (Pree et al., 1989).

In addition, the attraction of P. vulnus to the roots of growing bean seedlings is inhibited by treating the nematodes with solutions of fenamiphos at concentrations

below those necessary to inhibit motility and dispersion. It is possible that fenamiphos interferes with the attraction response of otherwise active and motile P. vulnus by disrupting a sensor control ganglia coordination function that allows nematodes to orient. When only the plants were treated with this nematicide, nematodes were attracted in the natural way and displayed no inhibitorial effects (Marban-Mendoza and Viglierchio, 1980b).

Fenamiphos-induced inhibition of motility, dispersion, and attraction may explain the observed disturbance in the nematode penetration of roots (Marban-Mendoza and Viglierchio, 1980c). Pratylenchus vulnus failed to penetrate bean roots with increased nematicide concentration, and other behavioral responses were also inhibited. Fenamiphos functioned as a nemastat by impairing the ability of H. schachtii J2 to penetrate cabbage roots when it was applied at 3.6 kg a.i./ha or as a split application of 3.6 kg a.i./ha at seeding and at 4 weeks after seeding. These fenamiphos applications also reduced the rate of juvenile development (Muchena and Bird, 1987).

Meloidogyne javanica and H. schachtii eggs were prevented from hatching by different fenamiphos concentrations. While 0.48 ug/ml did not affect the hatching of H. schachtii eggs, 0.01 ug/ml was enough to significantly depress the infectivity of both M. javanica and H. schachtii J2 (Greco and Thomason, 1980). The hatch of M. incognita eggs was inhibited with 2 ug/ml of fenamiphos (Payan et al., 1987).

Uses of fenamiphos in the control of plant parasitic nematodes.

Many studies indicate the benefits of fenamiphos in the control of several

nematode parasites of important crops. Meloidogyne incognita populations in the soil and root-gall indices in squash and cucumber were lower and yields were greater in plots treated with fenamiphos concentrations above 1.5 ug/g of soil in the 0-15 cm soil layer for 10 days (Johnson et al., 1982). After 1 year, 5.04 and 20.16 kg a.i./ha fenamiphos significantly reduced C. xenoplax and M. incognita populations and improved winter peach survival (Ritchie, 1984). The black shank/root-knot disease complex was controlled on all tobacco cultivars tested in a 2-year period, with the combination of 2.24 kg metalaxil/ha and 6.7 kg fenamiphos/ha. It was demonstrated that application of metalaxil + fenamiphos through irrigation water was as effective as application with a tractor-powered sprayer in controlling the disease complex (Csinos et al., 1986). Low root-gall indices and high yields from squash and corn indicated more effective M. incognita management when fenamiphos, at 6.7 kg a.i./ha, was applied at planting than at 2 weeks after planting (Johnson et al., 1986). In peanuts affected by M. arenaria and Macroposthonia ortatus, yields were increased with 2.8 kg a.i./ha applied before planting or 2 months after planting (Minton and Bell, 1981).

Neither adequate and consistent H. glycines control nor profitable soybean yields were achieved with 0.2 g a.i. fenamiphos/m row in soybeans double-cropped with wheat (Schmitt, 1987). In addition, microbial degradation reduced the observed effect of fenamiphos on H. glycines penetration of soybean roots when compared between treated and untreated plants (Sipes and Schmitt, 1989).

Fenamiphos at 22.4 kg a.i./ha reduced P. penetrans populations in alfalfa and

increased forage yields in the second and third season (Willis and Thompson, 1979). In apple and plum orchards, fenamiphos was less effective than carbofuran for controlling Xiphinema spp. but more effective in the control of Pratylenchus spp. populations (Rosenberger and Meyer, 1988). When fenamiphos at 20.2 kg a.i./ha was applied in both fall and spring or in spring only for the control of P. penetrans on apple, trunk diameter and shoot length were increased, suggesting the economic importance of this nematode in apple production (Santo and Wilson, 1990).

EXTRACTION TECHNIQUES AND EFFICIENCY

Nematode population estimates are dependent on the proportion of the total nematode population that is removed from a soil or plant tissue sample during extraction. This proportion is referred as the extraction efficiency (Ferris, 1987).

Extraction techniques differ in efficiency due to factors which the researcher does not know or is not able to control. Soil texture, pH and temperature, storage conditions and nematode species, among others, affect the precision in which nematodes are recovered by an extraction procedure. The effects of these factors on efficiency have been summarized in several reports (Merrifield, 1990; Kerr and Vythilingham, 1967; McSorley, 1987). Therefore, the search for procedure modifications that increase efficiency in nematode extraction is an important need in quantitative nematology (Viglierchio and Schmitt, 1983).

Extraction of nematodes from soil.

Barker *et al.* (1969) reported that the Baermann funnel is the most efficient soil extraction method for Pratylenchus. Basic requirements for this method are a funnel with a piece of rubber tubing attached to the stem that is closed by a spring or screw clip. The funnel is placed in a support and filled with tap water. Soil is placed on milk filters, tissue or butter muslin suspended on a screen support and submerged in the water in the funnel. Active nematodes pass through the filter or the cloth and sink to the bottom of the funnel stem. After a period of time, a small quantity of water containing the nematodes is collected (Hooper, 1986). The effects of temperature and duration of storage, sample volume, nematode species, funnel

filter material, soil compaction and extraction time on the efficiency of this method have been reviewed (Merrifield, 1990). Viglierchio and Schmitt (1983) and Merrifield (1990) suggested that the efficiency of extracting P. penetrans from soil by Baermann funnels is approximately 50 percent.

Extraction of nematodes from root tissue.

Various techniques have been used to obtain nematodes of the genus Pratylenchus from roots of plants. Perhaps the simplest is to place infected roots in a vessel containing water and collect the nematodes that move out of the roots into the water (Chapman, 1957). Unsuberized young avocado roots, were placed in Mason jars in moist condition and incubated at room temperature. Within a few hours, Radopholus similis and Pratylenchus spp. started accumulating within the small amount of water that drained from the roots to the bottom of the jar (Young, 1954). Plastic bags have been used as an alternative to jars because they are cheaper, easier to handle and do not require as much storage space (Tarjan, 1960). Incubation of infested root and soil samples in sealed polyethylene plastic bags for six to seven days at 24 C increased yield of R. similis as compared with yields from immediately processed samples (Tarjan, 1960a). Higher numbers of R. similis were obtained from roots incubated in polyethylene plastic bags than from roots incubated in glass jars for periods up to nine days at 24 C (Tarjan, 1969b). The same author improved nematode recovery of R. similis from chopped citrus roots by incubating roots for 2-3 weeks in polyethylene bags with the addition of 1-3% hydrogen peroxide (Tarjan, 1967). The optimum extraction time was found to be only 2 days for

recovery of Tylenchulus semipenetrans from citrus roots incubated in polyethylene bags with 3% hydrogen peroxide (Tarjan, 1972). The optimum concentration of hydrogen peroxide to recover greatest yields of R. similis and Helicotylenchus spp. from macerated banana roots was 10 ml of the 30% solution in 1 liter of tap water (Gowen and Edmunds, 1973).

A misting technique has been successfully used for extraction of endoparasitic nematodes from bulb tissue, leaves, stems, seeds and roots (Hooper, 1986). The mist chamber is an elaborate modification of the Baermann funnel which provides excellent aeration as an intermittent flow of water is sprayed continuously over the infested material (McSorley, 1987). Nematodes recovered by this method are usually more active than those extracted by other methods, possibly because of better oxygenation and because toxic decomposition compounds are washed away (Hooper, 1986). Some nematodes continue to develop in tissues in the mist chamber and emerge over long periods of time if extraction is prolonged (Barker, 1985).

EFFICIENCY OF EXTRACTING PRATYLENCHUS PENETRANS

INTRODUCTION

Nematode populations thresholds for plant injury are important for development of improved nematode control strategies and integrated pest management practices. Therefore, there is a need for standard nematode extraction techniques with optimum efficiency and associated correction factors to permit the determination of true field populations. These nematode extraction techniques should be easily manageable, accurate, rapid, inexpensive and independent of the nematode species.

Mist chamber and plastic bag methods for extraction of Pratylenchus penetrans from raspberry feeder roots were compared for ease and efficiency. An additional study examined the efficiency of P. penetrans extraction from soil by a modified Baermann funnel technique to determine appropriate correction factors for adjusting extraction counts to true field densities.

MATERIALS AND METHODS

Determination of Baermann Funnel Extraction Efficiency.

Fine sterile soil (100 g) was placed on Rapid-Flo milk filters which were supported by a piece of plastic window screen glued to a 4 cm-diameter 2-cm high ring of PVC pipe. The soil samples on their supported filters were then placed in tap water-filled funnels (13.5 cm diameter) with a piece of rubber tubing attached to the stem and closed by a spring or screw clamp.

Raspberry roots infested with P. penetrans were suspended on a 5 mm sieve and washed clean of soil and debris in a pressurized water stream from a hand held nozzle. Feeder roots were separated from woody roots and placed on wire screens inside 10 cm-diameter glass funnels with long stems inserted inside 15 cm-long glass test tubes. A fine mist of water was sprayed continuously over root samples. Active P. penetrans emerged and were recovered from the water in the bottom of the test tube after 48 hours. Nematodes collected from 15 funnels were poured into a beaker and the volume adjusted to 100 ml. The number of nematodes in the beaker was calculated by stirring the beaker contents and counting nematodes in 4 ml aliquots of the suspension and extrapolating for the total volume. Aliquots (4.5 ml) of the total suspension were poured into counting dishes and counted to determine the exact number in each aliquot. Each of 10 aliquots was then added to sterile soil placed on Baermann funnels as described above. Samples were extracted for five days, and the water level in each funnel was checked daily. About 200 ml of water

was then drained from the bottom of the funnel and poured through a 25 μ sieve to concentrate the nematodes. The nematodes were then rinsed off the sieve into a counting dish and counted.

The number of nematodes recovered were compared to the number initially added to determine extraction efficiency. Paired t-test was used to compare the log transformed numbers of P. penetrans initially added and the number of nematodes recovered.

Mist Chamber and Polyethylene Plastic Bag Root Incubation: Extraction Efficiency and Daily Recovery Rate.

Raspberry roots infested with P. penetrans were obtained from nontreated plots at field 1. Roots were suspended on a 5 mm sieve and washed clean of soil and debris in a pressurized water stream from a hand held nozzle. Feeder roots were separated from woody roots and thoroughly mixed to provide a root mass of relatively uniform nematode infestation.

A sub sample of feeder roots (4 g) were placed on wire screens inside 10 cm-diameter glass funnels with a long stem inserted inside a 15 cm-long glass test tube. The funnels were placed in a mist chamber where a fine mist of water was sprayed continuously.

Another sub sample of infested feeder roots (4 g) were sealed in thick polyethylene plastic bags of 0.95 liters capacity, and incubated in the dark at room temperature (20-22 C). Ten ml of 3% hydrogen peroxide was added to feeder roots to avoid oxygen shortage and to moisten all the roots thoroughly. Feeder roots were

remoisten with the solution after each day of recovery.

Nematodes recovered from the mist chamber and plastic bag incubations (placing roots from bags on a coarse screen and rinsing them off), were collected and counted daily until the yield of lesion nematodes had essentially ceased. Each extraction method was replicated 10 times. When the accumulation of debris in samples from plastic bags prevented counting immediately, samples were placed directly on milk filters in a Baermann funnels so nematodes would migrate through the filter and be collected in clean water for counting.

Differences between numbers of P. penetrans recovered from the mist chamber and plastic bag incubations on individual dates were determined with t-test after transformation to $\log_{10}(x + 1)$.

RESULTS

Determination of Baermann Funnel Extraction Efficiency.

Numbers of P. penetrans added and after five days of extraction by a modified Baermann funnel technique are summarized in Table 1. Final numbers of P. penetrans recovered after five days of Baermann funnel extraction were significantly ($P=0.0001$) less than initial numbers of nematodes added. Recovery ranged from 18.1 to 99.6%. Mean efficiency of recovery was 43.9% with a coefficient of variation of 21.7%.

Mist Chamber and Polyethylene Plastic Bag Root Incubation: Extraction Efficiency and Daily Recovery rate.

The daily recovery rate in mist chamber root extraction (MCRE) and plastic bag root incubation (PBRI) lasted 28 and 18 days, respectively. The highest recovery of P. penetrans from raspberry roots was obtained during the first day of processing for both techniques (Figure 1). There were no significant ($P=0.05$) differences in the number of nematodes between the two extraction methods for the first three days. The daily recovery rate for PBRI was significantly ($P=0.05$) less than the daily recovery rate from MCRE starting on the fourth day. In addition, nematode recovery from MCRE increased slightly on the tenth day. The mean total number of nematodes recovered during the entire extraction period was 887 and 1265 per gram fresh weight of raspberry root for PBRI and MCRE, respectively (Figure 2). Approximately 90% of the total P. penetrans were extracted after three and seven

Table 1. Numbers of Pratylenchus penetrans before and after Baermann Funnel Extraction for five days (N = 10).

