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AN INVESTIGATION INTO CULTURAL CONTROL OF THE ROOT-LESION NEMATODE, *PRATYLENCHUS PENETRANS* FILIPJEV & SCH. STEKHOVEN, WITH SPECIAL REFERENCE TO APPLE REPLANT DISEASE.

by

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B.Sc. (Agr.), McGill University, 1984.

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ABSTRACT

The root-lesion nematode (*Pratylenchus penetrans*) is a migratory endoparasite with a broad host range. A recent survey of apple orchards in British Columbia revealed that soil population densities of *P. penetrans* commonly exceeded levels reported to be damaging to young trees.

Pre-plant fumigation and a systemic nematicide application, for non-bearing trees, are the only registered control methods. The cost, unpredictability of control and potential negative side effects of broad spectrum fumigants, combined with increasing health and environmental concerns, illustrate the need for research into alternative control measures.

The purpose of this study was to evaluate alternative field practices that could be used by orchardists to control *P. penetrans*. In the summer of 1989, two field trials were conducted. In the first trial five treatments were evaluated in a noncropped field: marigolds (*Tagetes patula*), oats (*Avena sativa* var. Cascade) and a sorghum-sudangrass hybrid (*Sorghum sudanese X Sorghum bicolor* var. Pioneer Hybrid var. 998) all sown as cover crops. A clear polyethylene film mulch and hand weeding served as additional treatments. Post-treatment soil population densities of *P. penetrans* were significantly greater ($P < 0.05$) in the sorghum-sudangrass and the oat plots than in the hand weeded, clear plastic, or *T. patula* plots.

In the second experiment, four treatments were established in an orchard of poorly growing young apple trees with high root population densities of *P. penetrans*. Treatments were: mulching with clear or black polyethylene, intercropping with *T. patula*,

resulted in a significantly higher ($P < 0.05$) tree root population density of *P. penetrans* than did *T. patula* or the weeded control.

A greenhouse experiment was conducted to further evaluate the potential for intercropping *T. patula* with apple trees. Three population densities of *P. penetrans* (0, 4000, 8000 per 1.5 l pot) and presence or absence of a *T. patula* plant were incorporated into a factorial experiment. *T. patula* resulted in a significantly ($P < 0.05$) lower population density of *P. penetrans* in the soil, but not in the roots of the apple seedlings. Both the *T. patula* alone and inoculum levels of 4000 and 8000 *P. penetrans* resulted in significantly ($P < 0.05$) lower apple seedling dry weight and stemlength, than the controls.

Recommendations for further work on management of *P. penetrans* in apple replant situations are presented.

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1. INTRODUCTION

The root-lesion nematode, *Pratylenchus penetrans* Filipjev & Sch. Stekoven, has a significant role in certain replant problems of apple trees. A recent survey of 58 orchards in the Okanagan Valley of British Columbia revealed that 76.4% of soil samples and 69.8% of root samples from 127 sites contained *P. penetrans* (Vrain and Yorston 1987). Forty-one per cent of soils from apple tree root zones had population densities greater than 50 *P. penetrans* per 50 ml. This population density at planting has been demonstrated to reduce tree growth (Hoestra and Oostenbrink 1962).

The currently recommended methods for control of the root-lesion nematode in British Columbia are either pre-plant fumigation or a systemic nematicide applied to non-bearing apples trees (Adams 1987). Concern over human health and environmental risks of pesticide use, cost of treatment, and uncertainties associated with fumigation efficacy point to the need for alternative management strategies for *P. penetrans*.

This study examines the role of *P. penetrans* as a pathogen of apple trees (*Malus domestica* Borkh). Literature relevant to the development of an Integrated Pest Management (IPM) program for *P. penetrans* in apple orchards is reviewed and the results of two field experiments and a greenhouse experiment which evaluate potential cultural controls of *P. penetrans* are presented.

2. LITERATURE REVIEW

2.1 APPLE REPLANT DISEASE

The damage caused by *P. penetrans* on young apple trees is often considered to be part of the Apple Replant Disease complex. "Replant disease" refers to the poor growth of deciduous fruit trees such as apples, peaches, pears and cherries replanted on former orchard sites (Jaffee et al. 1982a).

The classification of apple replant problems as either Specific Apple Replant Disease (SARD) or Non-Specific Apple Replant (NSARD) is based on the crop preceding the replanted apple trees which exhibit symptoms of the disease. With SARD, the preceding crop is the same or a closely related species to that currently grown. With NSARD, the preceding crop is not the same or closely related species (Traquair 1984). The root-lesion nematode is widely reported to be one of the causal agents of NSARD (Hoestra and Oostenbrink 1962, Parker and Mai 1974, Mai and Abawi 1978).

In a recent review article Traquair (1984) concluded that the categorization of replant problems as either specific or non-specific has little merit due to the difficulty in establishing the causal agents against a background of other factors. In my opinion, the term replant disease is perhaps more confusing than enlightening as it is a very broad term applied to a complex of problems rather than to a specific problem. Therefore, the remainder of this paper avoids the terminology "SARD" and "NSARD" and focuses strictly on those replant problems in apples associated with *P. penetrans*.

2.2 THE ROOT-LESION NEMATODE

2.2.1 Description

Pratylenchus penetrans Filipjev & Sch. Stekhoven was identified by Cobb in 1917, who described it as a plant parasite of potatoes, violets and other hosts. All five stages of this tylenchid nematode are vermiform. Males and females are morphologically similar, except for sexual characteristics (Mai et al. 1977).

Proper identification of *P. penetrans* is difficult. The genus *Pratylenchus* contains approximately 40 described species, and knowledge of the extent of intraspecific variability is often lacking. Mai et al. (1977) observed that morphological characteristics among the offspring of a single female were so varied that some individuals possessed characters which overlapped with five described species. This report questions the validity of the use of certain characteristics previously used to identify *P. penetrans*.

2.2.2 Host range

Nearly 400 hosts of *P. penetrans* have been described from six continents (Corbett 1973, as cited by Mai et al. 1977). These include many valuable economic crops such as cereals, forages, fruit trees as well as a broad range of weed species (Townshend and Davidson 1960).

There are two reports of the occurence of different strains or pathotypes of *P. penetrans*. Olthof (1968) reported two strains of *P. penetrans* which were differentiated by their pathogenicity and reproductive potential on tobacco. Zepp and Szczygiel (1986) presented evidence suggesting the existence of different pathotypes of *P. penetrans* infecting temperate fruit trees. The existence of different pathotypes may be an important consideration in the evaluation of host plants, which will be considered later in this paper.

2.2.3 Life cycle and dispersal

P. penetrans is considered to be a migratory endoparasite, indicating that it moves freely in the soil, and feeds within the root tissues. Females lay eggs in clusters, primarily in the cortical tissue of roots. Males are required for fertilization. Apparently, all juvenile and adult stages are infective (Mai et al. 1977). However, adults and fourth stage larvae travel further (Jones and Mai 1964) and are found in greater proportions outside the roots (McGuidwin 1989) than earlier instars.

The duration of the life cycle of *P. penetrans* ranged from 30 days at 30° C to 92 days at 15° C on alfalfa (Mai et al. 1977). Mamiya (1971) reported developmental cycles of *P. penetrans* of between 30-31 days at 30° C and 86 days at 15° C in seedling roots of the conifer *Cryptomeria japonica* D. Don. However, at 33° C, *P. penetrans* development was inhibited, and eggs layed never reached advanced stages of development.

In laboratory studies, at temperatures of -12° C and -8° C resulted in > 90% mortality of *P. penetrans* in soil after 4 hours. At -4° C, 3.5 days were required for 50% mortality of *P. penetrans*. In the field , winter soil temperatures averaging between -1.1° to -0.8° in each of four different years resulted in 35% to 59% reduction in the population density of *P. penetrans* in the top 15 cm of soil. There was no change in the soil population density of the nematode at 15 cm to 30 cm, where freezing did not occur. In the fifth year of study, where the average soil temperature in winter did not go below 0° C, there was no decrease in the soil population over the winter (Kimpinski and Dunn 1985).

The population dynamics of *P. penetrans* in orchards has not been studied in detail but information from other host plants is pertinent. McGuidwin (1989) compared the relative distribution of *Pratylenchus scribneri* among the roots and soil habitats in corn (*Zea mays*) and potatoes (*Solanum tuberosum*) before, during and after the growing season. Results indicated that the

percentage of the total population recovered from the soil was about 20% during the growing season, and averaged about 50% at the beginning and the end of the growing season.

Colbran (1954) reported that root-lesion nematodes in soil from an apple orchard did not survive for more than 9 months in the absence of a host plant. McGuidwin (1989) reported that 50% of *P. scribneri* were found to overwinter in dead roots, and emphasized that this aspect should be taken into consideration when sampling.

The root-lesion nematodes primary means of dispersion is migration through the soil from root to root. They can be transported to new sites by the movement of soil adhering to cultivation equipment or workers boots, infected plants and occasionally by floodwater (Brown 1982).

2.3 HOST-PEST-ENVIRONMENT INTERACTIONS

2.3.1 Etiology and symptoms

Pitcher et al. (1960) determined that *P. penetrans* was a primary pathogen of apple trees since the nematode was able to invade, feed on and reproduce in apple feeder roots in the absence of all other potential pathogens.

The symptoms of damage due to *P. penetrans* in the field are stunting of trees and retarded shoot growth (Hoestra and Oostenbrink 1962). The stunting associated with *P. penetrans* infection is usually unevenly distributed throughout the orchard. These symptoms are not diagnostic. Visual symptoms on the feeder roots are necrotic lesions, dead rootlets, overall discolouration of the root system, and small root systems (Mai 1960). More specifically, Pitcher et al. (1960) described the symptoms of *P. penetrans* on apple seedlings as discoloration and necrosis of the epidermal and endodermal tissue in the feeder roots. Apple seedlings sustained *P. penetrans* in the root cortex for periods of a month or more without serious damage. Alternatively, peach seedlings showed more severe symptoms within a few hours of invasion (Mountain and Patrick 1959).

Apple and peach roots contain phenolic compounds which are broken down by enzymes secreted by the nematode to produce phytotoxic compounds and root discoloration. These phenolic compounds accumulate to high concentrations in peach roots, resulting in rapid and extensive necrosis and discoloration of damaged roots. In apple roots, phenolics are found primarily in the dermal and endodermal cells corresponding to the observed sites of root discoloration (Pitcher et al. 1960).

Merwin and Stiles (1989) conducted foliar nutrient analysis and showed that all nutrients were lower in apple seedlings grown in a nematode replant soil than those grown in the same soil after steam pasteurization. They concluded that impaired nutrient uptake was an important factor in this replant problem.

Physiological disorders observed in other plant species associated with *P. penetrans* infection include: loss of root cation exchange capacity, increased water tension in the stems and leaves, increased water loss from the leaves, a decrease in phosphorus content, and an increase in free phenols in the leaves (Mai et al. 1977). Evidence suggests that *P. penetrans* may reduce cold tolerance in cherries (Edgerton and Parker 1958).

2.3.2 Environmental factors

Biotic and abiotic factors effect the nematode population, its distribution and/or the manifestation of disease symptoms.

2.3.2.1 Associated pathogens

Interactions between nematodes and other pathogens is a common phenomenon (Powell, 1971). It has been demonstrated that *Pratylenchus* spp. interact synergistically with *Verticillium* spp. and *Trichoderma* spp. in certain crops (Vrain 1987, Powell 1971), and that *P. penetrans* predisposes other host species to bacterial infections (Vrain and Copeman 1987).

In apple seedlings, Pitcher et al. (1960) observed little injury in response to infection with surface aseptic nematodes over several months. This suggests that in apple orchards, other organisms may be involved in the manifestation of the disease symptoms. Further evidence to support this contention is the differential response of apple trees to broad spectrum and nematicidal fumigants. Jaffee et al. (1982a, 1982b) isolated pathogens in addition to *P. penetrans* from lesions on apple roots, which were found to cause additive rather than synergistic effects.