SAMPLE	NUMBERS / 100 G SOIL			PERCENTAGE
	INITIAL	FINAL	DIFFERENCE	OF RECOVERY (INIT/FIN)
1	231	230	1	99.6
2	234	73	161	31.2
3	281	208	73	74.0
4	244	56	188	23.0
5	206	42	164	20.4
6	199	36	163	18.1
7	196	86	110	43.9
8	209	60	149	28.7
9	244	150	94	61.5
10	212	81	131	38.2
MEAN	226	102*	123	43.9
(STD ERR)	(8)	(22)	(18)	(8)
(CV)	(3)	(22)	(14)	(22)

* Mean of numbers of P. penetrans after Baermann Funnel Extraction for five days was significantly less than initial numbers, according to paired-t test of $\log_{10}(\text{numbers}/100 \text{ g soil} + 1)$.

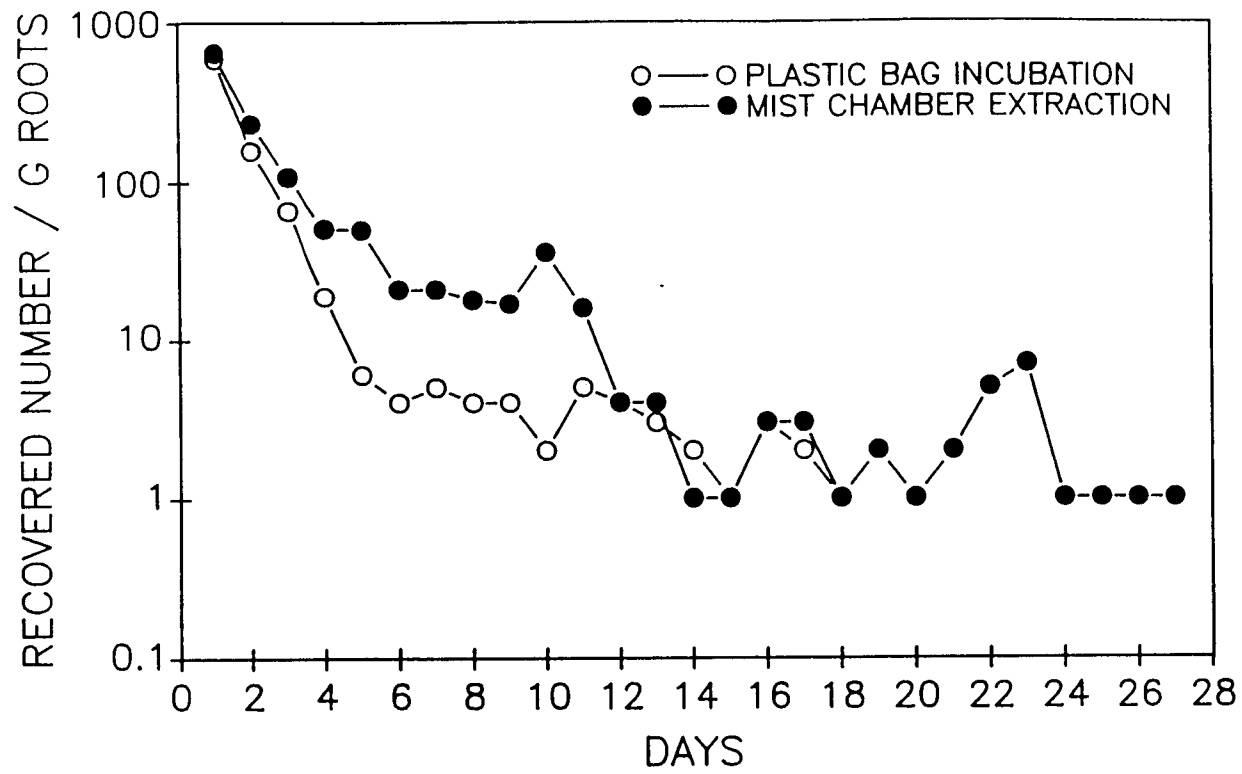


Figure 1. Recovery of *Pratylenchus penetrans* from raspberry roots.

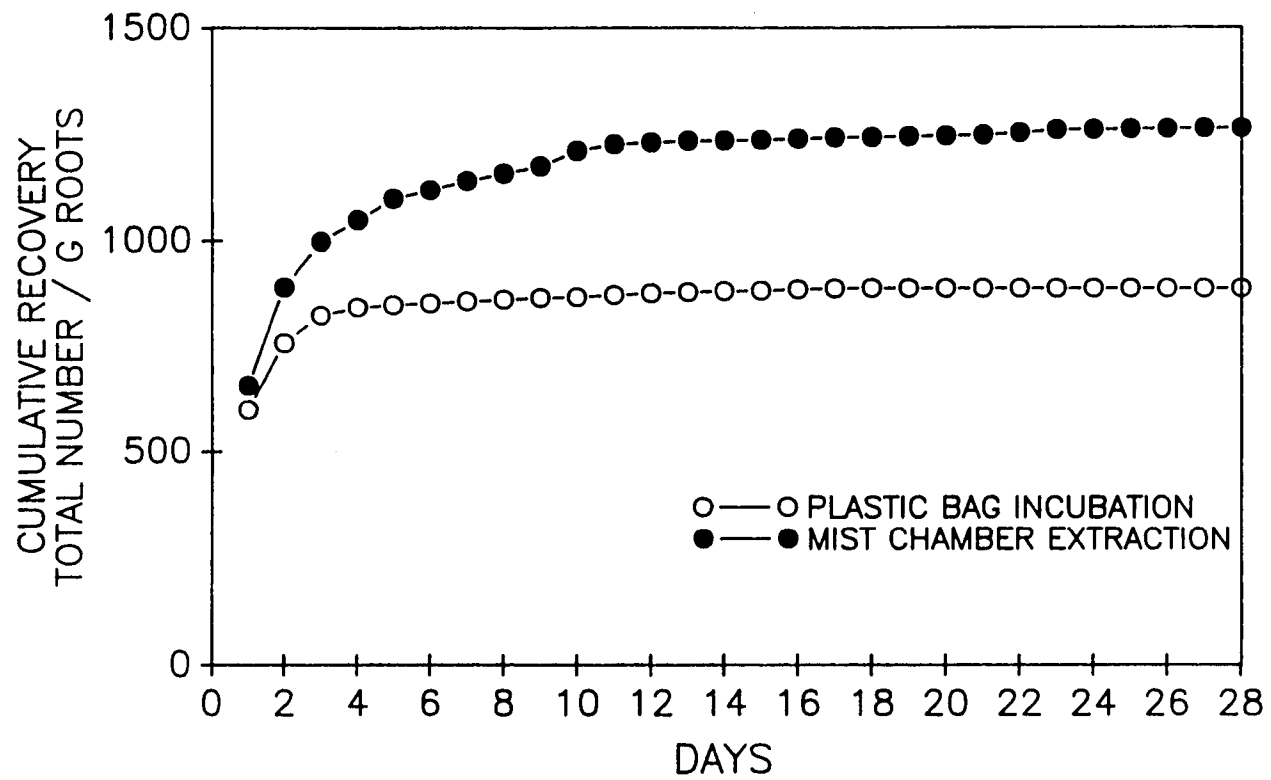


Figure 2. Cumulative recovery of *Pratylenchus penetrans* from raspberry roots.

days from PBRI and MCRE, respectively (Figure 3).

It was observed that, after the fifth day of extraction, most of the nematodes recovered from PBRI were juveniles, whereas adults and juveniles were recovered from MCRE for the entire extraction period.

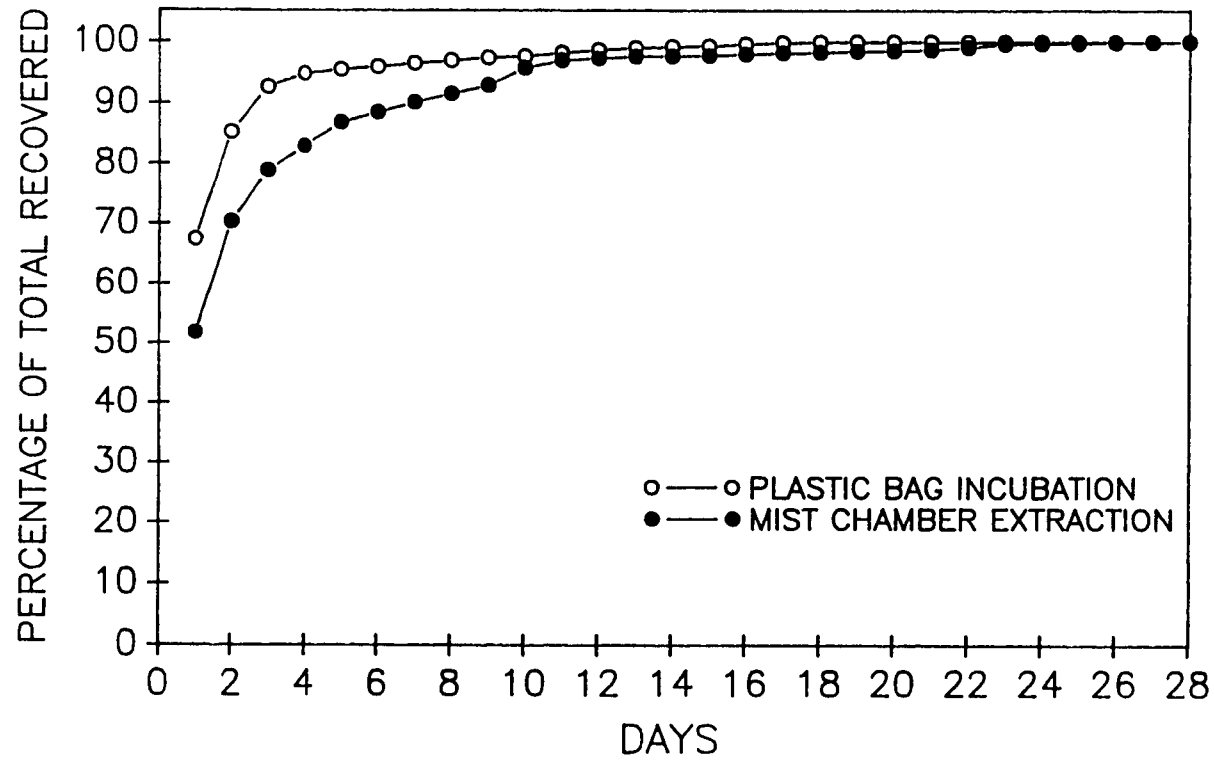


Figure 3. Cumulative percentage of recovery of *Pratylenchus penetrans* from raspberry roots.

DISCUSSION

Determination of Baermann Funnel Extraction Efficiency.

Almost half of the nematodes added to the sterile soil were not recovered by the Baermann funnel extraction process. The results of Merrifield (1990) and Viglierchio and Schmitt (1983), suggest that the efficiency of extracting *P. penetrans* with this method is approximately 50 percent. Several factors could affect this extraction efficiency value. The relative performance of motility-dependent extraction methods may vary with season (Barker *et al.*, 1969) and laboratory temperature during extraction on Baermann funnels may have an important effect on recovery (Kerr and Vythilingam, 1966). Since different nematode species and/or life stages may have different optimum temperatures for activity, temperatures present during the extraction period may delay or advance the time of recovery (Adams, 1965). Furthermore, extraction rates of the soil (or root) may differ for different life stages (MacGuidwin, 1989). However, the main factor that may regulate nematode yields from Baermann funnel extraction is the nematode mobility (Harrison and Green, 1976). Only active nematodes, with appropriate energy reserve will be able to migrate through soil and filters to accumulate at the bottom of funnels. Nematodes used in this experiment were previously extracted from roots and, although only visibly active individuals were selected, their energy supply may have been less than those normally found in soil. Nematodes maintain energy stores in the form of lipids (23 to 40% of the total dry weight) and carbohydrates. During

starvation in environments of low oxygen supply, lipids and carbohydrate are rapidly metabolized (Dropkin, 1989). Although a three-day Baermann extraction period appeared to be appropriate for extraction of P. penetrans from soil samples (Merrifield, 1990), the extraction duration of five days may have been inadequate to obtain high yields under the conditions in this study.

Finally, more examination of nematode recovery from extraction techniques needs to be conducted to determine appropriate correction factors for extraction efficiencies under different conditions.

Mist Chamber and Polyethylene Plastic Bag Root Incubation: Extraction Efficiency and Daily Recovery Rate.

The MCRE appears to be the best method for determining the number of nematodes in plant tissues (Barker, 1985). Total yields of P. penetrans extracted from raspberry roots by this method were significantly higher than PBRI. The extraction efficiency of MCRE was 30% higher than PBRI. The accumulation of debris that prevented the direct counting of samples was a major problem in ease and efficiency of PBRI. Samples often had to be reextracted through a Baermann funnel to clean them up enough to count. Nematodes were lost through both processes which reduced the efficiency of the procedure. Highest (and similar) yields were recovered from both PBRI and MCRE on the first day, suggesting that most of the active root population migrates immediately from the cut roots into the water environment. When roots are excised from a plant they begin to decay and

nematodes that are capable of migrating may leave their former nutritional source as soon as possible. The environmental conditions that the MCRE technique provides are better than in PBRI. Continuous mist over roots allows better oxygen supply, adequate moisture and removal of toxic substances produced by root decomposition (Hooper, 1986). The daily recovery for MCRE from raspberry roots lasted 28 days. Similarly, Wall and Chapman (1967) and Merrifield (1990) recovered P. penetrans from mist chamber extraction for 19 and 38 days, respectively. However, the daily recovery for PBRI lasted only 18 days. Daily recovery after three days was lower in PBRI than MCRE, but it cannot be concluded whether or not they were lost during the Baermann funnel reextraction.

It was noted that after the fifth day of incubation, the population from the PBRI was composed primarily of juveniles (J2 and J3). It appeared that the stressed conditions of this technique may have stimulated hatching of eggs present in the roots or prevented migration by adults. In contrast, MCRE populations were always composed of adults and juveniles even to the last day. Much of this additional recovery may be due to egg hatch (McSorley *et al.*, 1984) or possibly to reproduction within the roots before extraction. The efficiency of incubation procedures for short periods is considered to be low for many species of endoparasitic nematodes, since they will continue to emerge over long periods of time if incubation is continued (Chapman, 1957; Oostenbrink, 1960).

Approximately 90% of the total P. penetrans recovered was achieved after three and seven days of extraction for PBRI and MCRE, respectively. Even though

hydrogen peroxide was used to enhance oxygenation in PBRI, nematodes were observed to be less active than those coming from MCRE. Polyethylene plastic bags have some advantages that may solve several difficulties in root extraction systems (Tarjan, 1960). However, in this case, plastic bags were not better than the mist chamber to obtain highest yields of P. penetrans from raspberry roots.

**POPULATION DYNAMICS AND RESPONSE OF PRATYLENCHUS
PENETRANS ON RED RASPBERRY
TO FENAMIPHOS IN FOUR OREGON SOILS**

INTRODUCTION

The severity of the damage caused by Pratylenchus penetrans is proportional to their population density in both soil and roots (Jennings, 1988). Because this nematode is a migratory endoparasite and is found in soil and in plant roots, it is important to maintain both soil and root populations at a low level if optimum growth of raspberry plants is to be achieved. Red raspberry is an important small fruit crop in both Oregon and Washington and many fields are infested with Pratylenchus spp.

Seasonal fluctuations in soil and root populations of Pratylenchus spp. have been studied under various crop regimes by several workers but have not been examined for red raspberry in Oregon. Knowledge of population dynamics of nematodes is essential to determine proper timing for population estimates and determine when most damage to the plant may occur.

The behavior, mode of action and uses of fenamiphos in the control of Pratylenchus spp. have been studied under different crop regimes. However, the effects of fenamiphos on the populations of P. penetrans from red raspberry have not been examined in Oregon.

A population dynamics study of P. penetrans red raspberry grown in Oregon was conducted. The influence of fenamiphos on the population density of P. penetrans on red raspberry when delivered as a band or broadcast application was evaluated. Efficacy of fenamiphos applied mid fall or in the early winter, and to areas with and without ground cover was also examined.

MATERIALS AND METHODS

This study was initiated in October 1989 on red raspberry fields in Western Oregon. Several raspberry fields in the northern Willamette Valley of Oregon were surveyed for nematode population densities. Four fields with high densities of P. penetrans which represented different soil types and different growing areas were selected for this research.

Field 1 soil, located at Sandy, OR, was a silty clay loam (15% sand, 46% silt, 38% clay) with a 5.29% organic matter (OM) content. Field 2, located at Scholls, OR had a silt loam soil type (21% sand, 58% silt, 21% clay) with 3.25% OM. Field 3, located at Troutdale, OR, had a silt loam soil type (28% sand, 65% silt, 7% clay) and 2.55% OM. Soil in field 4, located at Sandy, OR was a silty clay (14% sand, 44% silt, 42% clay) with 7.1% OM.

Different treatments at each site were replicated five times in a complete randomized design. The treatments included fenamiphos treated plots with a band or broadcast method of nematicide application and control plots without nematicide application (Table 2). A nontreated buffer row was included between each of the rows containing experimental plots. In field 1, these treatments were applied to one set of plots with grass and weed ground cover in the aisles between rows and to one set of plots with ground cover removed.

Fenamiphos was applied to appropriate plots with a 11 l capacity CO₂ backpack sprayer. One pass using three nozzles on each side of the row was used

Table 2. Fenamiphos treatments and plot sizes at different raspberry fields in northwestern Oregon.

	FIELD 1	FIELD 2	FIELD 3	FIELD 4	
				WITHOUT GRASS GROUND COVER ¹	WITH GRASS GROUND COVER ²
FENAMIPHOS TREATMENTS	NON TREATED	NON TREATED	NON TREATED	NON TREATED	NON TREATED
	BAND- FALL ³	BAND-FALL	BAND-FALL	BAND-FALL	BAND-FALL
	-	-	BAND- WINTER ³	-	-
	BROAD- CAST-FALL	-	BROAD- CAST-FALL	BROAD- CAST-FALL	BROAD- CAST-FALL
PLOT SIZE (METERS)	27.5 X 5.5	27.5 X 5.5	27.5 X 5.5	18.3 X 5.5	18.3 X 5.5
LOCATION (NEAREST TOWN)	SANDY	SCHOLLS	TROUT- DALE	SANDY	SANDY

¹ Treatments in plots without ground cover between rows.

² Treatments in plots with grass ground cover between rows.

³ Fall and winter treatments were made on November 15 and December 28, 1989, respectively. Fenamiphos rate: 10 kg a.i./ha of treated area.

for broadcast applications and one nozzle on each side of the row was used for band applications. In broadcast applications, the entire area from the raspberry row to the center of the aisle between rows was treated on each side of the row. The width of the band application was 0.9 m on each side of the row. Fenamiphos was applied as NEMACUR 3, at a rate of 10 kg a.i./ha of treated area for both band and broadcast treatments, on November 15 when soil and air temperature averaged 9.5 and 10.3 C respectively. Field 3 also included a band treatment on December, 28 when air temperature averaged 8.9 C. Nontreated plots at all sites received no fenamiphos during the entire study period.

Soil and roots were collected from each plot monthly from October 19, 1989 to October 26, 1990. A soil probe was used to randomly collect nine (fields 1, 2, 3) or six (field 4) 2.5 x 30 cm soil cores from within the row and close to the plant. The soil cores from each plot were mixed into a single sample. Root samples were collected from three randomly selected plants from each plot. Root samples, 10 x 10 x 10 cm, were obtained with a clam shovel, roots were removed and bulked into a single sample.

Soil samples were thoroughly mixed and screened to remove roots. Nematodes were extracted from a 100 g soil sample using the modified Baermann funnel method described earlier. After five days, nematodes and approximately 200 ml water were drained from funnels into labeled bottles for storage until counting. Root samples were washed clean of soil and debris with a pressurized water stream from a hand held nozzle. Feeder roots were trimmed from woody roots, blotted dry,

weighed and placed in an intermittent misting chamber for seven days. Nematodes were then collected and poured into labelled bottles for storage until counting. When samples contained several hundred of nematodes, only a portion of the counting dish was examined and a conversion factor was used to determine the number of nematodes in the total area of the counting dish, and therefore, in the root or soil sample. Sample estimates were not corrected for extraction efficiency.

Nematode densities (number/100 g soil or number/g root) were transformed by $\log_{10}(x + 1)$ and analyzed with ANOVA. Differences in densities between sample dates within treatments were determined by a protected LSD procedure. All levels of significance are at $P < 0.05$ unless otherwise stated.

The effects of fenamiphos treatments on the seasonal mean densities of P. penetrans in soil and roots of red raspberry were evaluated by computing the average population density from December 1989 (one month after treatment) to September 1990 in each plot. Data were transformed ($\log_{10}(x + 1)$) before averaging, analyzed with ANOVA and statistical separation of means was determined with a protected LSD procedure.

RESULTS

Effects of ground cover on Pratylenchus penetrans populations.

There was no significant effect of ground cover on soil ($P=0.163$) or root ($P=0.800$) populations of P. penetrans on red raspberry at field 4. However, the interaction between ground cover and fenamiphos treatments was statistically significant for soil populations ($P=0.012$), but not for root populations ($P=0.914$). Since there was no effect of cover on densities from the nontreated plots, soil populations from control plots with and without cover were combined into a single site ($N=10$) for analysis of population dynamics in nontreated areas. In all evaluations of fenamiphos treatments on soil populations however, field 4 was treated as two sites ($N=5$). Root populations from plots with and without cover were combined into one site ($N=10$) for all analyses.

Population dynamics of P. penetrans on red raspberry.

Significant ($P<0.001$) differences between sites and sampling dates were found in nontreated soil populations of P. penetrans in red raspberry. Population changes over time in the four sites are illustrated in Figures 4 and 5.

Soil populations declined markedly between October and December, 1989 in all sites and continued to decline more slowly until February, 1990 in all but site 3. In site 1, soil densities were significantly less in February than in October, but began to increase again in April and attained a seasonal (1990) peak in September. Population densities remained lower during 1990 than those observed in October,

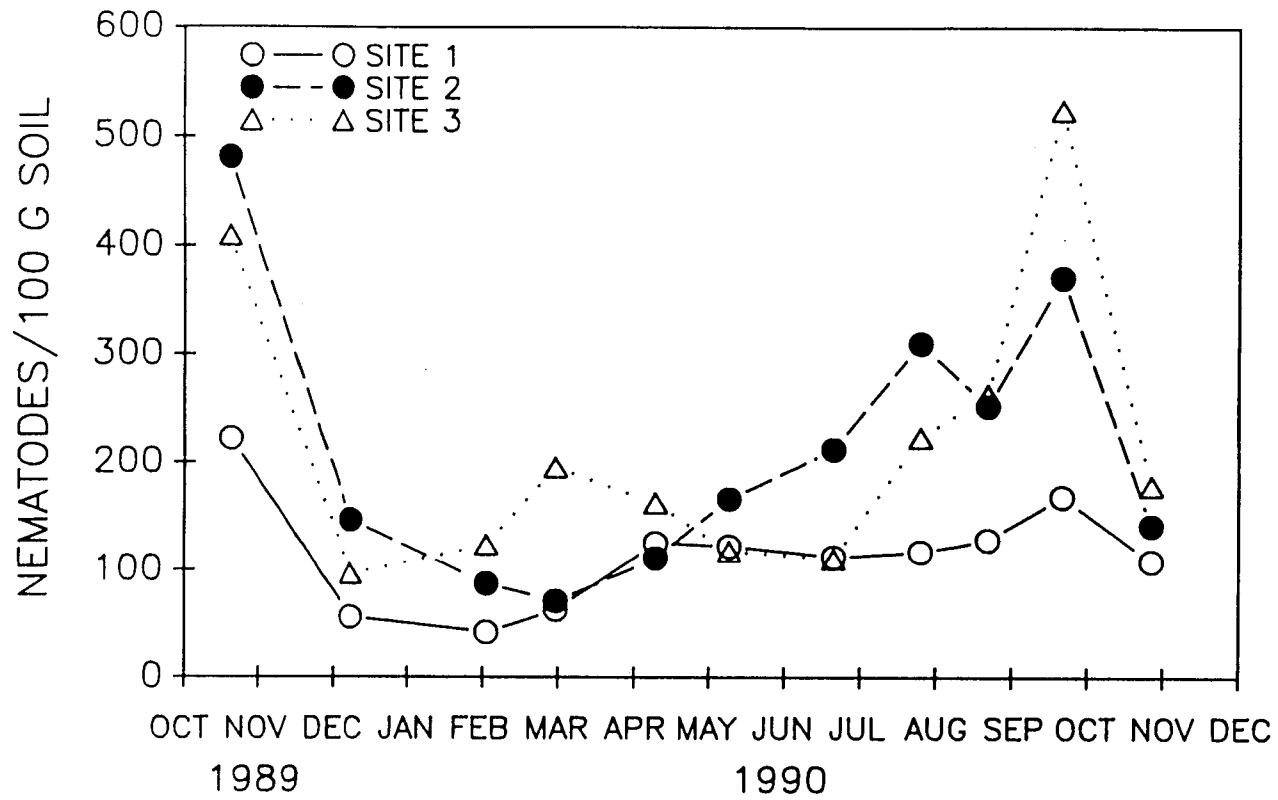


Figure 4. Population dynamics of *Pratylenchus penetrans* in soil from three raspberry fields in Northwestern Oregon.