Associations of species of phytopathogenic nematodes with *P. penetrans* are common (Mai et al. 1977). However, *P. penetrans* is the only species believed to play a significant role in apple replant problems (Mai and Abawi 1978).

2.3.2.2 Apple rootstocks

Hoestra and Oostenbrink (1962) suggested that differential susceptibility to nematode damage may exist in different rootstock - scion combinations. Commercial apple trees are produced by grafting the budwood or "scion" of a desirable cultivar onto a root system or "rootstock" which imparts certain growth characteristics to the tree. The Malling (M) rootstocks, named after the East Malling Research Station in England where they were developed, are used extensively in British Columbia orchard production. Hoestra and Oostenbrink (1962) reported that seven Malling rootstocks tested supported high levels of *P. penetrans*. However, the extent of reduction in height and branch growth between inoculated and uninoculated rootstocks varied. For example in Malling four (M IV), growth was reduced by 51% compared to M VII in which growth was reduced by 11%. The authors suggested that this difference was due to tolerance in the M VII, but their data lacks a measure of variance or test of significance. Their observation has been supported also by empirical observations: Parker and Mai (1974) concurred that M that the response of VII was less to soil fumigation with 1,3-dichloropropene-dichloropropane than many of the other Malling

rootstocks. They observed that deeply rooted root-stocks appeared to respond less to fumigation and suggested that this relationship may be the cause of the apparent tolerance of some of rootstocks.

Extensive screening or breeding for rootstock resistance to *P. penetrans* has not been undertaken. Host resistance occurs most frequently where there is a complex host-parasite interaction, such as those between sedentary endoparasites like *Meloidogyne* and *Heterodera* and their hosts (Howard and Cotten 1982). The parasite-host interaction between *P. penetrans* and its host is less specialized which could account for the relatively few reports of resistance.

2.3.2.3 Soil moisture

Jaffee and Mai (1979b) compared growth of rootstocks inoculated with *P. penetrans* to uninoculated at water tensions of 40 kPa and 1014 kPa . They observed reductions in growth due to each of the factors individually, but there was no interaction between them. Mai et al. (1977) reported the rate of *P. penetrans* population growth was greatest at a moderate range of soil moisture tensions (between 10 to 100 kPa) and was least at very low or very high water tensions (Mai et al. 1977).

2.3.2.4 Soil types

Hoestra and Oostenbrink (1962) observed *P. penetrans* to be more prevalent in sandy soils than in clay soils. Mai and Abawi (1981) also reported that higher populations of *P. penetrans* were found in coarser soils. The fact that pore diameter restricts nematode movement through soil (Jones 1982), could account for the observations reported above. Winoto Suatmadji (1969) suggested that a heavier soil might interfere with a hypothesized host detection mechanism of *P. penetrans*, such as a CO_2 gradient. His experimentation failed to provide evidence for the existence of such a mechanism.

2.3.2.5 Cultural practices

The effects of cultural practices on *P. penetrans* will be discussed in the subsection of Integrated Pest Management dealing with cultural controls (See Section 2.4.4).

2.4 INTEGRATED PEST MANAGEMENT

"Integrated Pest Management (IPM) is an interdisciplinary science dealing with the development, evaluation, and implementation of pest control strategies that result in favorable economic, ecological and sociological consequences" (Bird 1980). The main components of the decision process in nematode IPM are the value of the predicted damage and the cost of the management alternative. To this end, Ferris (1980) argues that the relative immobility of nematodes is advantageous for the development of predictive models.

The fundamental relationship between plant parasitic nematodes and the growth and yield of perennial plants is a function of pre-plant nematode population densities (Barker and Olthof, 1976). Although accidental re-introduction and buildup of initial undetectable nematode populations must be considered, newly planted trees appear to be more easily damaged and have a greater susceptibility to infection. Rackham et al. (1975) reported that six years after preplant fumigation with 1,3,-dichloropropene, *P. penetrans* population densities in apple roots were similar in the treated and unfumigated plots on the same site. However, 11 years after treatment, the trees in the control plots were only a third the size of those in the fumigated plots. Cameron et al. (1986) presented evidence suggesting that equivalent root population densities of *P. penetrans* in pears are more damaging to newly planted trees than to older established trees. In greenhouse trials, Jaffee and Mai (1979a) observed that the younger the apple seedlings at time of inoculation, the greater the infection by and reproduction of *P. penetrans* in the root system.

Characterization of nematode-host interactions for the development of an effective IPM strategy is dependent on (Barker and Olthof 1976) :

1) precise measurements of host response;

2) reliable methods of monitoring the nematode population;

3) considerations of environmental factors.

The first two factors will be examined with regard to the host-parasite relationship of the apple-lesion nematode; environmental factors have already been discussed to some extent in section 2.3.2.

2.4.1 Measurement of host response

Hoestra and Oostenbrink (1962) demonstrated, through linear regression of plant growth against the log of pre-plant population densities of *P. penetrans*, a highly significant negative correlation between shoot growth and nematode densities in two different experimental fields with four varieties of apple. Heavy nematode infestation (130-330 nematodes per 100 ml of soil) reduced shoot growth by greater than 50%. Extrapolation to zero *P. penetrans* population density (no plant response) suggests significant losses at even a relatively low level of 33 *P. penetrans* per 100 ml of soil.

Seinhorst (1965) argued that the extrapolation of plant response from a high range of nematode densities to zero could easily lead to an overestimation of damage at lower densities. He indicated that two phenomena invalidated extrapolation to zero. First, plants may have more roots than are needed to support the amount of shoots they produce; therefore, not all root tissue may be of equal importance. Second, plants have the ability to replace lost roots. These two phenomena contribute to a tolerance level below which detectable damage would not occur. Seinhorst (1965) argued that alternative exponential model, based on the theoretical considerations presented above

is more appropriate than a linear regression model. In a later paper, Oostenbrink (1972) defended the use of the linear regression model as a predictive tool, giving numerous empirical examples of data which fit the model. He defended his contention that extrapolation to zero is valid, by saying that a horizontal curve must theoretically precede the sloping regression to account for the plant's ability to sustain or compensate for a light infection. Ferris (1980) argues that validity and predictability are the important qualities of a model to be used for pest management decisions.

In summary, the tolerance of apples to the root lesion nematode has not been accurately determined. With the exception of Hoestra and Oostenbrink (1962), the response of apple trees to *P. penetrans* has only been demonstrated indirectly through the use of nematicides, or from trials conducted in greenhouses.

A full discussion of the relationship between nematode population densities and crop responses is given in a review by Barker and Olthof (1976) and point out that data from greenhouse trials have poor transferability to the field.

2.4.2 Monitoring of nematode populations

The horizontal distribution pattern of nematodes is generally patchy (Goodell and Ferris 1980, McSorley and Parrado 1982, Barker 1985). The patchy distribution is attributed to numerous factors including unevenly occurring environmental factors (e.g. hosts, roots, soil type) and the tendency for some species to aggregate independently of environmental factors (Elliot 1971). The vertical distribution of nematodes varies greatly depending upon crop, nematode species and soil type (Brodie 1976).

The clumped distribution of phytoparasitic nematodes in the soil is not a random, normal distribution but a contagious distribution. There are numerous mathematical models which can be fitted to contagious distributions. The populations are characterized by a variance to mean ratio of greater than one

(Elliot 1971). Hence, the variance and sampling error increase with population density (Ferris 1984). The distribution which is most commonly applied and is perhaps the most useful for nematode counts is the negative binomial distribution (Proctor and Marks 1974, Goodell and Ferris 1980, McSorley and Parrado 1982). Noe and Campbell (1985) questioned certain assumptions of the negative binomial distribution and investigated several more direct analysis of spatial distribution in an attempt to define more efficient sampling plans.

Soil sampling is commonly done using a cylindrical core sampler. Due to the distribution of nematodes and the time required to process each sample, several composite samples are taken at random per unit area. This gives data from which it is impossible to separate sampling from biological variance (Southwood 1978), unless numerous bulk samples are taken from the same area. There are numerous mathematical models from which the number of cores required for a given level of precision can be estimated (Karindinos 1976, Southwood 1978). Use of these models requires estimates of the mean and a parameter of the negative binomial distribution ($\underline{k}$) which is a measure of clumping. Elliot (1971) suggests that a sample size of greater than 50 observations is required for an accurate estimate of $\underline{k}$.

For advisory purposes, a bulked sample of twenty 2.5 cm diameter cores taken to a depth of 20 cm is generally recommended for an area no larger than 2 hectares (Goodell and Ferris 1981). Proctor and Marks (1974) demonstrated that for a plot of 100 m^2, estimates of *P. penetrans* densities, based on one subsample of a 20 core sample, were of very low precision. They concluded that to achieve a precision such that 95% of the possible estimates of nematode density per 0.01 ha plot lay within 20% of the true mean density, this would require five subsamples from each of five 40 core samples per plot, for a total time expenditure of about 7 hours. Goodell and Ferris (1981) examined sampling optimization for advisory purposes on

five nematode species and concluded that for *Pratylenchus minyus* the recommended advisory sampling program would give an estimate within 15% of the true mean. The differences in the species of *Pratylenchus* under investigation and the methodology used contribute to the widely divergent evaluations of the 20 core samples recommended for advisory purposes.

The wide differences in the estimated errors reported above illustrate Barker's (1985) contention that more investigation into the inherent error in sampling and extraction techniques is required.

2.4.3 Chemical control

The chemicals currently available in Canada for control of *P. penetrans* are preplant fumigants or a systemic nematicide for use on non-bearing apple trees (Adams 1987). The fumigants belong to the two pesticide groups listed below (Hague and Gowen 1987), registered fumigants are listed after their pesticide grouping: 1) *Halogenated aliphatic hydrocarbons* - Methyl bromide, 1,3-dichloropropene (1,3-D) mixtures (Telone II), chloropicrin. 2) *Methyl isothiocyanate* (MIT) precursor compounds - dazomet (Basamid) and metham sodium (Vapam) - and methyl isothiocyanate mixture (MIT) and 1,3-D (Vorlex). The systemic nematicide registered in Canada is oxamyl from the Oxime-carbamate group (Hague and Gowen 1987). Details on the specifics of application and efficacy of these different compounds are reviewed in numerous articles (Van Berkum and Hoestra 1979, Adams 1987, Hague and Gowen 1987, Vrain 1987), A general discussion of how fumigation works and the factors effecting successful fumigation follows.

2.4.3.1 Fumigation

Fumigants are introduced into the soil, where they are quickly transformed into a gas. The gas moves through the air spaces in the soil and dissolves into the film of water surrounding the soil particles where nematodes and other

microorganisms are found. The nematicidal effect of the fumigant is dependent upon an "effective dose" which is a concentration x time product (Hague and Gowen 1987). Factors affecting the efficacy of fumigants are (Van Berkum and Hoestra 1979, Hague and Gowen 1987, Vrain 1988): 1) composition and structure of the soil; 2) soil temperature; 3) soil moisture content; 4) physical properties of the fumigant.

In general, loosely-structured soils enable easy diffusion of the fumigant gases and, as a result, may require tarping. It has been demonstrated that diffusion of gas through the soil is slower in peaty soils, and in wet, heavier soils (Leistra 1972 and 1973, cited by Van Berkum and Hoestra 1979). Higher rates of fumigant may be required in soils with a high organic matter content as the fumigant is bound to the organic matter. The percentage of blocked pore spaces and the moisture holding capacity is usually higher in fine textured, clay soils (Van Berkum and Hoestra 1979).

Soil temperature will effect diffusion of the fumigant through the soil. If the temperature is too high, the fumigant vapour will disperse too rapidly. If the temperature is too low, diffusion is too slow and the optimal concentration x time products will not be reached. The optimum temperature for fumigation is between 10° C and 20° C (Van Berkum and Hoestra 1979).

The moisture content of the soil is critical for successful soil fumigation. Too low water content may result in excessive diffusion and escape of the fumigant prior to achieving lethal dosages. Conversly, excessive soil moisture will result in poor diffusion, due to vapours dissolving in water. This results in uneven fumigant distribution throughout the soil (Van Berkum and Hoestra 1979, Hague and Gowen 1987, Vrain 1988).