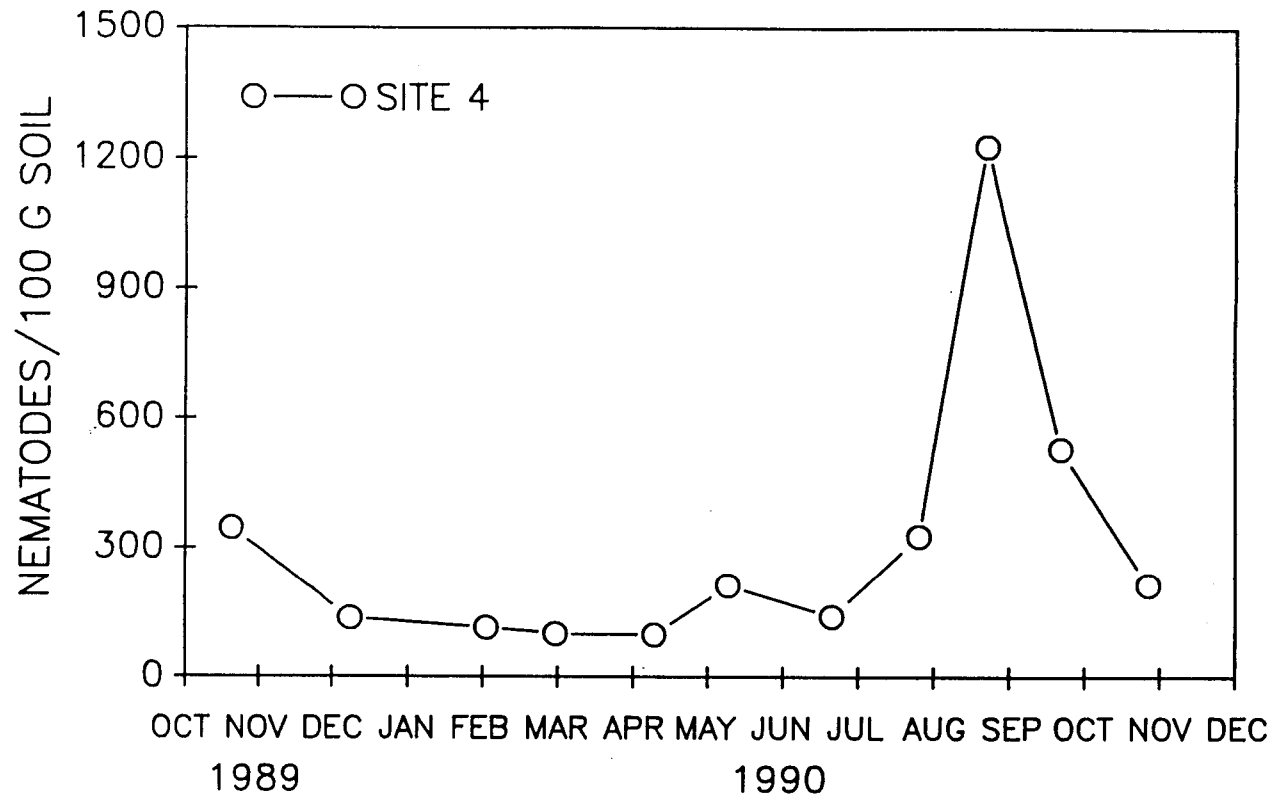


Figure 5. Population dynamics of *Pratylenchus penetrans* in soil from red raspberry in Northwestern Oregon.

1989 and did not increase in the same proportion as the other three sites. Population densities in site 2 increased steadily from March until September and then declined in October. Densities were significantly less from December through April, 1990 than in October 1989, and significantly higher in September 1990 than in January and February. Soil populations in field 3 were significantly less in December and January, 1990 than in October, 1989. A small peak occurred in March and then numbers declined through June. As in field 2, populations increased steadily to reach a peak in September and then declined rapidly. Densities in September were significantly higher than those in December, January, May and June. After the winter decline, soil densities of P. penetrans in field 4 remained low until July, 1990 and then increased rapidly to peak populations in August (Figure 5). Numbers declined rapidly between August and October in all plots. Soil populations were significantly higher in August than on all dates except October, 1989 and September, 1990.

The effect of site was not significant ($P=0.674$) for root populations of P. penetrans on red raspberry, so populations from all sites were averaged for each date. Unlike soil populations, nematode densities in roots remained stable between October and March, and then increased until June (Figure 6). Populations in June were significantly higher than levels from October, 1989 to March, 1990. Numbers declined slightly in July, but increased to attain peak population in August before declining steadily until October. Peak densities in August were significantly higher than for all dates before June 1990. Population dynamics of P. penetrans in soil and

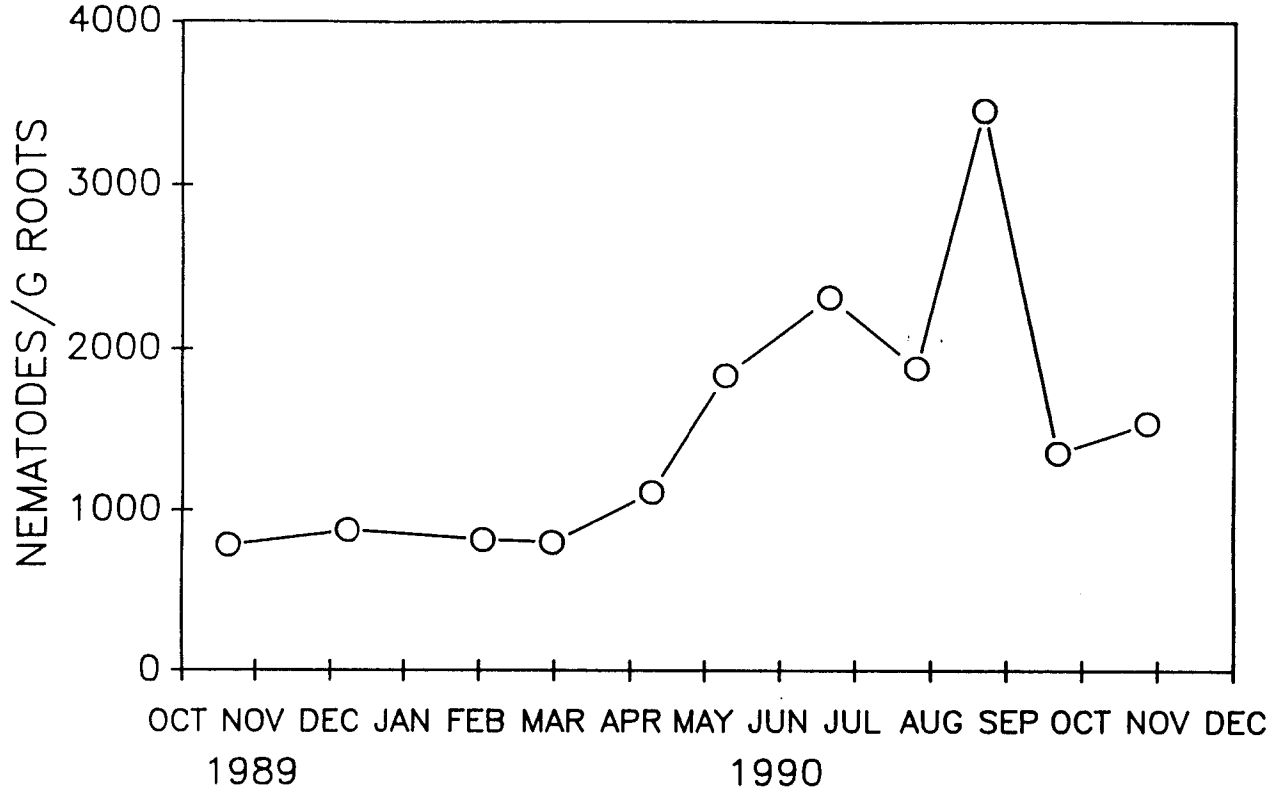


Figure 6. Population dynamics of Pratylenchus penetrans in roots from red raspberry in Northwestern Oregon.

roots from all treatments at all fields are illustrated in appendix I.

Effectiveness of fenamiphos in the control of P. penetrans in red raspberry.

There was no significant difference in efficacy for control of soil ($P=0.421$) and root ($P=0.687$) populations of P. penetrans on red raspberry when fenamiphos was applied in mid fall or early winter at field 3. Similarly, there was no significant difference in control of soil ($P=0.434$) and root ($P=0.612$) populations of P. penetrans on red raspberry when fenamiphos was delivered as a band or broadcast application in the fall (fields 1, 3 and 4). Therefore, only effects of fall band applications are discussed further. Dates on which populations in treated plots were significantly different from nontreated plots for band and broadcast applications in each of the four fields are given in tables in appendix II.

When soil populations in nontreated and band treated plots were analyzed, there were significant ($P<0.001$) effects of site, date, treatment and the interactions of site X date and site X treatment. Therefore the effects of treatment were examined at each site individually. Band applications of fenamiphos in the fall did not significantly reduce soil populations at field 1 ($P=0.072$) or in field 4 plots without cover ($P=0.983$), but did significantly reduce densities at field 2 ($P=0.007$), field 3 ($P<0.001$), and plots with cover at field 4 ($P<0.001$).

Seasonal mean densities (December 1989 to September 1990) of P. penetrans from band fenamiphos treated areas were numerically less than from nontreated plots in all instances for soil populations (Table 3) but were only significantly

Table 3. Effect of fenamiphos application on soil seasonal mean densities (December 1989-September 1990) of Pratylenchus penetrans on red raspberry, cv. Willamette, in northwest Oregon (N=5).

FENAMIPHOS TREATMENT	FIELD 1	FIELD 2	FIELD 3	FIELD 4	
				COVER	NO COVER
NON- TREATED	104 ¹ (8)	192 (15)	202 (16)	315 (12)	327 (26)
BAND-NOV	75 (4)	136 (10)	137 (17)	175 (6)	264 (24)

¹ Seasonal mean number of Pratylenchus penetrans/100 g soil and (standard error).

($P < 0.001$) lower than nontreated controls in areas with ground cover at field 4.

When the effects of band application of fenamiphos on root populations were analyzed across all sites, there were significant ($P < 0.001$) effects of treatment, date and the site X date interaction. However, there were no significant effects of site ($P = 0.796$) or the site X treatment ($P = 0.462$) and date X treatment ($P = 0.091$) interactions, so data were averaged across all sites for further analysis.

The average populations in nontreated and band applied fenamiphos plots from all four fields for each sample period is illustrated in Figure 7. Seasonal mean density of *P. penetrans* in band-fenamiphos treated areas (1006/g root) from all fields were significantly ($P < 0.001$) lower than in nontreated areas (1605/g root). Similarly, root densities were significantly lower in band treated plots than in nontreated plots on December 1989, May, Jun and August, 1990.

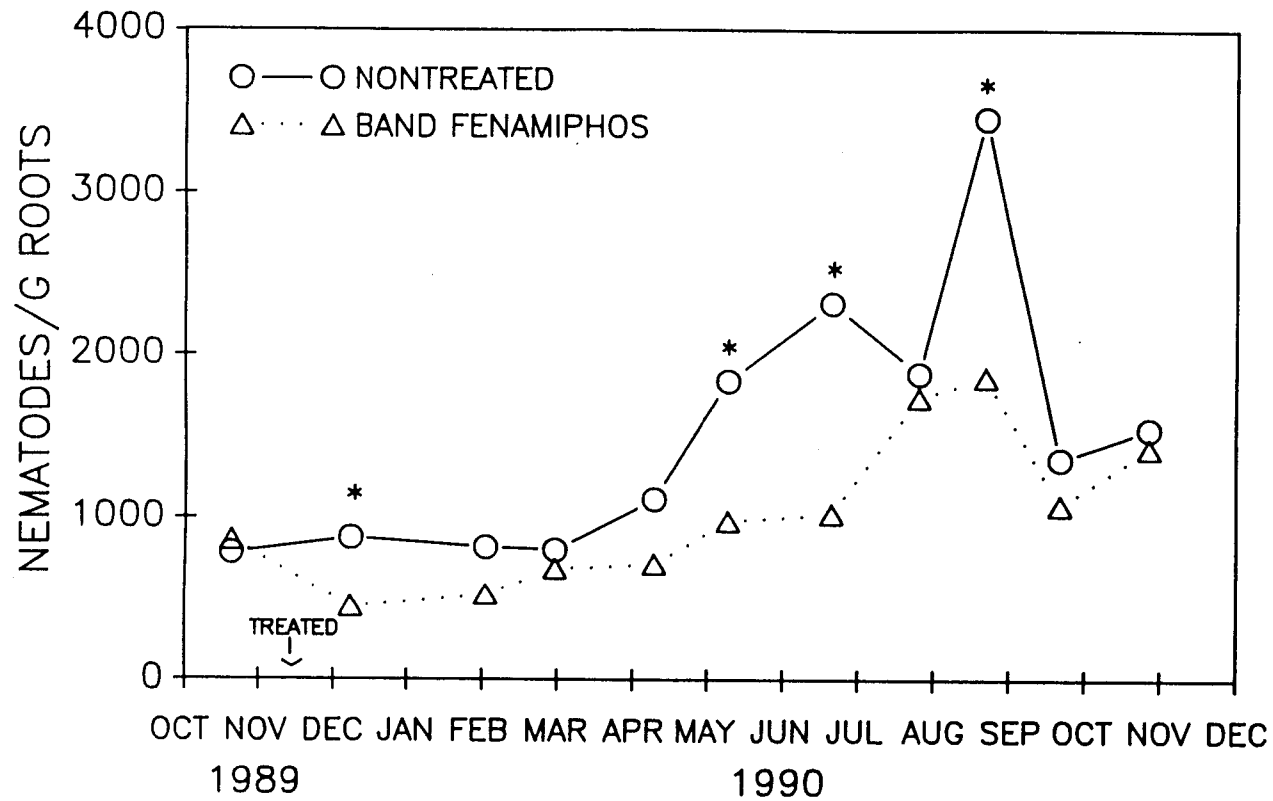


Figure 7. Population dynamics of *Pratylenchus penetrans* in red raspberry roots from nontreated and band applied fenamiphos plots in Northwestern Oregon.