The physical properties of the fumigant contribute to the efficacy of fumigation. Each chemical has a constant ratio of fumigant concentration in soil water to fumigant concentration

in soil air at a given temperature. For example, the weight ratio water : air for 1,3-D at 20° C is 18, whereas under the same conditions the ratio for MIT is 92, five times that of 1,3-D. Therefore, MIT requires a higher volume fraction of air filled pores to diffuse as successfully as 1,3-D. (Van Berkum and Hoestra 1979).

The factors presented above illustrate the need for care and sophistication for achieving success in fumigant applications. The soil structure, moisture and temperature parameters define a narrow window of timing for successful application.

A distinction is made in the literature between "Multipurpose fumigants" or "broad spectrum biocides" and "True nematicides" or "nematicidal fumigants"(Van Berkum and Hoestra 1979, Mai and Abawi 1981, Hague and Gowen 1987). Fumigants in the first category include methyl bromide, dazomet, metham sodium and the MIT 1,3-D compound Vorlex. The activity of fumigant nematicides is more restricted than that of the broad-spectrum fumigants. However, 1,3-dichloropropene at high rates of application are fungicidal. Fungicidal activity has been reported against *Sclerotium rolfsii*, some *Fusarium* species and species of *Phytophthora* (Rodriguez-Kabana and Curl 1980). Several workers have observed a greater growth response of fruit trees to broad spectrum fumigants than to fumigant nematicides (Mai and Abawi 1981, Jensen and Buszard 1988). This provides additional support to the concept of *P. penetrans* being only one of several organisms involved in the apple disease complex.

Several workers have used economic studies of the plant response to nematicidal fumigants as a justification for control. Arneson and Mai (1976) determined that preplant fumigation with Vorlex was cost effective within a two-year period. Mai and Abawi (1981) cite two examples where the return on investment over seven and nine-year periods was positive. Contrary to these reports, Cameron et al. (1986), working in pear orchards, determined that despite yield increases over a

10-year period in response to fumigation, the monetary return barely paid for the cost of fumigation. The problem with studies such as these is that the economic values used are often relevant only to the particular time and place where they were conducted and, therefore, can not be readily extrapolated to other situations.

2.4.3.2 Disadvantages of chemical use

There are several disadvantages to the use of soil fumigants. All of the fumigants reported here have a moderate to high mammalian toxicity (Adams 1987). This factor combined with their volatility can make them very hazardous to the applicator. Methyl bromide is an extreme example because at ambient temperatures it is a colourless, odourless gas that is highly toxic to humans. Both methyl bromide and methyl isothiocyanate may remain in the soil (Hague and Gowen 1987) and residues are found in ground water (Vrain 1987).

Certain potential draw-backs to the use of fumigants have been reported in crops other than apple and are of concern. It has been demonstrated that the use of broad spectrum fumigants in citrus orchards can result in stunting and chlorosis in the newly planted trees because of inadequate nutrition brought about by the death of mycorrhizal fungi (Kleinschmidt and Gerdemann, 1972).

Hussey and Roncadori (1982) cited two studies, one conducted in cotton and the other in rough lemon where the simultaneous presence of vesicular-arbuscular (VA) mycorrhizal fungi and migratory endoparasitic nematodes resulted in improved growth of the host. In cotton, the VA mycorrhizae reduced the number of nematodes in the host plant, possibly by making the host less suitable for the parasite. In rough lemon, the beneficial effects were due to the presence of VA mycorrhizae greatly outweighing the growth retardation attributed to the nematode, without reducing the nematode population in the roots.

Fumigation can be expensive. In British Columbia, the cost per hectare of fumigation ranged between $ 620 for Telone II B and $4000 for a methyl bromide-chloropicrin mixture in 1988 (Vrain 1988).

2.4.4 Cultural control

According to Brown (1982) cultural methods are "attempts to adapt husbandry practices so as to minimize losses due to nematodes." He divides cultural control of nematodes into four categories: 1) crop rotation 2) prevention of spread; 3) selection of healthy propagating materials; and 4) the influence of manuring. This categorization fails to identify other cultural methods, such as intercropping, fallow, and the use of plastic mulches which were examined experimentally in this study. Crop rotation, intercropping, fallow and the plastic mulches will be examined in some detail later.

The ubiquitous presence of *P. penetrans* in the soils of temperate regions makes prevention of spread within an area difficult. If fumigation is used to reduce nematode populations in soil, care should be used to reduce the transfer of soil in from other fields; however, this is considered almost impossible and certainly impractical with farm machinery (Brown 1982).

The selection of uninfested fruit tree rootstocks in Canada is difficult since there are no regulations for nurseries to keep their rootstocks free of *P. penetrans*. Selection of vigorously growing replant trees is a recommended practice.

2.4.5 Crop rotation

The criteria by which the success of crop rotation schemes can be measured were developed in the early part of this century (Bessy 1911 as cited by Nausbaum and Ferris 1973) and are still considered valid today. The crop rotation must: 1) prevent development and reproduction of the parasite; 2) at least pay for the expense of working the land, as well as rent, taxes, etc.; 3) enrich the land or at least not impoverish it; and 4)

make such vigorous growth as to choke out susceptible weed hosts.

Considerable work has been done on potential orchard cover crops and their status as hosts of *P. penetrans* . This section will consider crops with potential for use in orchards either as intercrops or in rotations in two parts: i) grass crops and ii) marigolds (*Tagetes* spp.) and other compositae.

2.4.5.1 Grasses

Table 2.1 summarizes the status of numerous temperate or semi-tropical crops as hosts of *P. penetrans*. The ratings given in the table are based upon comparisons among species evaluated by each researcher. Due to different experimental conditions, establishment of a common rating system was impossible. Nevertheless, plants rated with (*) indicate they support low levels of *P. penetrans* appear to have promise as cover or intercrops. These include some cultivars of oats (*Avena sativa*), tall fescue (*Festuca arundinacea*), creeping red fescue (*Festuca rubra*), sudangrass (*Sorghum vulgare*) and sorghum - sudangrass hybrids (*Sorghum sudanese* x *Sorghum bicolor*).

Table 2.1 also reveals conflicting reports on the host status of a number of species. Oats (*Avena sativa*), for example, have conflicting evaluations which can be explained by three factors: 1) the use of cultivars with varying levels of resistance, e.g. "Saia " oats appear to have greater resistance than most oat cultivars; 2) possible presence of different pathotypes of *P. penetrans*; and 3) the use of different experimental procedures including inoculation, extraction and different growing conditions. Townshend et al. (1984) reported wide discrepancies in the tolerance of species to *P. penetrans* between evaluations conducted under greenhouse and field conditions.

Table 2.1 Some of the plant species reported as potential cover crops for use in *Pratylenchus penetrans* management.

Species	Rating	Reference
1. Oats		
Avena sativa	***	MacDonald and Mai(1963)
(cv. Saia)	*	Colbran (1979)
(cv. Saia)	*	Townshend (1989)
(cv. Woodstock)	**	Townshend (1989)
2. Bromegrass		
Bromus spp.	**	MacDonald and Mai (1963)
(cv. Redpatch)	**	Willis et al (1982)
3. Orchard grass		
Dactylis glomerata	*	Bird (1968)
	**	Willis et al (1982)
4. Tall fescue		
Festuca arundinacea	*	Townshend et al. (1984)
(cv. Oregon B)		
5. Creeping red fescue		
Festuca rubra	* - **	Townshend et al. (1984)
(various)	**	Marks and Townshend(1973)
6. Perennial rye grass		
Lolium perenne	*	Bird (1968)
		Marks and Townshend(1973)
7. Rye		
Secale cereale	**	Colbran (1979)
	***	Dunn and Mai (1973)
	**	Marks and Townshend(1973)
8. Sorghum		
Sorghum vulgare	*	Bird (1968)
var. sudanese		
(cv. Piper)	*	MacDonald and Mai (1963)
	*	Dunn and Mai (1973)
	***	Marks andTownshend(1973)
	**	Marks et al. (1973)
9. Sorghum-sudangrass hybrid		
Sorghum sudanese x	*	Bird (1968)
Sorghum bicolor	**	Dunn and Mai (1973)
(cv. Zulu)	***	Colbran (1979)
	**	Johnson and Burton(1973)

* supported low levels of P.penetrans
** supported medium levels of P.penetrans
*** supported high levels of P.penetrans

2.4.5.2. Marigolds and other compositae

Plants in the genus *Tagetes*, commonly referred to as marigolds, have long been reported to have nematicidal properties, and considerable research has been undertaken to determine their efficacy as a rotation crop for nematode control.

Some of the most extensive field work conducted on the nematicidal effects of *Tagetes* spp. was by Winoto Suatmadji (1969), who reported the suppression of populations of species of *Pratylenchus*, *Meloidogyne*, and *Tylenchorhynchus*. Populations of the genus *Pratylenchus* were suppressed by *T. patula*, *T. erecta* and *T. minuta* in descending order of effectiveness. After several months of growth *T. patula* reduced field populations of *P. penetrans* to near zero, while in fallow soil two years were required to reduce populations to the same level.

Increased growth of tobacco was reported following a rotation of marigolds which contrasted with the reduced growth of tobacco following a crop of rye (*Secale cereale*), a good host of *P. penetrans* (Miller and Aherns 1969).

Merwin and Stiles (1989) reported that apple seedlings grown in soil following growth of *T. patula* cv. Sparky, treatment with Oryzalin herbicide, and growth of Astro oats, respectively, had dry weights of 97%, 68% and 43% that of seedlings grown in steam sterilized soil. They attributed the significantly greater plant growth response associated with *T. patula* in this bioassay to lower soil populations of *P. penetrans*. In an earlier study (Winoto Suatmadji 1969), the growth of apple seedlings was slightly reduced following a crop of *Tagetes* in a soil not infested with *P. penetrans*. This reduction in growth was attributed to a reduction of soil nitrogen after a *Tagetes* crop.

Daulton and Curtis (1963) observed that each of *T. patula*, *T. erecta* and *T. minuta*, effectively reduced soil populations of *Meloidogyne javanica* and resulted in higher tomato yields the following year.

Gommers and Voorin't Holt (1976) reported that 70 out of 175 species of compositae tested effectively suppressed populations of *P. penetrans*. Hijink and Winoto Suatmadji (1967) reported that certain species from several genera of compositae, such as *Helenium*, *Gaillardia*, and *Eriophyllum*, were as effective as *Tagetes* spp. in reducing reproduction of *P. penetrans*.

2.4.5.2.1 Mode of action.

The mode of accion of marigolds has been studied in depth by Uhelnbroek and Bijloo(1958), Winoto Suatmadji (1969) and Gommers (1973).

Winoto Suatmadji (1969) observed that leachates from potted *T. patula*, red clover (*Trifolium repens*), apple trees, and control soil without plants did not effect *P. penetrans*. Soil remained only slightly nematicidal after the removal of *T. patula* roots. Penetration of *T. patula* roots appeared to be less than that of a susceptible host red clover. This finding was challenged by Gommers (1973) who reported the same degree of penetration of *T. patula* by *P. penetrans* as in a susceptible host, *Avena sativa*. Nematodes in the genera *Pratylenchus* and *Meloidogyne* which penetrated *T. patula* died or did not reproduce once in the roots (Winoto Suatmadji 1969). Root extracts of *T. patula* demonstrated nematicidal properties which killed *P. penetrans in vitro* (Winoto Suatmadji 1969). Winoto Suatmadji (1969) concluded that the endoradicular effects were primarily responsible for the nematicidal effect of *Tagetes*. The histological reaction of *T. patula* roots to infection by *P. penetrans* was very slight. The resulting lesions were small, dark, necrotic and contained only 1 to 3 nematodes which were often dead, dying or twisted (Winoto Suatmadji 1969).

Uhelnbroek and Bijloo(1958), cited by Winoto Suatmadji(1969), were the first workers to isolate nematicidal thiophenes from the roots of *Tagetes*. They identified the principal nematicidal compounds to be alpha-terthienyl and 5-(3-buten-1-ynyl)-2, 2,-bithienyl.

A close relationship was shown between *P. penetrans* suppression and the chemotaxonomy of 70 of the 175 species of compositae reviewed by Gommers and Voorin't Holt (1976). Those species of compositae which suppressed nematodes contained thiophenes, principally alpha-terthienyl and 5-(3-buten-1-ynyl)-2,2'-bithienyl. Gommers (1973) reported that the nematicidal thiophenes present in the roots of *Tagetes* were photo-activated by ultra-violet light. There was no conclusion as to how these compounds were activated within the roots of *Tagetes*.

2.4.5.2.2 Practical aspects

One of Bessy's (1911) criteria for judging the success of a crop rotation was that it would at least pay the expense of working the land, as well as rent, taxes, etc. Winoto Suatmadji (1969) concluded that the value of *Tagetes* as a source of organic matter, stains, therapeutics or other chemicals limited its potential for use as an economic control of nematodes. Assuming: 1) certainty that preplant populations of *P. penetrans* are above threshold; 2) other replant organisms are absent; and 3) the establishment and management of marigold plantings is feasible on a large scale; I would argue that with a potentially high value perennial crop, such as apples, that a rotation of one year out every 10 to 15 years could be economically viable. The three assumptions listed above illustrate the need for more research in these areas.

Winoto Suatmadji (1969) recommended that a breeding program be developed for *Tagetes* to combine its strong nematicidal effects with more useful agronomic properties. In the Dutch climate, they recommend that *Tagetes* be planted in the autumn to precede a main crop. Spring planting was not recommended due to the slow establishment of *T. patula* in cooler

climates. The fact that apple harvest occurs late in the fall, and replanting is most successful when conducted in the early spring suggest that a complete growing season would be needed to grow *Tagetes* in British Columbia.

2.4.5.2.3 Intercropping

Intercropping of *Tagetes* spp. with numerous crops to control several different nematodes has resulted in mixed results with regard to nematode control and crop yield.

The report most relevant to this study was that of Hoestra and Oostenbrink (1962) who under-seeded young apple trees with red clover, apple seedlings, marigolds and maintained fallow control plots. The *Pratylenchus* spp. population was high (865/100 ml of soil) for red clover, intermediate for apples (320/100 ml) and fallow (300/100 ml) and low for *Tagetes patula* (160/100 ml). For a study conducted which was conducted over a single growing season, the results are encouraging. A measure of the root population densities of *P. penetrans* would aid in the evaluation of this practice.

Other reports of limited success in intercropping with *Tagetes* have also been published. According to Rhode (1962 as cited by Mai et al. 1977) interplanting marigolds with potatoes reduces populations of *P. penetrans* when *Tagetes* are grown at 23 cm from potatoes but not when grown at 46 cm. This indicates that the proximity of *T. patula* to the crop may be an important factor for successful intercropping. This experiment also suggests that if there is any preferential attraction of *P. penetrans* to *T. patula*, that it may be limited by distance. Motsinger et al. (1977) believed there was random penetration of *P. penetrans* into both tomato and marigold roots. From greenhouse trials, they reported fewer *Meloidogyne* spp. mature females in tomato roots intercropped with *T. patula* than in tomatoes grown alone. In field trials, there was no significant difference in nematode population densities between the two treatments. Laboratory experiments by Winoto Suatmadji (1969) suggested that *P. penetrans* host selection was random. If this

is true, it would suggest that intercropping would most likely be of limited success in reducing nematode populations.

The following reports confirm the limitations of intercropping *Tagetes* spp. with different crops to control several species of nematode. Tarjan (1960) reported that interplanting *T. erecta* with grapefruit seedlings in the greenhouse significantly reduced populations of *Radopholus similis* in the soil in three out of four tests and in the roots in two out of four tests. However, under-seeding citrus trees in large field plots resulted in no significant difference in nematode populations. Miller and Aherns (1969), intercropped marigolds with strawberries, tomatoes and gladiolas to control *P. penetrans* and found that the marigolds behaved as a weed by competing with the crop and reducing rather than increasing yields. Hackney and Dickerson (1975) reported that intercropping of marigolds with tomatoes did not reduce numbers of *Meloidogyne* spp. or *Pratylenchus alleni* in the roots.

2.4.6 Fallow

In Ontario, Marks et al. (1973) determined that preplant herbicides were effective in reducing soil populations of *P. penetrans*. Moreover, hand weeding until the end of July, at which time the land was allowed to revert to weeds and maintained by mowing, resulted in large root-lesion nematode buildups. Merwin and Stiles (1989) reported a significant reduction in soil populations of *P. penetrans* following a treatment with the herbicide Oryzalin. However, this did not resukt in a significant growth response in an apple seedling bioassay.

Townshend and Davidson (1960) reported that 55 common weed species in Ontario strawberry plantings were good hosts of *P. penetrans*. This suggests that complete weed control is required for fallow to be effective. There are no reports in the literature on the effects of periodic disruption, such as cultivation, on reproduction of *P. penetrans*.

Clean cultivation around trees may increase infection by *P. penetrans*, presumably by removing alternate hosts in which the nematodes may reproduce. Hoestra and Oostenbrink (1962) observed that nematode population density in the roots of cleanly cultivated apple trees was high, while population densities in the surrounding soil was close to zero. On a clay soil, they observed that in the cultivated area, nematode population density per gram of apple root was one order of magnitude higher than root population density where grass was grown. Egunjobi (1968) determined the soil populations of *Pratylenchus* spp. in apple orchards to be greatest in the top 10 cm of soil, reducing to one fifth to a third that density in the next 10 cm. A stronger association of *P. penetrans* with grass roots than with apple tree roots was proposed to explain this observation. An equally valid interpretation may be that the higher nematode population is associated with the greater availability of both grass and apple roots for reproductive sites rather than any host preference. Weed control with herbicides after peach tree establishment did not result in a reduction of *P. penetrans* apparently because the peach trees were effective hosts (Marks et al. 1973).

2.4.7 Plastic mulches

The use of plastic mulches is becoming increasingly prominent in modern agriculture as a way to improve crop yields (Stapleton and DeVay 1986). In young apple orchards, black plastic mulches have been shown to significantly increase vegetative growth and fruit yields more than herbicide treatments (Mage 1982). The manner in which plant growth is increased is due to an effect of both warming of the soil and improved nutrient uptake. The effect of mulching on vegetative growth appears to be most pronounced in the first year after application. Mage (1982) reported that the nitrogen content of apple leaves from mulched trees was significantly higher than from unmulched trees in the first two years of growth.

Other benefits to be considered from the use of plastic mulches are increased soil moisture retention, weed control, soil temperature management, protection against soil erosion and improvement of soil tilth (Stapleton and DeVay 1986).

Reports in the literature of the effects of mulching on nematodes can be divided into two broad areas: solarization and environmental modification.

2.4.7.1 Solarization with clear plastic mulch

Stapleton and Devay (1986) define solarization as "the thermal, chemical and biological changes in soil caused by solar radiation when covered by clear plastic film, especially when the soil has a high moisture content."

Stapleton and Devay (1986) list the principle factors which influence the efficacy of solarization:

1. Soil preparation; a smooth soil surface is required to allow close proximity of the plastic to the soil by reducing the insulating effect of air between soil and the plastic film.

2. Soil characteristics; dark soils transfer more radiation than light colored soils.

3. Soil moisture; thermal sensitivity of soil borne microflora and fauna and conduction are increased by irrigating before mulching.

4. Film type and characteristics; Clear transparent polyethylene, 20 to 25 microns thick, is usually employed because of its low cost, high strength and effectiveness.

Katan (1984) discusses in detail the mechanisms through which solarization controls plant disease. The three major factors contributing to improved growth are: 1) the thermal inactivation of pathogens; 2) enhancement of biological controls; and 3) increased plant growth beyond pest control.

Solarization has been used to successfully control a wide range of soil-born pests including bacteria, fungi, weeds, mites and nematodes (Katan 1984, Stapleton and Devay 1986). Perhaps the most detailed study of solarization on nematodes was conducted on ten field sites in California by Stapleton and Devay (1983). Solarization for 4-6 weeks resulted in soil population density reductions of 42 to 100 % of seven phytoparasitic nematode genera, including *Pratylenchus spp.* Additional sampling 7-10 months later often indicated a further reduction of nematode populations perhaps due to the enhancement of biological controls. The effectiveness of solarization was reduced at increasing depths. Plant growth increased following solarization. However, the authors could not correlate this with a reduction in nematode numbers. The inability of solarization to completely eradicate nematodes makes its value as a control for nematodes questionable in the opinion of the authors (Stapleton and Devay 1983).

2.4.7.2 Environmental modification with black plastic mulch

This section deals with enhancement of biological controls and increased growth response through the utilization of black plastic mulches.

Colbran (1979) in Australia reported almost complete control of *P. penetrans* with black polythene film for a period of 4 months. This reduction was significantly greater than the reduction achieved by clean cultivation. Apple tree shoot growth following black polythene was significantly higher than following a cover crop mixture of maize, oats, rye, blue lupin, sudan grass and cowpea. However, it was not higher than following clean cultivation.

Miller and Waggoner (1963) reported that the number of *P. penetrans* in the soil under young apple trees was reduced significantly by mulching with a black plastic film. Additionally, they observed an increase in the fungus *Rhizoctonia solani* in plots covered with the black plastic

mulch. However, when the *Rhizoctonia* was suppressed by the use of the fungicide pentachloronitrobenzene (PCNB) under the mulch, the nematode populations were not reduced. From these observations, they hypothesized that the mode of action of the mulch was to modify the environment as to encourage growth of microorganisms predacious on *P. penetrans*.

Miller (1977) found that a black plastic mulch used on tomatoes significantly reduced *P. penetrans* populations to 60% of that in the controls in the first year . However, in the second year, the population of *P. penetrans* in the roots actually increased to about 170% of the control.

Many other workers have reported a growth response to mulching of crops with black plastic, but few have reported on the effects on nematodes. Johnson et al. (1977) reported a reduction in the population densities of *Criconemoides ornatus* under a film mulch, but the treatment did not affect crop yields.

Jensen and Buszard (1988) reported an excellent response of replanted apples in an apple replant disease area to a black plastic mulch treatment, but no observations were made on *P. penetrans* populations. A Vorlex treatment included in the same experiment resulted in a significant increase in total branch length and total number of branches over the untreated control, but this was significantly less than the response to treatment with plastic mulch. Without measurement of the *P. penetrans* populations, it is not clear if the plastic mulch had any effect on nematode populations. These results collectively illustrate that black plastic mulch can have an effect that goes beyond controlling the nematode component of replant problems in apples.

This literature review has demonstrated that *P. penetrans*, which is widespread in British Columbian orchards, can be a serious pest of fruit trees. Historically chemical nematicides have been used to control *P. penetrans*. Due to increasing public

concern over the use of toxic pesticides, the problems associated with their usage and the availability of promising non-chemical controls gave impetus to the field study described in the following section. Although a substantial amount of work has been done using marigolds as a rotational crop, little work has been done on intercropping. The use of plastic mulches to reduce nematode populations has shown some promise for nematode control. Initially the focus of this project was to evaluate intercropping marigolds with apples and raspberries and the use of plastic mulches on these same crops to determine their effects on populations of *P. penetrans*. Unfortunately, due to the death of the majority of raspberry plants at one field site and unavailability of a second site for a repitition of the apple intercropping experiment, the focus of the experiments was changed. The final experimental plan of orchard, noncropped and greenhouse experiments was designed to accommodate available options as well as to seek empirical support for the use of alternatives to chemicals for the control of *P. penetrans* in British Columbia.

3. MATERIALS AND METHODS

3.1 NEMATODE EXTRACTION TECHNIQUES

Nematode extraction from the soil and root samples in all three experiments was conducted within five days of sampling. Samples were stored in a cold room at 10° C until they were processed for extraction.