DISCUSSION

Seasonal changes of Pratylenchus penetrans populations.

Numbers of P. penetrans in soil decreased dramatically between October and December in all four fields. Although, fenamiphos applications occurred in November (or December), even nontreated plots experienced the same decrease. This suggests that some other factor besides nematicide treatment, such as natural seasonal mortality, depressed soil populations. Soil populations remained low through the winter, increased in density during spring to mid-summer and reached their highest peak toward the end of the season. These patterns are similar to populations of P. penetrans, from red and black raspberry in Maryland which were lowest in May and then increased to reach a peak in July and August (Golden and Converse, 1965).

Populations of P. penetrans in roots did not decrease between October and December 1989 as did soil populations and reached their highest numbers during spring and summer at all fields. This may indicate that soil populations experience high winter mortality and that most P. penetrans overwinter in roots. These results are similar to those from strawberries, where P. penetrans soil populations declined after July and were lowest in January, but root populations remained stable from October through January and increased from March through July (DiEdwardo, 1961). Goheen and Williams (1955), studied the seasonal fluctuations of P. vulnus in the roots of six varieties of blackberry and one black raspberry in North Carolina.

Maximum populations of P. vulnus in the roots occurred early in June, but during the summer, populations decreased markedly and stayed at a low level, with minor fluctuations during the fall and winter. Milder climates in North Carolina may allow this species to attain peak densities earlier in the year than in Oregon.

Through spring and summer, high variability of numbers of P. penetrans in red raspberry roots was obtained from all plots. These fluctuations may be related to root growth pattern of this perennial plant in that period as discussed by DiEdwardo (1961) for nematodes associated with strawberry roots. During fruiting in late June and July, root growth declines, because plant resources are allocated to the reproductive stage of the plant. Because of this lack of growth, nematodes may accumulate in roots, producing higher densities per root volume. After fruiting, roots begin to grow again and nematodes are diluted by this root expansion and smaller counts per root volume are obtained. Nematodes then migrate from soil to new roots and reproduction occurs in nematodes already present in older roots. The progeny colonize the new roots and higher numbers per root volume are obtained again. All this process is favored by appropriate soil moisture content and warmer temperatures during late spring, summer and even early fall. Additional work is needed on the relationship between root growth and nematode population dynamics in perennial fruit crops like raspberry and other caneberries. Kable and May (1968), suggested that seasonal fluctuations of P. penetrans may be largely due to variations in soil moisture. In the present study, the effect of moisture on P. penetrans population was not determined.

Seasonal changes in densities of Pratylenchus spp. have been studied in many annual crops (Ferris and Bernard, 1961; Olthof, 1967; Barker, 1968; Bird, 1977), but less is known about the seasonal behavior of these nematodes in perennial crops. Considering the differences, particularly in root growth and physiology, between these two types of crops, conclusions made from one crop type to the other may not be valid. Population studies of P. penetrans under field grown raspberries showed that populations could increase over 130% per year (McElroy, 1979). At this rate, 10 nematodes per 100 g of soil at planting would increase to a population that would cause severe damage to raspberry in 3 to 4 years. Furthermore, nematodes in the current study, were present in soil and raspberry roots throughout the year and, although densities were low compared to population peaks, they were well above the damage threshold of 21 per 100 g of soil (Sheer and Garren, 1987).

Effects of fenamiphos on Pratylenchus penetrans populations.

Band application is generally as effective as broadcast application if the plant rows are spaced more than 61 cm (Sasser, 1989). Similarly, the current study found that band and broadcast applications, at the same rate per treated area, were equally effective. This should be true, since nematodes, by themselves, move only a few cm a year (Dropkin, 1989). It is unlikely that many nematodes present beyond the width of band application (0.9 m) reach plants roots. In addition, densities of nematodes in the aisle, even in areas with ground cover, were substantially lower than populations in the raspberry row. Initial samplings were made from all aisles in field 4 on several dates, but densities were too low to provide meaningful data and

sampling was discontinued.

Mid fall and early winter band application dates of fenamiphos, appeared to be equal in effectiveness for control of P. penetrans on red raspberry. This may be attributed to the fact that populations decline (soil) or remain stable (roots) during this period. Therefore application at any time during the label period of October 1 to December 31 may be equally effective. Efficacy may be improved if applications could also be made before populations increase in summer, but most Oregon raspberry growers do not irrigate so proper incorporation may not be possible at that time.

Seasonal mean densities were always numerically less in fenamiphos treatments than in nontreated areas but statistical differences were only observed for soil populations in field 4, and when root populations were averaged across all fields. High variance in populations estimates and low sample size may have prevented statistical support for the trends which were observed. Although soil populations at field 4 were significantly less in band-fenamiphos treatments on plots with ground cover, there was no statistical difference between treatments from plots without ground cover. These results may have been due to greater variance in population estimates made from areas without ground cover. Seasonal mean densities from soil in nontreated plots were nearly identical in both areas but the standard error was almost 6 times higher in plots without cover. Although there was no significant effect of cover on population density, it may affect nematode distribution which could affect the precision of sample estimates. The effects of ground cover in perennial crops on

nematode distribution and nematicide efficacy needs to be evaluated further.

An additional observation that can be made from population dynamics data is that, when populations increased in spring and summer, this increase was delayed and/or the population peak less in fenamiphos treatments (see Figure 7 and appendix 1). Indeed, many of the significant differences between treatments were noted during peak population periods. That population levels are less in fenamiphos treatments nearly one year after application is remarkable considering the short half life (1 month) of the nematicide. Thus, although the reduction in populations by fenamiphos during winter and spring may not always have been statistically significant it may have been biologically significant. It may be necessary for nematicides to reduce nematode populations to a much lower level than achieved in this study before statistically significant differences would be obtained during the period of low density.

Limited studies on movements of non-volatile nematicides in soil indicate that they move downward as a result of rainfall or irrigation (Rohde *et al.*, 1980). The low water solubility of fenamiphos (0.04-0.07%) prevents rapid leaching from sandy loam soils in the absence of excess amounts of water (<9.0 cm up to 15 days after application) and allows sufficient time for adsorption by the plant of an adequate nematicidal concentration (Johnson *et al.*, 1981). Fenamiphos and/or its degradation products do not leach to any great extent in soils and may be the least likely of the contact or systemic nematicides to reach groundwater in the major grape growing areas of California (Mobay Chemical Corporation, 1985). In Oregon and

Washington, large amounts of winter rainfall follow fall fenamiphos applications. The potential for this volume of water to dilute the concentration of fenamiphos in the soil and wash it through the soil profile before it has had sufficient exposure with nematodes may reduce efficacy and needs to be examined. In any event however, greater reductions in nematode populations than were observed in this study would be desirable for optimum raspberry production. Therefore, further work may be needed to increase the efficacy of fenamiphos in these types of soils.

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APPENDICES

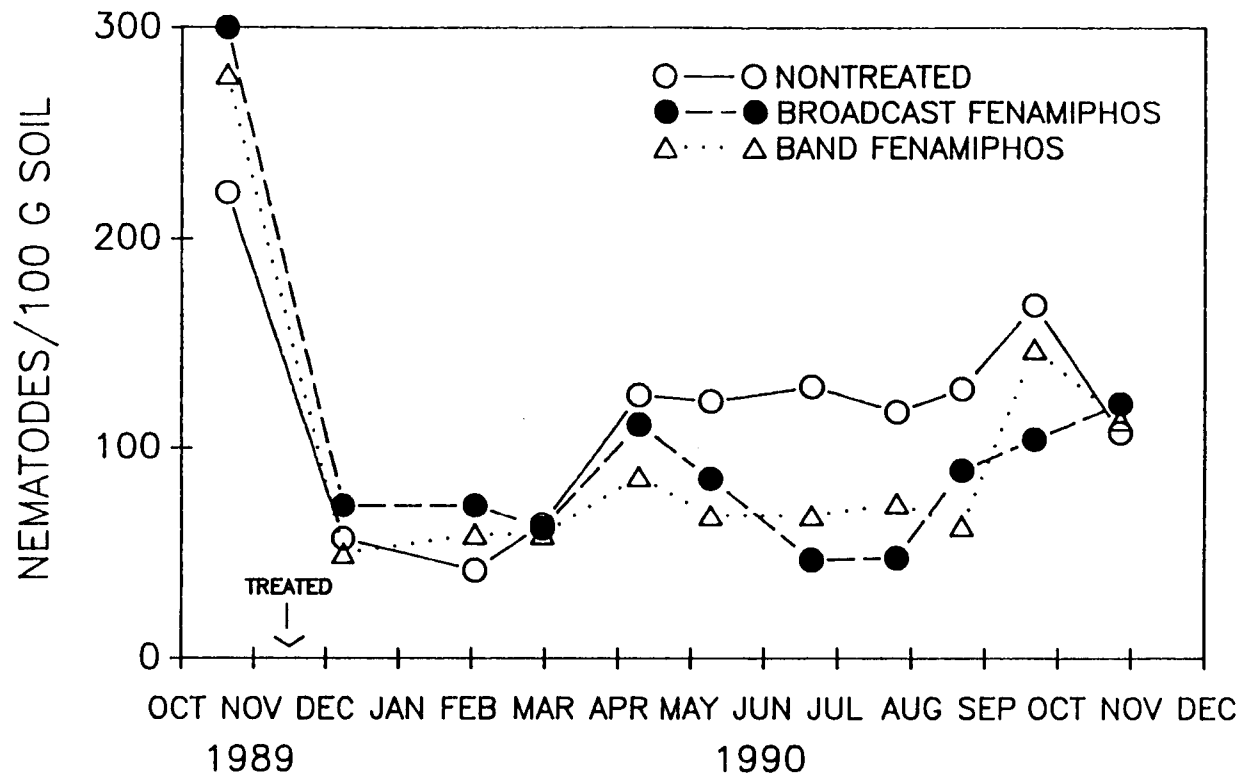


Figure 1. Soil population dynamics of *Pratylenchus penetrans* in red raspberry from field 1, Sandy, OR.

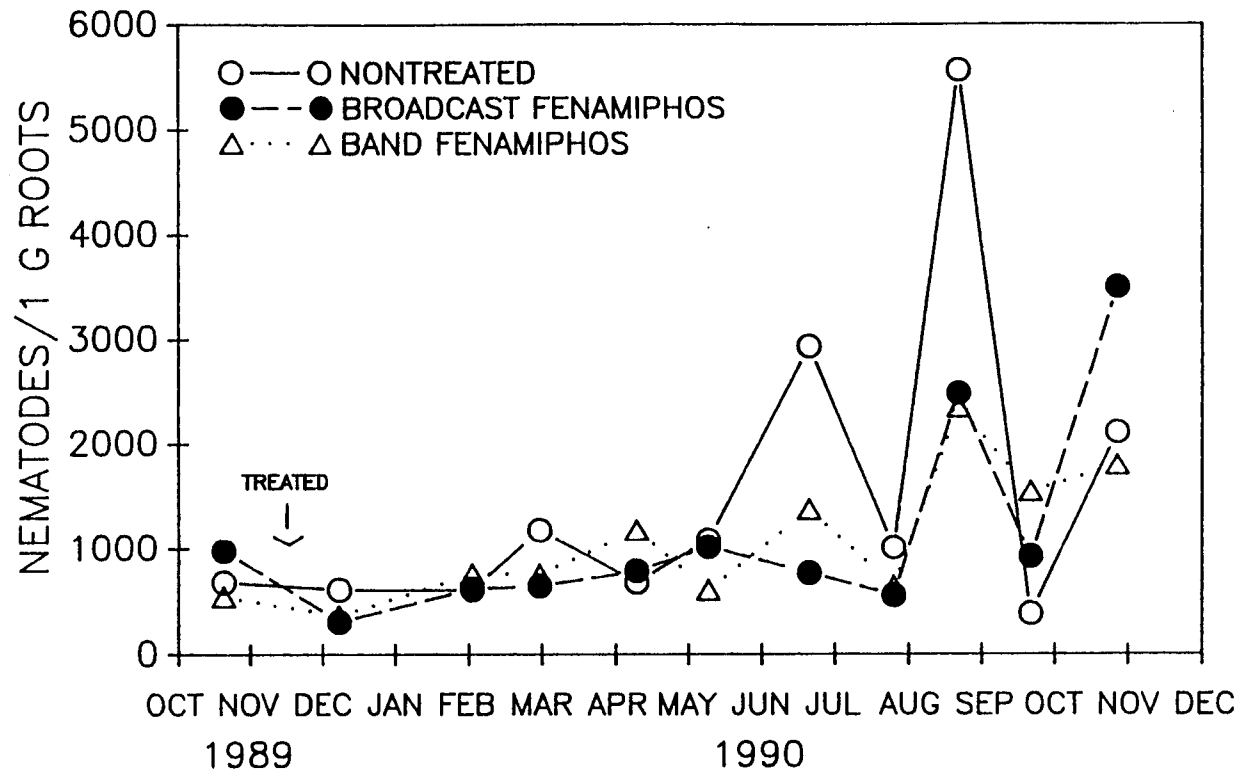


Figure 2. Root population dynamics of Pratylenchus penetrans in red raspberry from field 1, Sandy, OR.