The modified Baermann pan technique (Townshend 1963) was used to extract nematodes from soil. Materials for this procedure included a coarse sieve (10 mesh, 2.0 mm aperture), a 50 cc beaker, three-ply paper tissue, plastic screens (17 cm diameter) supported on three 5 mm legs and aluminium pie pans (20 cm diameter) to accommodate the screens.

Each soil sample was thoroughly mixed and then sieved through the coarse sieve onto a large pan to remove all roots, rocks and to reduce the size of larger soil clumps. Small quantities of soil were taken randomly with a teaspoon from the sieved sample and combined to make up a 50 ml volume for extraction. This soil was then spread uniformly on a three ply tissue paper superimposed on a plastic screen. The screen was then placed in a pie plate and sufficient water was added to bring the meniscus slightly above the screen without completely submerging the soil sample. The Baermann pans were stored at room temperature in large plastic bags to minimize evaporation for a period of 7 d.

After 7 d, the nematode suspension in the pie pan was poured into a fine sieve (20 micron nylon mesh), and the bottom of the plastic screen and the pie plate were rinsed onto the sieve to assure complete recovery of nematodes. A squeeze bottle was used to rinse the nematodes from the screen into a sample jar with a minimum volume of water.

A misting chamber was used to extract nematodes from the root samples (Seinhorst 1950). The following materials were

used: 15 ml conical centrifuge tubes , glass funnels (100 mm diameter), misting baskets (80 mm diameter) and a misting chamber.

Roots were separated from the soil sample with the coarse sieve and washed off with pressurized tap water. After fresh weights were recorded, the roots were chopped into 2-4 cm lengths and placed into a misting basket. The misting basket was placed on a glass funnel which emptied into a 10 ml tube. This entire apparatus was then placed into the chamber where a fine mist was applied for 1 minute at every 10 minute interval. The nematodes that emerged from the roots were washed into the centrifuge tube; the nematodes gathered at the bottom of the tube, which allowed the excess water from misting to run off the top. After a period of 7 d, the nematodes are washed from the centrifuge tubes into vials where they were stored in a refrigerator for counting.

The procedure used for identifying and counting nematodes was the same for both soil and root samples. The counting vials were agitated to suspend the nematodes randomly. A 3 ml aliquot was removed and placed in a grided plexiglass counting dish. All different stages of *P. penetrans* nematodes were counted under a dissecting microscope. The total volume of the counting vial was measured and from this counts were converted to number of nematodes per vial, equivalent to the number per 50 cc of soil or root weight. The count per root weight was then converted to count per gram of roots. All nematode counts were generously performed by Mr. Rob Favrin, a technician in Dr. Vrain's laboratory at Agriculture Canada Research Station Vancouver.

All nematode counts for both experiments were log transformed and subjected to analysis of variance. Fisher's LSD (least significant difference) test was used to compare means following a significant ANOVA.

3.2 FIELD EXPERIMENTS

Two field experiments, a noncropped and orchard experiment, were conducted to evaluate five cultural control methods for *P. penetrans*. In this section, a description of the treatments; *T. patula*, black and clear polyethylene mulches, handweeding and cereals is followed by the details of each experiment.

3.2.1 Marigolds

French marigold, *T. patula* var. Petite Harmony, seed was obtained from similar experiments conducted by Dr. Vrain and Integrated Crop Management Inc. (a pest management consulting and research company in the Okanagan Valley) in a previous year. Seed bed preparation consisted of both rotovation by tractor and hand hoeing. All plots were then raked to produce a fine, even seedbed and seed was manually broadcast at a rate of 8 kg per ha. The seed was lightly covered by raking and rolled with a lawn roller. Irrigation was necessary for the establishment of the marigolds and was applied according to the availability of water. The restricted availability of water was a result of the limited water resource of commercial operations where the plots were located. Weeding was done manually every 2 weeks for the first 2 months until the marigolds were large enough to shade out competition. Uneven establishment of the marigold stands at both sites required manual re-seeding in mid-June.

3.2.2 Plastic mulches

The plastic mulches used were made of polyethylene. The clear polyethylene was 50 microns thick and the black polyethylene was 150 microns thick. Land preparation for the black and clear plastic mulch treatments was conducted in an identical fashion to that of the marigold treatments. The plots were irrigated the day before the mulches were laid. The polyethylene films were laid out by hand and anchored in a 15 cm trench surrounding the plot by refilling with soil. In the noncropped experiment two sheets of clear polyethylene were glued together by a construction glue PL300. The glue however

deteriorated mid-way through the season and was replaced by a water-resistant duct tape.

3.2.3 Hand weeding

All control plots were weeded every 2 weeks for the duration of the field season.

3.2.4 Cereals

The oat and sorghum-sudangrass treatments were included in the noncropped experiment only. They were seeded with a Precision Garden Seeder Model-1001 B, from Earthware Products Inc. Bristol IN. USA 46507. The sorghum-sudangrass, "Pioneer Hybrid var 998", was seeded at a rate of 56 kg ha^{-1} with 50 cm between rows. The oats var. "Cascade", were seeded at a rate of 120 kg ha^{-1} with 20 cm between rows.

3.2.5 Noncropped experiment

The objective of this experiment was to determine the effects of five cultural practices on soil populations of *P. penetrans* in the absence of a crop.

3.2.5.1 Site selection and description

Preliminary samples of twenty cores were taken randomly from two separate areas of a field that had a previous history of *P. penetrans* infestation. Results indicated a *P. penetrans* population of 212 nematodes per 50 ml of soil in one of the two areas, which was considered to be a sufficiently high population density for the establishment of a field trial.

The site was established at "The Ginseng Farm" in Armstrong situated in the northern Okanagan Valley, located approximately 50 28'N and 119 14'W. The farm is owned and operated by Pat Aguilar. The site has a western exposure and a slope of one to two percent. The exact cropping history of the site is unavailable as the farm was purchased in 1987 and records prior to that are unavailable. However, it is reputed to have been planted to cereals, predominantly oats. In the summer of 1987,

it was planted to wheat, and in the spring of 1988, to oats. Following the harvest of the oats, the land was kept fallow by discing approximately every 2 weeks during the rest of the growing season until the experiment was established in the spring of 1989.

3.2.5.2 Experimental design.

Plots 4 x 5 m were established in a randomized complete block design. The blocks were perpendicular to the slope of the field and each treatment was replicated five times. Treatments were assigned randomly within each block. The five treatments tested on this site were: i) french marigolds *T. patula* var. Petite Harmony; ii) 50 micron clear polyethylene film; iii) a sorghum - sudangrass hybrid cultivar"Pioneer hybrid variety 988"; iv) an oat cultivar "Cascade" and v) a hand weeded control.

3.2.5.3 Sampling

Each plot was sampled three times during the experiment. The initial sampling was prior to the application of the treatment on May 31st, a mid-season sampling on August 20th and a final sampling at the end of the growing season on October 10th. A single bulked soil sample, comprised of 20 randomly selected cores taken with a 2.5 cm diameter Oakfield sampling tube to a depth of 20 cm, was taken from each plot. Root samples from the oats and sorghum-sudangrass treatments were comprised of small portions of roots from ten randomly chosen plants within the plot, the outer three rows were not sampled. Samples were put into labelled plastic bags and placed in a styrofoam ice chest. Within 24 hours samples were transported to Vancouver where they were stored at 10^{o} C until extraction.

3.2.5.4 Soil temperatures

Soil temperature data was recorded in the solarization and control plots at depths of 5 cm, 10 cm, and 20 cm with an Omni

Data Logger from Omnidata International, North Logan Utah. Maximum, minimum and average soil and air temperatures were recorded every 2 hours during most of the month of August.

3.2.6 Orchard experiment

The objective of this experiment was to determine the effect of four cultural practices on the rhizosphere and root populations of *P. penetrans* in replanted apple trees.

3.2.6.1 Site selection and description

Criteria for site selection were: a) apple trees replanted in a former orchard within the last 4 years b) poor tree growth c) at least 80 apple trees, to allow for an experimental design incorporating a plot size of four trees, four treatments and five replicates and, where possible, d) a known infestation of *P. penetrans*.

Sites meeting the first three criteria were sampled to determine the population density of nematodes. 20 soil cores each taken to a depth of 20 cm with a 2.5 cm diameter Oakfield sampling tube, were collected randomly from an area of approximately 80 trees, thoroughly mixed and a subsample was removed for nematode extraction. Nematodes extracted from each soil sample were identified and counted.

Twenty sites were sampled between May 6 and 29, 1989. The site with the highest *P. penetrans* counts was chosen for the experiment.

The site chosen had an initial nematode count of 160 *P. penetrans* per 50 ml of soil, and was located in the unincorporated town of Oyama in the northern Okanagan Valley, approximately 50 05'N and 119 22'W. The orchard was owned and managed by Leo Giroux.

The previous crop, a 30 year old orchard of mixed apple varieties with a 10 x 10 m spacing was removed in the summer of 1985. The site was replanted in the spring of 1986 to Antonovka

Spur Macs with a 5 x 3 m spacing and rows established in a north-south orientation. Vegetation was allowed to regrow naturally between rows after the new trees were established. The experimental site was located at the north end of the block.

3.2.6.2 Experimental design

A randomized complete block design was chosen for this experiment. Each plot consisted of four adjacent trees within a row. The treatments covered the ground between each tree and extending a meter past the end trees in the plot, and a meter on either side of each tree. The area between rows was defined by a herbicide strip and bordered by the ground cover of predominantly *Agropyron repens* and a mixture of other weeds. Blocking was determined on the basis of the different slopes present on the site. The four treatments tested at this site were: i) french marigolds, *T. patula* var. Petite Harmony; ii) clear polyethylene film, 50 microns thick ; iii) black polyethylene film, 150 micron thick and iv) a hand-weeded control. Each treatment was replicated five times and randomized within each block.

3.2.6.3 Sampling

Each plot was sampled three times during the experiment: the initial sampling prior to the application of the treatment on May 30th, a mid season sampling August 20th and a final sampling at the end of the growing season on October 10th.

Twenty soil cores were taken randomly at the driplines of the four trees in each plot. Cores were taken with a 2.0 cm diameter Oakfield sampling tube to a depth of 20 cm. Soil cores were bulked, thoroughly mixed, put into labelled plastic bags and placed in a styrofoam ice chest while still in the field. Within 24 hours samples were transported to Vancouver where they were stored at 10 C.

and placed in a styrofoam ice chest while still in the field. Within 24 hours samples were transported to Vancouver where they were stored at 10 C.

Root samples were bulked from the four trees in each plot. Initially, root samples taken with the soil sampler were to be used. However, this provided insufficient material. Therefore, a trowel was used to remove roots from the four quadrants around each tree to a depth of 20 cm and those roots were added to the soil sample for extraction.

3.3 GREENHOUSE EXPERIMENT

The objective of this experiment was to determine the effect of intercropping marigolds with apple seedlings on an established population of *P. penetrans* both in the apple seedling roots and surrounding soil.

3.3.1 Treatments

Stratified MacIntosh apple seeds were donated by the Agriculture Canada Summerland Research Station. These seeds were planted into 7.5 cm pots with a 1:1:1 greenhouse potting mix of soil: peat: pearlite, and maintained until they reached approximately 20 cm in height. Roots were washed of soil and the total plant weight was recorded. Plants were repotted, a single seedling per pot, into 1.5 l of soil in 15 cm pots with one of the following treatments:

1) 0 *P. penetrans* per pot
2) 4000 *P. penetrans* per pot
3) 8000 *P. penetrans* per pot
4) 0 *P. penetrans* and one *T. patula*
5) 4000 *P. penetrans* and one *T. patula*
6) 8000 *P. penetrans* and one *T. patula*.

The nematode inoculum was prepared using the following procedure: potted raspberry plants were inoculated with infested field soil and grown over the summer. The plants were then cut back and the soil and roots were stored at 10° C. The *P. penetrans* inoculum was prepared by homogenizing approximately

80 liters of soil and raspberry roots. From this volume four 50 ml samples were taken at random and were extracted using the modified Baermann pan method. The mean number of nematodes was used as the population density of the inoculum. The inoculum soil was then mixed with a quantity of uninfected greenhouse soil to achieve the desired nematode population density.