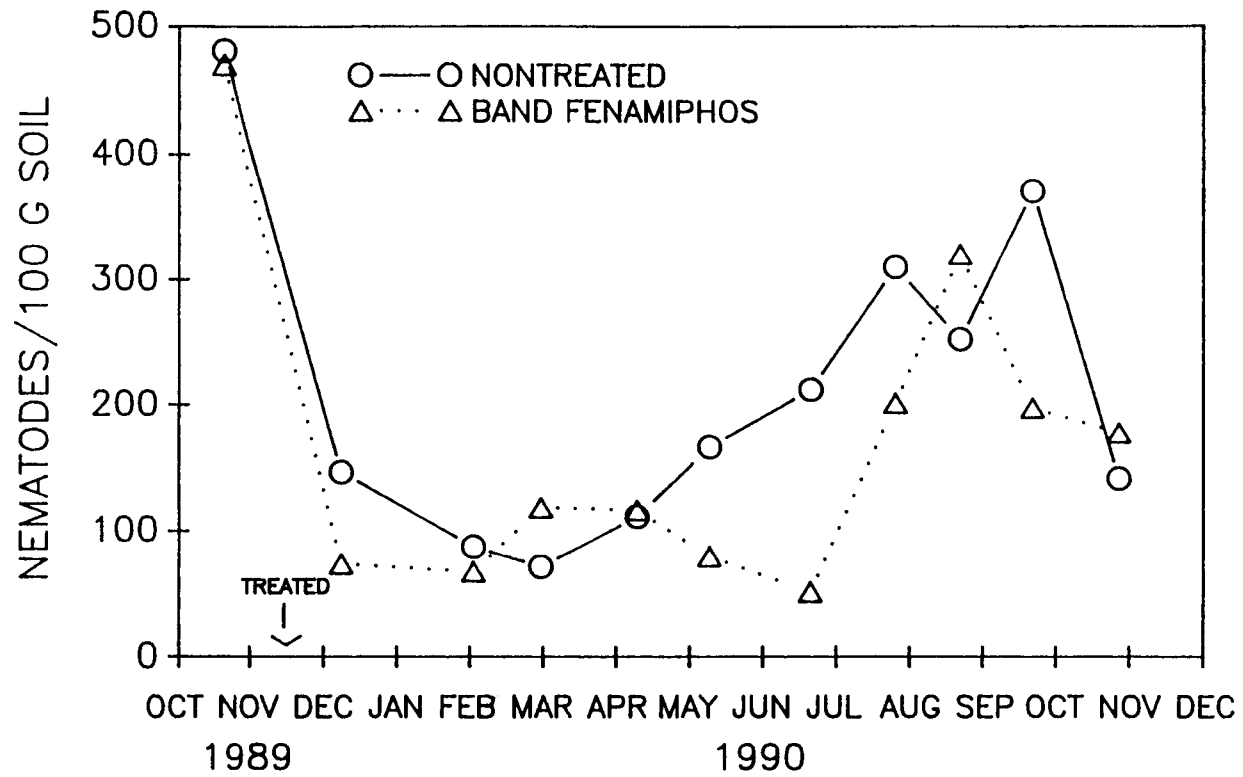


Figure 3. Soil population dynamics of *Pratylenchus penetrans* in red raspberry from field 2, Scholls, OR.

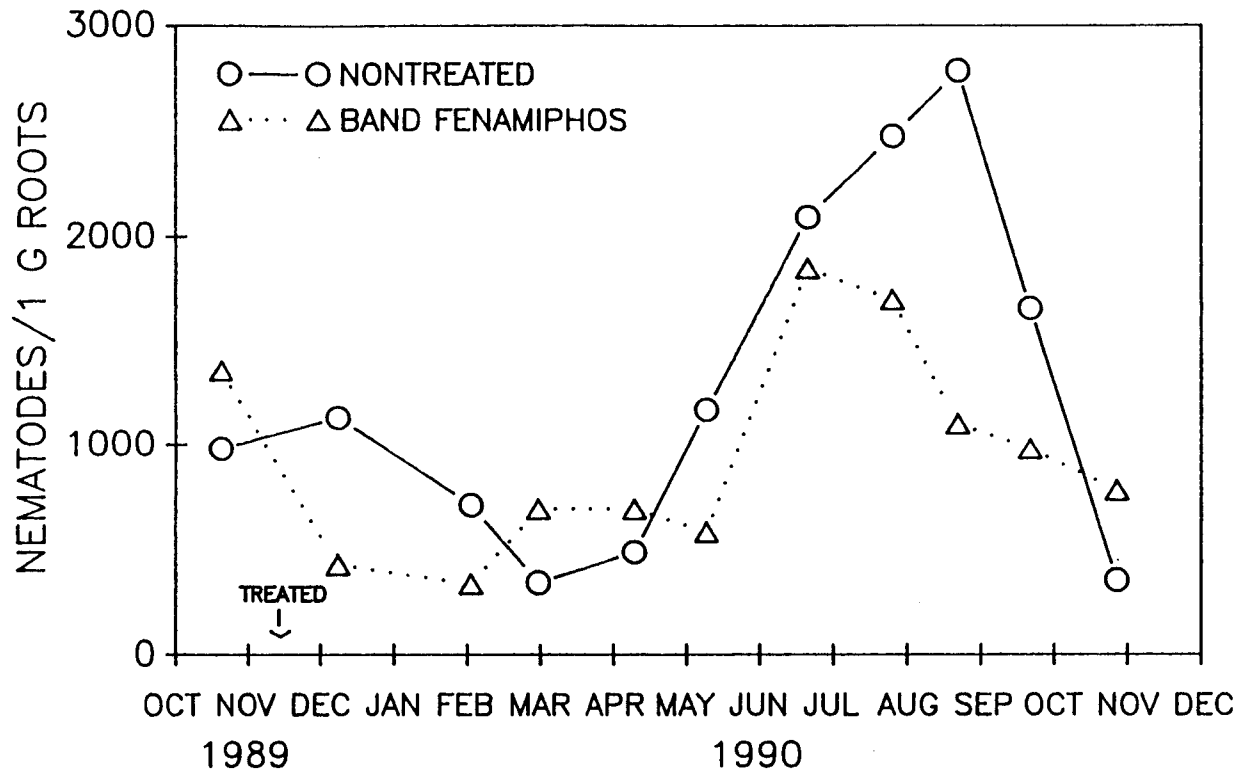


Figure 4. Root population dynamics of *Pratylenchus penetrans* in red raspberry from field 2, Scholls, OR.

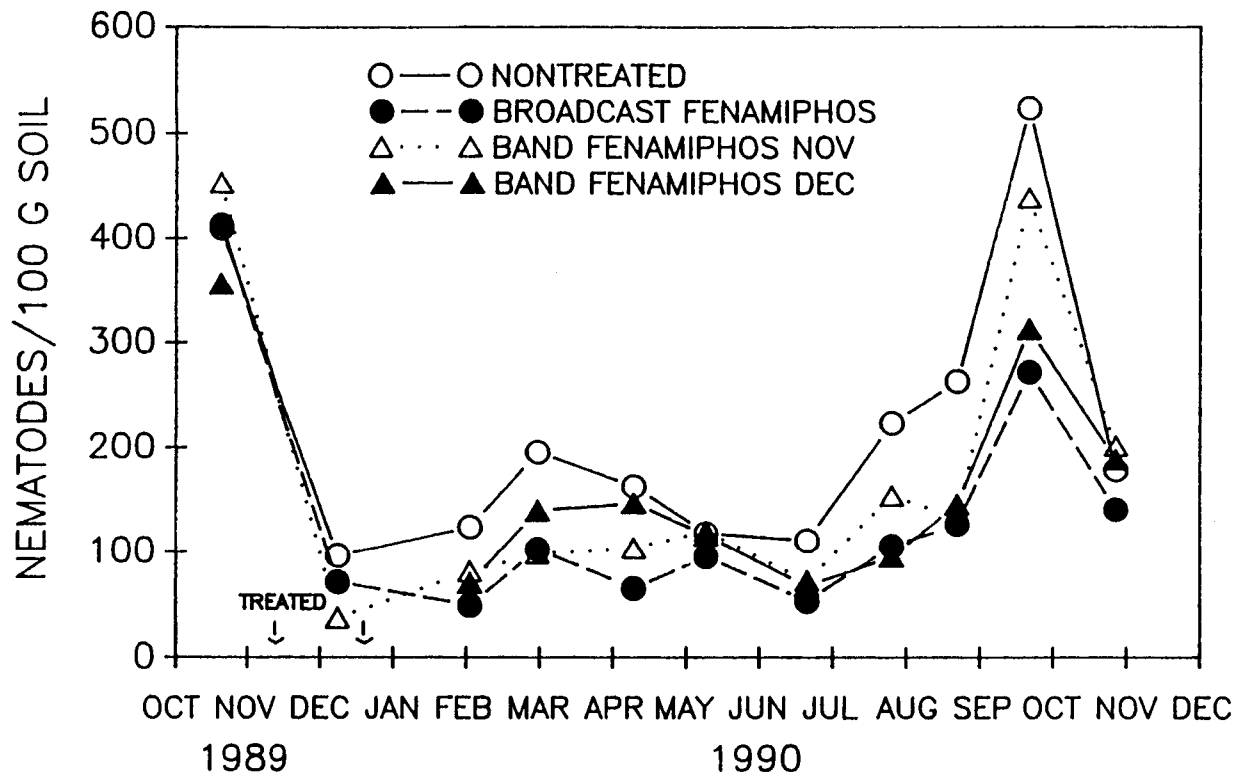


Figure 5. Soil population dynamics of *Pratylenchus penetrans* in red raspberry from field 3, Troutdale, OR.

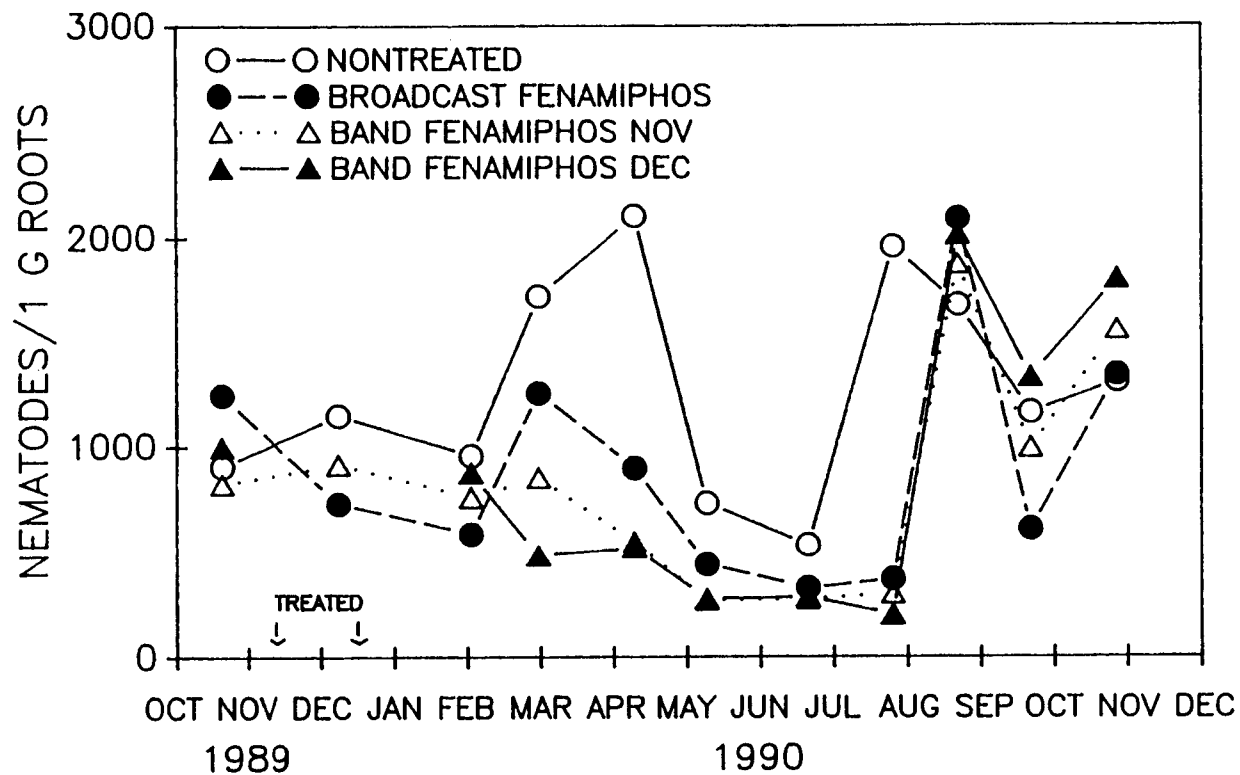


Figure 6. Root population dynamics of *Pratylenchus penetrans* in red raspberry from field 3, Troutdale, OR.