The soil, used for the treatments requiring a zero nematode population, was prepared by freezing the infested soil at -12° C for a 12 h period to kill the nematodes. Post freezing extraction revealed that 10 to 15 *P. penetrans* per 50ml of soil survived the freezing process.

Three seeds of French marigolds, *T. patula* var Petite Harmony, were planted in each pot of the appropriate treatment but were then thinned to one per pot after 2 weeks.

The experiment was maintained in a greenhouse at the Agriculture Canada Research Station in Vancouver. Greenhouse temperature was maintained at 21° C with a range 20 - 26° C. The plants grown at 21° C, with a range 20 - 26° C, under florescent light with 17000 lux, initially for a 10 h period beginning at noon each day and later for a 16 h period as all plants were growing poorly under the initial light regime. All pots were fertilized with a liquid 20-20-20 fertilizer at 3 week intervals.

3.3.2 Experimental design

The experimental design was a 2 x 3 factorial (marigolds and nematodes) experiment, with 10 replicates per treatment in a randomized complete block design. Blocking was achieved by grouping apple seedlings of common weights together, treatments were then randomized within these blocks.

3.3.3 Sampling

The experiment was left to run for 3.5 months at which time the height of the apple seedlings was measured and the plants harvested. Roots were washed of soil and blotted dry.

The wet weight of stems and roots of each apple seedling was taken. After weighing, all the fine feeder roots were taken from each pot and extracted in the mist chamber. From each pot 50 ml of soil were extracted using the modified Baermann pan technique.

Nematode counts were log transformed to stabilize variance. Nematode counts and plant measurements were subject to analysis of variance. A randomized block model assuming no interaction between treatments and blocks was used (Neter et al. 1990).

4. RESULTS

4.1 NONCROPPED EXPERIMENT

The number of *P. penetrans* per 50 ml of soil was significantly higher in the solarization plot prior to treatment (Table 4.1). Soil population densities of *P. penetrans* were significantly greater than in the weeded control in the sorghum-sudangrass and oats plots at both the mid-season and final sampling dates (Table 4.1). On the final sampling date the sorghum-sudangrass plots had a significantly larger soil population density of *P. penetrans* than the oat plots, this difference was not significant at the mid-season sampling . Solarization (mulching with clear plastic) resulted in lower nematode densities than did *T. patula*, oats or sorghum-sudangrass cover crops at the mid-season sampling. However, this population density was not significantly lower than that of the weeded control. Results from the final sampling date indicate no significant differences between *P. penetrans* counts in the solarization, *T.patula* cover crop and weeded control (Table 4.1).

At the mid-season, root population densities of *P. penetrans* were higher in the oats than in the sorghum-sudangrass hybrid but not significantly (Table 4.2). *T. patula* roots were not harvested due to the small size of the *T. patula* plants at this time. The post-treatment sampling yielded significantly different ($P < 0.05$) root population densities of *P. penetrans*, listed in increasing order of magnitude they are *T. patula* , Sorghum-sudangrass and oats (Table 4.2).

4.2 ORCHARD EXPERIMENT

In this experiment there were no significant ($P < 0.05$) treatment effects on the soil population density of *P. penetrans* (Table 4.3).

Table 4.1 Noncropped Experiment: Mean soil population of Pratylenchus penetrans in the soil at pre-treatment (May), mid-season (August) and post-treatment (October) in the solarization, Tagetes patula, sorghum-sudangrass and control treament plots.

P. penetrans per 50 ml of Soil.

	Pretreatment (1)		Mid-Season (1)		Posttreatment	
Treatments	Mean (2)	95% C.I.	Mean (2)	95% C.I.	Mean	95% C.I.
Solarization	189 b	$127 < \bar{x} < 280$	23 a	$9 < \bar{x} < 57$	47 a	$26 < \bar{x} < 86$
Marigolds	164 ab	$110 < \bar{x} < 243$	66 bc	$27 < \bar{x} < 163$	44 a	$24 < \bar{x} < 79$
Weeded Control	145 a	$98 < \bar{x} < 216$	31 ab	$12 < \bar{x} < 75$	70 a	$39 < \bar{x} < 127$
Oats	136 a	$91 < \bar{x} < 202$	97 c	$39 < \bar{x} < 293$	256 b	$141 < \bar{x} < 463$
Sorghum	94 a	$63 < \bar{x} < 139$	126 c	$51 < \bar{x} < 310$	494 c	$273 < \bar{x} < 895$

(1) Geometric means and 95% confidence intervals (C. I.) are derived from log transformed values.
(2) Means within columns followed by a common letter are not significantly different at P = 0.05, by protected LSD.

Table 4.2 Noncropped Experiment: The mean number of *Pratylenchus penetrans* per gm of root from oat, sorghum-sudangrass and *Tagetes patula* at mid-season (August) and post-treatment (October).

	Mid-Season(1)		Posttreatment	
	Mean	95% C.I.	Mean(2)	95% C.I.
Marigolds	N.A.		5 a	$1 < \bar{x} < 38$
Sorghum x sudangrass	424	$203 < \bar{x} < 886$	94 b	$13 < \bar{x} < 675$
Oats	627	$275 < \bar{x} < 1430$	548 c	$77 < \bar{x} < 3926$

(1) Geometric means and 95% confidence intervals (C.I.) are derived from log transformed values.
(2) Means within columns followed by a common letter are not significantly different at P = 0.05, by protected LSD.

Table 4.3 Orchard Experiment: Soil population density of Pratylenchus penetrans at pre-treatment (May), mid-season (August), and post-treatment (October).

P. penetrans per 50ml of soil.

	Pre-treatment (1)		Mid-season (1)		Post-treatment (1)	
Treatments	Mean	95% C.I.	Mean	95% C.I.	Mean	95% C.I.
Tagetes patula	86	$47 < \bar{x} < 159$	6	$2 < \bar{x} < 22$	27	$9 < \bar{x} < 77$
Black Plastic Mulch	72	$39 < \bar{x} < 133$	18	$5 < \bar{x} < 67$	47	$16 < \bar{x} < 137$
Clear Plastic Mulch	96	$52 < \bar{x} < 177$	30	$8 < \bar{x} < 111$	55	$19 < \bar{x} < 159$
Weeded Control	67	$36 < \bar{x} < 123$	28	$8 < \bar{x} < 102$	28	$10 < \bar{x} < 80$

(1) Geometric means and 95% confidence intervals (C.I.) are derived from log transformed values.
Note : There were no significant treatment effects (P= 0.05).

In the apple tree roots, prior treatment, the population density of *P. penetrans* was significantly lower in the control plots than the treatment plots (Table 4.4). Root samples were not taken from the *T. patula* plots in the mid-season to avoid damaging the *T. patula* plants which were poorly established at this time. The number of *P. penetrans* extracted from the apple tree roots at the mid-season were significantly lower ($P < 0.05$) under the clear plastic than in the control plot. Population density of *P. penetrans* under the black plastic mulch did not differ significantly from the other two treatments. This effect was not apparent at the post-treatment sampling. Post-treatment *P. penetrans* population densities were significantly higher in the roots from under the black plastic mulch than in the weeded control or the *T. patula* plots (Table 4.4) .

4.3 GREENHOUSE EXPERIMENT

A significantly lower soil population density of *P. penetrans* was observed in pots with *T. patula* grown concurrently with the apple seedling (Table 4.5, Figure 1.). The relationship between inoculation level and the number of *P. penetrans* per 50 ml of soil is positive and differences were significant at $P < 0.05$ (Table 4.5, Figure 1.).

There was no significant difference between the apple seedling root population densities of *P. penetrans* grown with or without *T. patula* (Table 4.6, Figure 2.). The root population density of nematodes was significantly lower at the zero *P. penetrans* inoculum level than at the higher levels of inoculum. The difference in root population densities of nematodes between the 4000 and 8000 *P. penetrans* per pot level of inoculum was not significant

There were fewer *P. penetrans* per pot with the *T. patula* plant than without (Table 4.7). There were significantly ($P < 0.05$) fewer nematodes in those pots inoculated withe the frozen inoculum (zero nematodes) than those inoculated at 4000 and 8000 which were not significantly different from each other (Table 4.7).

Table 4.4 Orchard Experiment: Mean population densities of *Pratylenchus penetrans* in apple roots at pre-treatment (May), mid-season (August) and post-treatment (October) in the Tagetes patula, black and clear plastic mulches and control plots.

P. penetrans per gm of root.

	Pre-treatment (1)		Mid-season		Post-treatment	
Treatment	Mean (2)	95% C.I.	Mean	95% C.I.	Mean	95% C.I.
Tagetes patula	507 b	211 < $\overline{x}$ < 1216	N.A.	N.A. N.A.	102 a	50 < $\overline{x}$ < 209
Black Plastic Mulch	696 b	290 < $\overline{x}$ < 1671	150 ab	91 < $\overline{x}$ < 250	328 b	160 < $\overline{x}$ < 671
Clear Plastic Mulch	481 b	201 < $\overline{x}$ < 1156	100 a	60 < $\overline{x}$ < 165	146 ab	71 < $\overline{x}$ < 299
Control	175 a	73 < $\overline{x}$ < 422	248 b	150 < $\overline{x}$ < 413	127 a	56 < $\overline{x}$ < 356

(1) Geometric means and 95% confidence intervals (C.I.) are derived from log transformed values.
(2) Means within each column followed by a common letter are not significantly different at P = 0.05, by protected LSD.

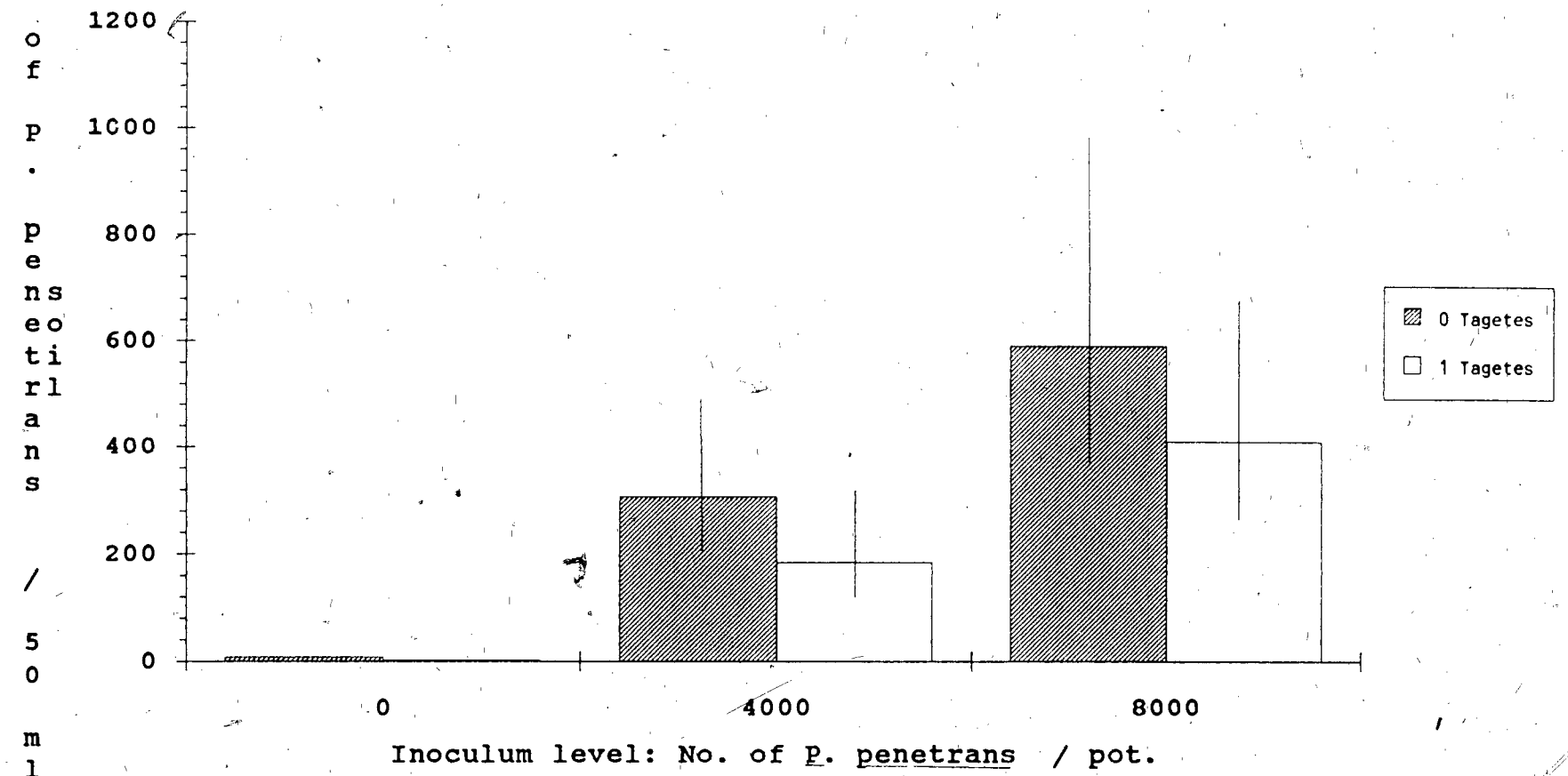
Table 4.5 Greenhouse Experiment: The influence of Tagetes patula on the final population density of Pratylenchus penetrans, expressed as the mean number per 50 ml of soil, at three inoculum levels.