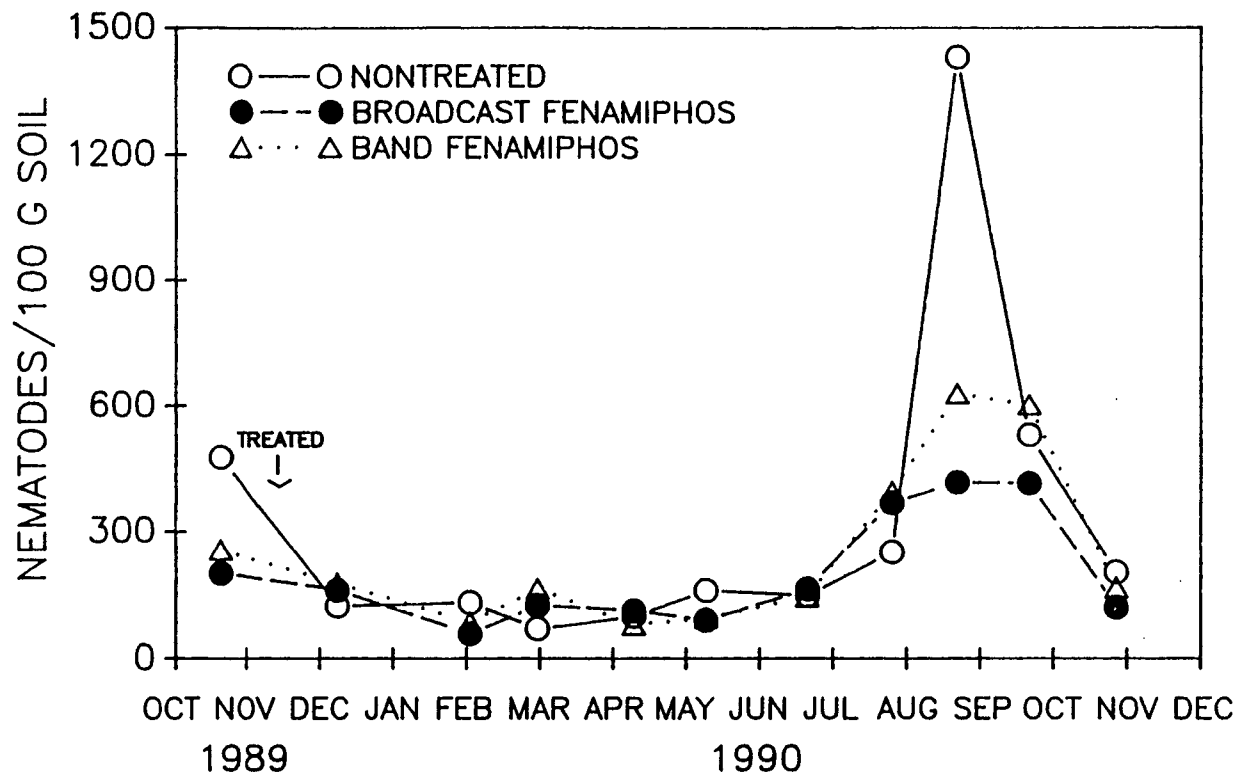


Figure 7. Soil population dynamics of *Pratylenchus penetrans* in red raspberry from clean tillage plots at field 4, Sandy, OR.

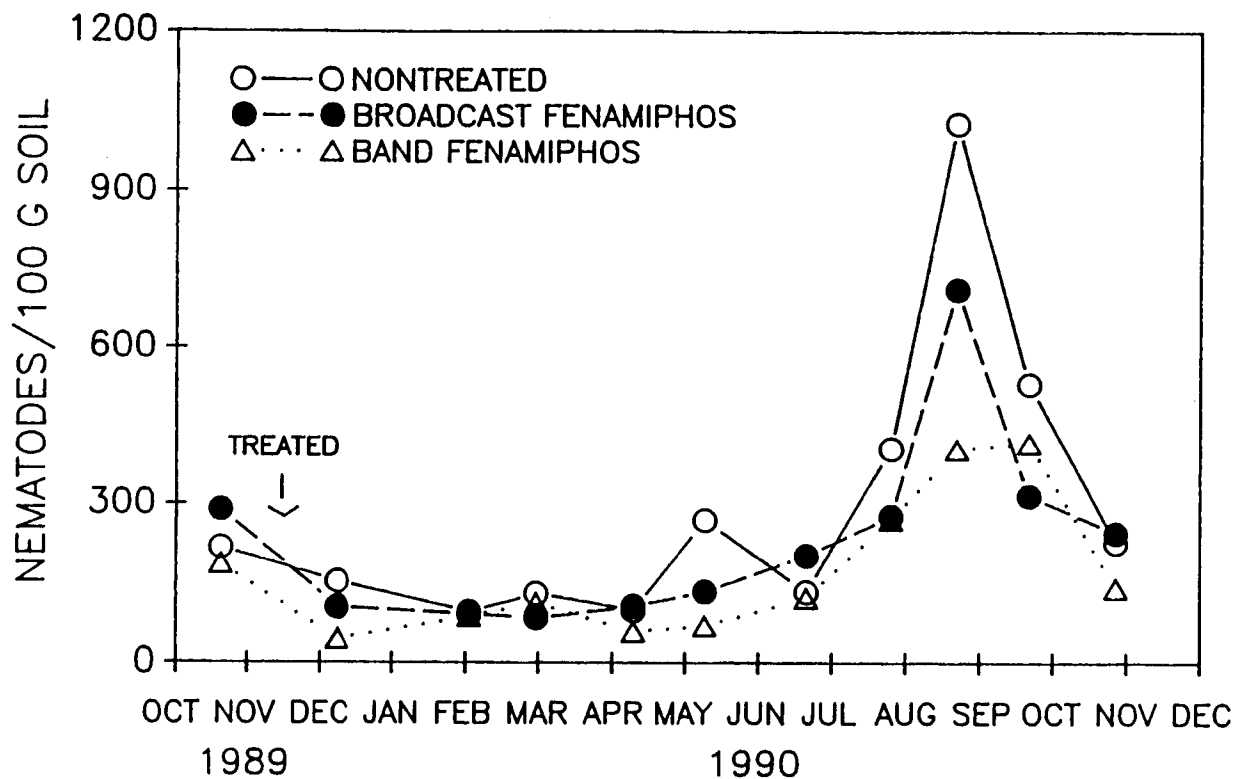


Figure 8. Soil population dynamics of *Pratylenchus penetrans* in red raspberry from weed and grass covered plots at field 4, Sandy, OR.

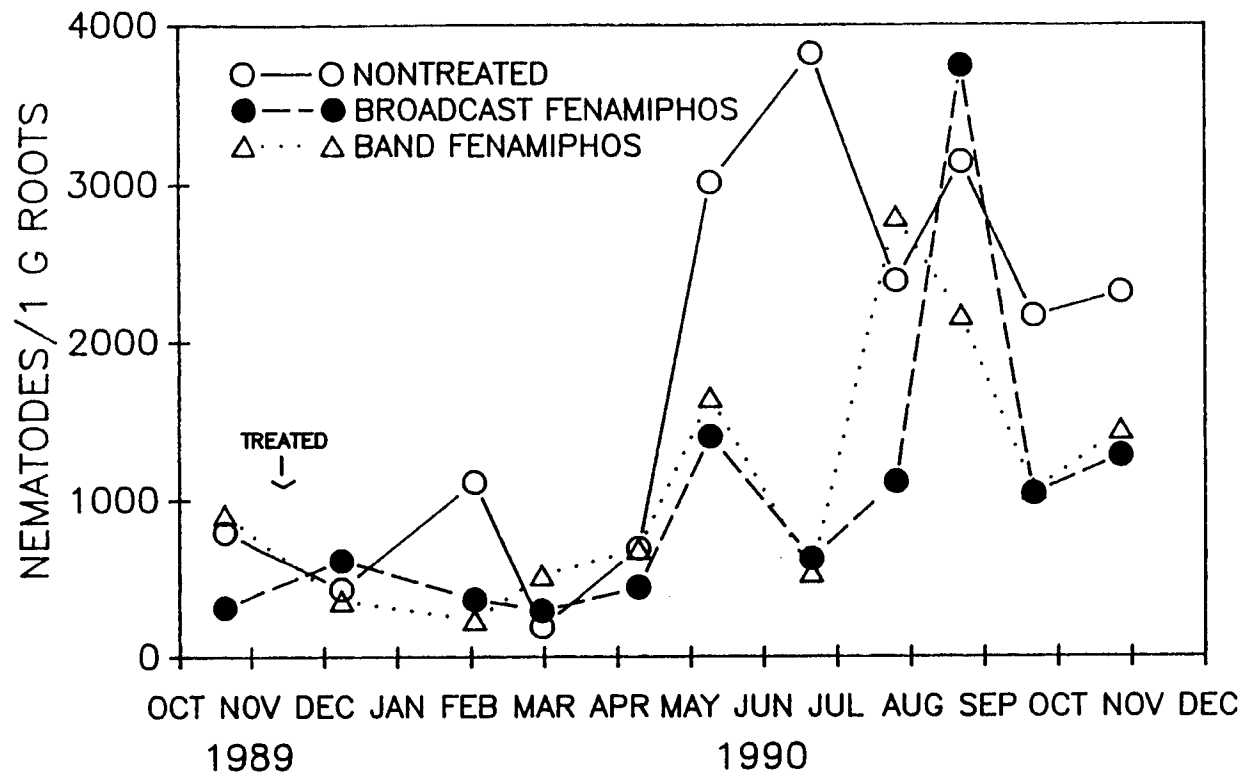


Figure 9. Root population dynamics of *Pratylenchus penetrans* in red raspberry from clean tillage plots at field 4, Sandy, OR.

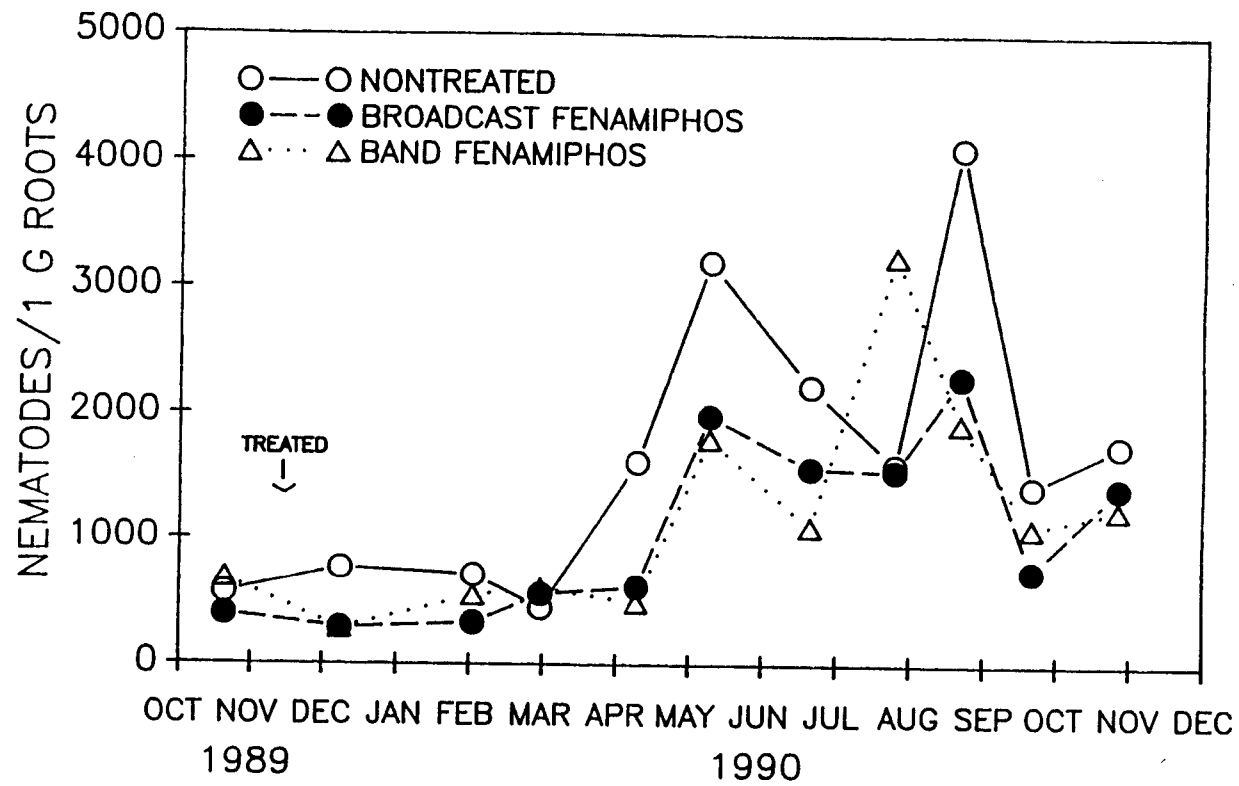


Figure 10. Root population dynamics of *Pratylenchus penetrans* in red raspberry from weed and grass covered plots at field 4, Sandy, OR.

Appendix II

Table 1. Effect of fenamiphos on *Pratylenchus penetrans* soil populations in red raspberry cv. Willamette at field 1, in Sandy, OR, 1989-1990 (N=5).

SAMPLING DATE	TREATMENTS		
	NONTREATED	BROADCAST	BAND
OCTOBER	222(47) ¹	306(83)	278(46)
DECEMBER	56(30)	72(20)	49(18)
JANUARY	41(9)	72(12)	58(18)
FEBRUARY	63(8)	61(8)	58(8)
APRIL	125(38)	111(13)	86(18)
MAY	122(31)	85(24)	67(19)
JUNE	129(36)	46(17)	67(23)
JULY	117(20)	47(14)	73(17)
AUGUST	128(57)	89(10)	62(22)
SEPTEMBER	168(39)	104(26)	147(26)
OCTOBER	107(36)	121(35)	113(32)

¹ The $\text{Log}_{10}(\text{number of } P. \text{ penetrans}/100 \text{ g soil} + 1)$ transformation was used for statistical analysis. Means (standard error) are presented.

cont. Appendix 2

Table 2. Effect of fenamiphos on *Pratylenchus penetrans* root populations in red raspberry cv. Willamette at field 1, Sandy, OR, 1989-1990 (N=5).

SAMPLING DATE	TREATMENTS		
	NONTREATED	BROADCAST	BAND
OCTOBER	678(107) ¹	977(387)	541(60)
DECEMBER	608(174)	307(80)	362(88)
JANUARY	608(155)	620(165)	758(216)
FEBRUARY	1174(422)	648(42)	754(286)
APRIL	677(50)	788(275)	1172(502)
MAY	1079(526)	1013(332)	602(242)
JUNE	2928(458)	765(242)*	1367(459)*
JULY	1004(245)	557(209)	637(209)
AUGUST	5567(35)	2480(616)	2342(684)
SEPTEMBER	382(92)	920(244)	1536(728)
OCTOBER	2107(588)	3498(1717)	1792(851)

¹ The $\log_{10}(\text{number of } P. \text{ penetrans/g root} + 1)$ transformation was used for statistical analysis. Means (standard error) are presented.

* Mean population density was significantly ($P < 0.05$) less than nontreated control, according to paired-t test, on that sample date.

cont. Appendix 2

Table 3. Effect of fenamiphos on *Pratylenchus penetrans* soil populations in red raspberry cv. Willamette at field 2, Scholls, OR, 1989-1990 (N=5).

SAMPLING DATE	TREATMENTS	
	NONTREATED	BAND
OCTOBER	481(109) ¹	470(74)
DECEMBER	146(46)	73(24)
JANUARY	87(20)	67(14)
FEBRUARY	71(29)	118(16)
APRIL	111(22)	116(29)
MAY	166(61)	79(10)
JUNE	212(62)	51(10)*
JULY	310(58)	201(14)
AUGUST	252(59)	320(140)
SEPTEMBER	371(99)	197(35)
OCTOBER	141(36)	177(81)

¹ The $\log_{10}(\text{number of } P. \text{ penetrans}/100 \text{ g soil} + 1)$ transformation was used for statistical analysis. Means (standard error) are presented.

* Mean population density was significantly ($P < 0.05$) less than nontreated control, according to paired-t test, on that sample date.

cont. Appendix 2

Table 4. Effect of fenamiphos on *Pratylenchus penetrans* root populations in red raspberry cv. Willamette at field 2, Scholls, OR, 1989-1990 (N=5).

SAMPLING DATE	TREATMENTS	
	NONTREATED	BAND
OCTOBER	978(213) ^{1,2}	1357(350)
DECEMBER	1128(440)	421(81)
JANUARY	707(374)	330(140)
FEBRUARY	337(23)	689(221)
APRIL	482(166)	688(265)
MAY	1166(372)	578(286)
JUNE	2092(439)	1847(590)
JULY	2479(516)	1698(560)
AUGUST	2788(755)	1096(331)
SEPTEMBER	1656(592)	975(269)
OCTOBER	349(81)	777(92)

¹ The $\log_{10}(\text{number of } P. \text{ penetrans/g root} + 1)$ transformation was used for statistical analysis. Means (standard error) are presented.

² Mean population density was not significantly different, according to paired-t test, between treatments on any sample date.

cont. Appendix 2

Table 5. Effect of fenamiphos on *Pratylenchus penetrans* soil populations in red raspberry cv. Willamette at field 3, Troutdale, OR, 1989-1990 (N=5).

SAMPLING DATE	TREATMENTS			
	NON TREATED	BROADCAST	BAND	
			NOVEMBER	DECEMBER
OCTOBER	409(55) ¹	413(103)	453(97)	356(82)
DECEMBER	96(38)	71(13)	37(12)	
JANUARY	123(35)	49(14)*	81(42)*	70(30)*
FEBRUARY	195(28)	102(19)*	98(36)	139(3)
APRIL	162(41)	65(16)	103(21)	146(26)
MAY	117(31)	95(29)	119(44)	115(32)
JUNE	110(36)	53(22)*	72(43)*	67(18)*
JULY	223(45)	105(31)*	153(54)	95(37)*
AUGUST	263(137)	126(54)*	132(60)*	145(53)
SEPTEMBER	524(135)	272(52)	439(161)	313(61)
OCTOBER	178(41)	140(35)	201(79)	188(75)

¹ The $\log_{10}(\text{number of } P. \text{ penetrans}/100 \text{ g soil} + 1)$ transformation was used for statistical analysis. Means (standard error) are presented.

* Mean population density was significantly ($P < 0.05$) less than nontreated control, according to paired-t test, on that sample date.

cont. Appendix 2

Table 6. Effect of fenamiphos on *Pratylenchus penetrans* root populations in red raspberry cv. Willamette at field 3, Troutdale, OR, 1989-1990 (N=5).

SAMPLING DATE	TREATMENTS			
	NON TREATED	BROADCAST	BAND	
			NOVEMBER	DECEMBER
OCTOBER	903(150) ¹	1243(635)	821(156)	996(372)
DECEMBER	1144(250)	728(146)	913(95)	
JANUARY	954(194)	583(211)	757(320)	874(240)
FEBRUARY	1719(462)	1252(196)	851(185)	479(147)*
APRIL	2103(577)	897(356)	543(165)*	517(163)*
MAY	729(373)	437(151)	267(95)	273(90)
JUNE	529(162)	325(76)	272(57)	282(48)
JULY	1956(1119)	367(117)	297(42)	199(74)*
AUGUST	1674(490)	2092(726)	1872(528)	2014(736)
SEPTEMBER	1158(221)	607(177)	991(457)	1326(366)
OCTOBER	1307(178)	1340(708)	1555(787)	1802(677)

¹ The $\log_{10}(\text{number of } P. \text{ penetrans/g root} + 1)$ transformation was used for statistical analysis. Means (standard error) are presented.

* Mean population density was significantly ($P < 0.05$) less than nontreated control, according to paired-t test, on that sample date.

cont. Appendix 2

Table 7. Effect of fenamiphos on *Pratylenchus penetrans* soil populations in red raspberry cv. Willamette with grass and weed ground cover at field 4, Sandy, OR, 1989-1990 (N=5).

SAMPLING DATE	TREATMENTS		
	NONTREATED	BROADCAST	BAND
OCTOBER	216(47) ¹	289(93)	185(23)
DECEMBER	152(28)	104(19)	44(8)*
JANUARY	95(29)	90(19)	86(26)
FEBRUARY	129(54)	84(12)	111(28)
APRIL	98(29)	106(54)	58(26)
MAY	268(134)	133(52)	69(17)
JUNE	132(44)	201(54)	121(41)
JULY	405(80)	276(41)	269(88)
AUGUST	1027(57)	710(235)	404(165)*
SEPTEMBER	529(141)	315(31)	416(72)
OCTOBER	226(63)	244(52)	140(25)

¹ The \log_{10} (number of *P. penetrans*/100 g soil + 1) transformation was used for statistical analysis. Means (standard error) are presented.

* Mean population density was significantly ($P < 0.05$) less than nontreated control, according to paired-t test, on that sample date.

cont. Appendix 2

Table 8. Effect of fenamiphos on *Pratylenchus penetrans* soil populations in red raspberry cv. Willamette without ground cover at field 4, Sandy, OR, 1989-1990 (N=5).

SAMPLING DATE	TREATMENTS		
	NONTREATED	BROADCAST	BAND
OCTOBER	476(147) ¹	200(42)	255(80)
DECEMBER	123(54)	160(39)	176(44)
JANUARY	131(46)	58(20)*	85(11)
FEBRUARY	70(12)	125(37)	163(35)
APRIL	99(25)	112(27)	79(20)
MAY	159(60)	90(15)	95(20)
JUNE	147(62)	163(46)	145(31)
JULY	250(50)	368(101)	395(43)
AUGUST	1431(358)	417(193)*	628(261)*
SEPTEMBER	529(37)	415(80)	601(221)
OCTOBER	202(50)	120(25)	165(43)

¹ The \log_{10} (number of *P. penetrans*/g root + 1) transformation was used for statistical analysis. Means (standard error) are presented.

* Mean population density was significantly ($P < 0.05$) less than nontreated control, according to paired-t test, on that sample date.

cont. Appendix 2

Table 9. Effect of fenamiphos on *Pratylenchus penetrans* root populations in red raspberry cv. Willamette with grass and weed ground cover at field 4, Sandy, OR, 1989-1990 (N=5).

SAMPLING DATE	TREATMENTS		
	NONTREATED	BROADCAST	BAND
OCTOBER	565(107) ¹	393(122)	679(152)
DECEMBER	760(295)	286(126)*	281(70)
JANUARY	704(188)	328(108)	543(244)
FEBRUARY	444(147)	556(285)	598(228)
APRIL	1612(439)	612(268)	486(178)
MAY	3199(1467)	1982(856)	1797(832)
JUNE	2218(847)	1566(536)	1095(464)
JULY	1591(713)	1544(561)	3249(690)
AUGUST	4106(823)	2293(700)	1922(402)*
SEPTEMBER	1419(241)	741(181)*	1088(253)
OCTOBER	1750(384)	1416(593)	1240(374)

¹ The $\log_{10}(\text{number of } P. \text{ penetrans/g root} + 1)$ transformation was used for statistical analysis. Means (standard error) are presented.

* Mean population density was significantly ($P < 0.05$) less than nontreated control, according to paired-t test, on that sample date.

cont. Appendix 2

Table 10. Effect of fenamiphos on *Pratylenchus penetrans* root populations in red raspberry cv. Willamette without ground cover at field 4, Sandy, OR, 1989-1990 (N=5).

SAMPLING DATE	TREATMENTS		
	NONTREATED	BROADCAST	BAND
OCTOBER	793(208) ¹	317(107)	908(658)
DECEMBER	431(115)	615(248)	359(68)
JANUARY	1113(530)	367(106)	236(55)
FEBRUARY	193(62)	295(86)	520(171)
APRIL	691(341)	444(130)	687(304)
MAY	3014(920)	1402(330)	1648(699)
JUNE	3823(872)	624(236)*	533(278)*
JULY	2386(1069)	1115(369)	2795(1166)
AUGUST	3143(600)	3745(993)	2164(604)
SEPTEMBER	2163(373)	1040(212)*	1058(271)
OCTOBER	2315(475)	1280(452)*	1443(185)

¹ The $\log_{10}(\text{number of } P. \text{ penetrans/g root} + 1)$ transformation was used for statistical analysis. Means (standard error) are presented.

* Mean population density was significantly ($P < 0.05$) less than nontreated control, according to paired-t test, on that sample date.