Mean and 95% Confidence Interval. (1)

	Inoculation level: number of P. penetrans per pot							
	0		4000		8000		Row Means (3)	
No Tagetes	9	$5 < \bar{x} < 15$	308	$178 < \bar{x} < 530$	591	$343 < \bar{x} < 1018$	116 b	$84 < \bar{x} < 161$
With Tagetes	3	$2 < \bar{x} < 5$	185	$104 < \bar{x} < 329$	411	$239 < \bar{x} < 708$	58 a	$42 < \bar{x} < 80$
Column Means (2)	5 a	$3 < \bar{x} < 7$	239 b	$163 < \bar{x} < 351$	439 c	$335 < \bar{x} < 724$		

(1) Geometric means and 95% confidence intervals (C.I.) are derived from log transformed values.
(2) Means within row followed by a common letter are not significantly different at P = 0.05, by protected LSD.
(3) Means within column followed by a common letter are not significantly different at P = 0.05, by protected LSD.

Figure 1. Greenhouse experiment: The influence of Tagetes patula on the final soil population density of Pratylenchus penetrans, at three inoculum levels.



Vertcal bars represent the 95% confidence interval of each mean.

Table 4.6 Greenhouse Experiment: The effects of *Tagetes patula* on the final population density of *Pratylenchus penetrans*, expressed as the mean number per gram of apple seedling root, at three inoculum levels.

Means and 95% Confidence Intervals. (1)

	Inoculum: No. of *P. penetrans* per pot							
	0		**4000**		**8000**		**Row Means**	
Control	**59**	$34 < \overline{x} < 101$	**1473**	$857 < \overline{x} < 2533$	**2024**	$1074 < \overline{x} < 3813$	**562**	$419 < \overline{x} < 753$
Tagetes	**63**	$37 < \overline{x} < 108$	**1361**	$760 < \overline{x} < 2436$	**1609**	$899 < \overline{x} < 2880$	**516**	$385 < \overline{x} < 693$
Column Means (2)	**61a**	$43 < \overline{x} < 87$	**1416b**	$983 < \overline{x} < 2040$	**1811b**	$1229 < \overline{x} < 2668$		

(1) Geometric means and 95% confidence intervals (C.I.) are derived from log transformed values.
(2) Means within row followed by a common letter are not significantly different at P=0.05, by protected LSD.

Figure 2: Greenhouse experiment: The influence of Tagetes patula on the final root population density of Pratylenchus penetrans, at three inoculum levels.

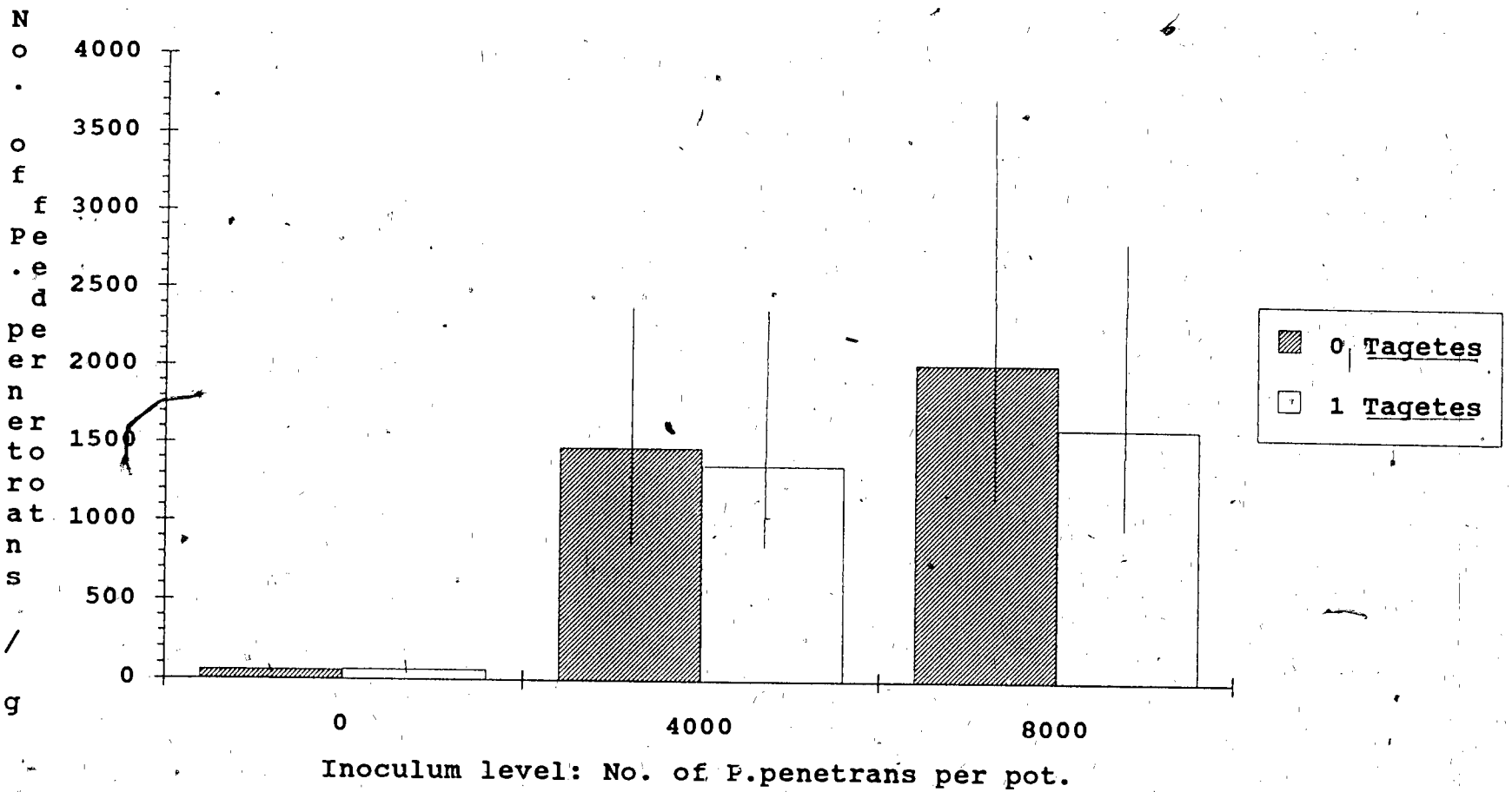


Table 4.7 Greenhouse Experiment: The effects of Tagetes patula on the total number of Pratylenchus penetrans per pot, at three inoculum levels.

Means and 95% Confidence Intervals. (1)

	Inoculum: No. of P. penetrans per pot							
	0		4000		8000		Row Means	
Control	**1331**	899 < $\bar{x}$ < 1970	**24712**	16691 < $\bar{x}$ < 36586	**36093**	24378 < $\bar{x}$ < 53435	**10152a**	8094 < $\bar{x}$ < 12733
Tagetes	**1131**	763 < $\bar{x}$ < 1674	**18333**	12383 < $\bar{x}$ < 27141	**25285**	17078 < $\bar{x}$ < 37434	**7838a**	6249 < $\bar{x}$ < 9830
Column Means(2)	**1227a**	950 < $\bar{x}$ < 1584	**21454b**	16610 < $\bar{x}$ < 27709	**29929b**	23172 < $\bar{x}$ < 38655		

(1) Geometric means and 95% confidence intervals (C. I.) are derived from log transformed values.
(2) Means within row followed by a common letter are not significantly different at P=0.05, by protected LSD.

The dry weight of the total apple seedling was significantly greater ($P < 0.05$) when grown without the companion plant *T. patula* (Table 4.8). Analysis of variance of both the root and stem components of the total plant weight indicated the stem weight to be more severely affected than the root weight. There was a significant interaction ($P < 0.05$) between *T. patula* and the three inoculum levels of *P. penetrans* on stem dry weight. The interaction effect on total seedling dry weight was also near to the standard level of significance ($P < 0.06$, Figure 3.). The effects of different levels of nematode inoculum on plant mean weights are also presented in Table 4.8. The negative relationship between plant dry weight and the inoculum level were significant at $P < 0.05$.

Apple seedling height decreases significantly ($P < 0.05$) with higher levels of nematode inoculum and when grown together with *T. patula* (Table 4.9). The interaction between the treatments is significant ($P < 0.05$).

Table 4.8 Greenhouse Experiment: The influence of Tagetes patula with three inoculum levels of Pratylenchus penetrans on apple seedling dry weight.

Means and Standard Errors.

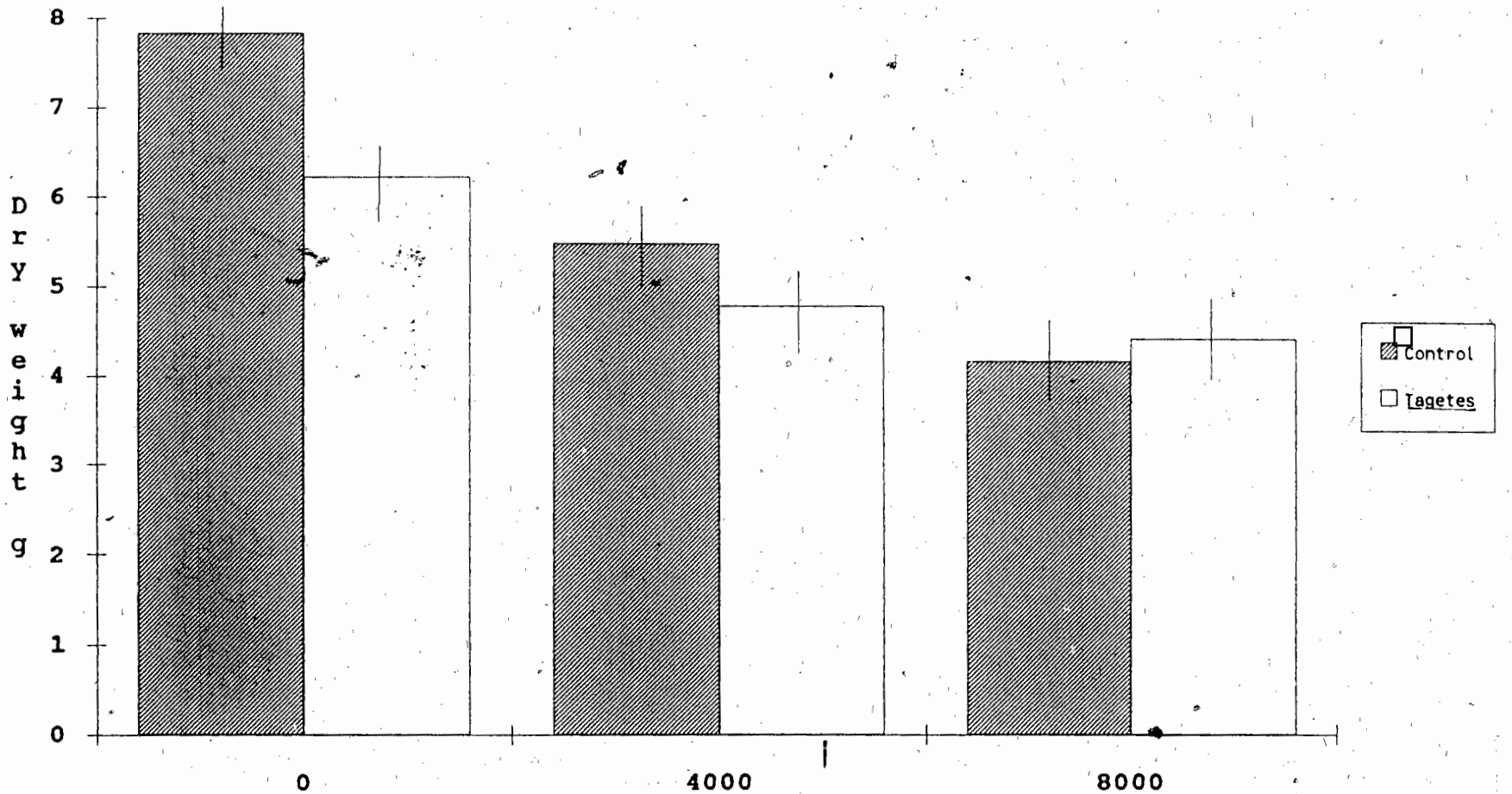
	Total Plant (1) Dry Wt. gm			Root Dry Wt gm			Stem Dry Wt gm		
Without Tagetes	5.82 b	±	0.23	2.46	±	0.14	3.37 b	±	0.17
With Tagetes	5.15 a	±	0.23	2.51	±	0.14	2.63 a	±	0.17

(1) Means within colums followed by a common letter are not significantly different at P = 0.05, by protected LSD.

Nematode 0	7.03 c	±	0.29	3.56 b	±	0.31	3.47 b	±	0.21
Nematode 4000	5.14 b	±	0.29	2.19 a	±	0.31	2.96 ab	±	0.21
Nematode 8000	4.29 a	±	0.29	1.72 a	±	0.31	2.57 a	±	0.21

(1) Means within colums followed by a common letter are not significantly different at P = 0.05, by protected LSD.

Figure 3: Greenhouse experiment: The influence of Tagetes patula on apple seedling dry weight, at three levels of Pratylenchus penetrans inoculum.



Vertical bars represent two standard errors.

Table 4.9 Greenhouse Experiment: The effects of Tagetes patula on the stem length of apple seedlings at harvest, expressed in cm, at three inoculum levels of Pratylenchus penetrans.

Mean and Standard Error.

	Inoculation level: number of P. penetrans per pot			
	0	4000	8000	Row Means(3)
No Tagetes	38.4 ± 2.7	23.8 ± 2.7	22.5 ± 2.7	28.2 b ± 1.6
With Tagetes	22.5 ± 2.7	18.9 ± 2.7	20.5 ± 2.7	20.6 a ± 1.6
Column Means(1)	30.4 b ± 1.9	21.4 a ± 1.9	21.5 a ± 1.9	

(1) Means within row followed by a common letter are not significantly different at P=0.01, by protected LSD.
(2) Means within column followed by a common letter are not significantly at P=0.01, by protected LSD.

5. DISCUSSION

The first section of the discussion is on the sampling procedure used to estimate *P. penetrans* populations. This is followed by a discussion of the results obtained from each of the three experiments.

5.1 SAMPLING

The distribution of phytoparasitic nematodes in the soil is often clumped (Goodell and Ferris 1980, McSorely and Parrado 1982, Barker 1985). The results of both field experiments and the greenhouse experiment were characterized by variances much larger than the means for each treatment. This is an indication of a clumped or contagious distribution (Elliot 1971). Plotting treatment variances against the treatment means on a log-log scale (Southwood 1978) resulting in a positively sloped straight line with a slope significantly greater than one indicates a dependence of the variance on the mean. A variance-to-mean ratio of greater than one is a common relationship that describes the distribution of many organisms (Southwood 1978, Elliot 1971). Dependence of the variance on the mean is also a violation of one of the assumptions of the analysis of variance (Elliot 1971, Sokal and Rohlf 1973, Southwood 1978). Mathematical transformations of the data can be applied to meet the assumptions of ANOVA. Classification of the distribution to choose an appropriate normalizing transformation requires a large number of samples from the same population. The time expenditure required per nematode count makes this process very difficult. In nematology, logarithmic transformations, based upon sampling and distribution studies (Procter and Marks 1974, Goodell and Ferris 1981, Francl 1986), are often applied to nematode counts (Townshend et al. 1984, Vrain and Copeman 1987, Merwin and Styles 1989).

For the nematode count data, obtained in each of the experiments, I plotted the log variance of each treatment against the log mean of each treatment and fitted a regression

line. The slope of the lines were tested and determined to be greater than one. Based upon this information, and the precedence in the literature, all *P. penetrans* count data were log transformed.

The geometric means were calculated by taking the anti-logarithms of the means of the transformed counts to which the analysis of variance was applied. Presentation of data as back-transformed means is recommended by Elliot (1971) and Sokal and Rohlf (1973). The 95% confidence intervals are derived by multiplying the standard error of each mean, calculated from the transformed data, by the tabulated t statistic based on the number of observations per mean. The resulting value is added and subtracted from the mean to calculate the upper and lower confidence intervals respectively. Back-transforming these confidence intervals, by taking anti-logarithms, results in the uneven confidence intervals presented in the tables. The standard error terms used in these calculations are determined by taking the square root of the error mean square term from the analysis of variance divided by the number of observations per mean. The 95% derived confidence intervals are presented along with the geometric means as an indication to the reader of the variability found in the nematode counts between plots.

Francl (1986) reported that the frequency distribution of nematode counts from a set of subsamples, taken from a bulked field sample, would at best be random. If this observation is valid for my own samples (it wasn't tested) then my own sampling procedure may have been improved greatly by the extraction of numerous subsamples from the bulked field samples. This would have improved the estimate of the mean for each plot; thereby reducing the experimental error term and providing a measure of sampling error. The disadvantage to subsampling is the considerable investment of time required in preparation and counting of each additional subsample.

The significantly different population densities prior to the application of treatments, observed in the soil in the

noncropped experiment (Table 4.1) and the apple tree roots in the orchard experiment (Table 4.4), may be a result of sampling error or a chance occurrence.

5.2 MARIGOLDS

The results from the uncropped experiment, which indicated no significant difference in soil population densities of *P. penetrans* in the *T. patula* or the weeded treatment (Table 4.1), are contrary to many reports of the near eradication of soil populations of *P. penetrans* by *T. patula* (Winoto-Suatmadji 1969, Vrain 1989 (pers. comm.), Merwin and Stiles 1989).

The average number of *P. penetrans* per gram of *T. patula* root was 5 per gram (Table 4.2) which is very low compared with the numbers extracted from sorghum-sudangrass hybrid and the Oat cultivar treatments, which were 94 and 548 per gram of root respectively. Based on the literature, this suggests that *P. penetrans* was not reproducing in *T. patula*. A possible explanation for the sustained densities of *P. penetrans* in the *T. patula* plots is the small number of weeds which continually proliferated between weedings and provided suitable alternative hosts for *P. penetrans* reproduction. Another possible explanation for the unimpressive results is the *T. patula* cultivar, "Petite Harmony", which was reported by Rickard and Dupree (1978) to be ineffective at suppressing soil populations of *Meloidogyne spp*. This suggests that the cultivar used may not be a preferred cultivar for nematode control. However, Vrain (pers. comm. 1989) observed a reduction in soil densities of *P. penetrans* following a culture of this variety, thus supporting the explanation that *P. penetrans* proliferated in the weeds.

In the orchard experiment, the failure of *T. patula* to reduce soil population densities of *P. penetrans* (Table 4.3) may be explained by the presence of the apple trees. With large population densities of *P. penetrans* in apple roots, any subtle reduction in population soil densities resulting from *T. patula* intercropping could be buffered by the nematodes moving into the

soil close to the roots. This result conflicts with that of Hoestra and Oostenbrink (1962), who reported a significantly lower soil population in the rhizosphere soil of apple trees intercropped with *T. patula*.

Population densities (Table 4.4) of *P. penetrans* in the apple root were not significantly affected by the intercropping of *T. patula* when compared with the weeded control. There is little evidence in the literature suggesting a preferential attraction of nematodes to *T. patula* roots as opposed to other types of roots. Winoto-Suatmadjii's (1969) tested the attraction of *P. penetrans* to different host plants in an agar medium. Results from his experiment suggested there was no influence of roots from either good or poor host plants on the movement of *P. penetrans*. If true, or assuming there is an equal attraction of *P. penetrans* to the roots of *T. patula*, and the roots of other plants, then intercropping might prove somewhat effective if both plants are planted simultaneously. In this scenario, nematodes entering the *T. patula* root would be unable to reproduce and eventually this could result in a significant overall decrease in the total *P. penetrans* populations. This hypothesis was tested in the greenhouse experiment where apple seedlings and *T. patula* seeds were planted simultaneously into soil inoculated with *P. penetrans*.

In the greenhouse experiment, there were significantly lower *P. penetrans* densities in the soil with *Tagetes* intercropped (Table 4.5). There was a tendency for fewer *P. penetrans* to be in the roots of apples with *T. patula* intercropped than without, but this result was not significant.

The calculation of the number of nematodes per pot is biased but is included for discussion purposes. The most important bias is that the root density was multiplied by the total root fresh weight to determine the number of *P. penetrans* in the entire root system. *P. penetrans* were extracted only from the fine feeder roots in each pot, while fresh weight was a measurement of the entire root mass including the larger roots

which are less suitable for *P. penetrans* reproduction. Since I have no estimate of the feeder to larger root ratio for each pot, a simple multiplication of population density by total fresh weight will result in an over estimation of the number of nematodes in each root system. This overestimation may be even greater in those pots whose fine feeder root system was largely destroyed by the nematode population. Nevertheless, as *T. patula* did not effect the apple root dry (Table 4.8) or fresh weight, the calculated value gives us an estimate of the effect of *T. patula* on the entire population per pot. Although reduction in the total number of *P. penetrans* per pot was not significant at $P < 0.05$ it would have been at $P \leq 0.1$. Even if this reduction were statistically significant it is unlikely to be biologically or agronomically significant.

The reduction in seedling growth observed with the intercropping of *T. patula* (Table 4.8 and Table 4.9) is of serious concern. Whether this is a competition or allelopathic effect cannot be determined by this experiment. If it is a competition effect, then it may have been caused by the greenhouse conditions where root growth was severely restricted. Results demonstrating a significant positive growth response of apple seedlings following *Tagetes* in rotation (Winoto-Suatmadji 1969, Merwin and Stiles 1989) suggest that if the effect observed here is allelopathic, there is no persistent toxicity in the soil.

There was a significant interaction between *T. patula* and the different densities of *P. penetrans* inoculum on final stem length (Table 4.9) and stem dry weight (Table 4.8). The near significant ($P < 0.06$) interaction between marigold and nematode effects can be seen graphically in Figure 3. This interaction is explained by *T. patula* reducing the growth of the apple trees as a result of competition, but there is no additive negative effect of *P. penetrans* to this competition effect at these levels of inoculation. Determination of the true extent of this competition effect would require a similar trial be conducted in the field.

The results of the orchard and greenhouse experiments suggest that intercropping *Tagetes* with apple trees is ineffective at controlling *P. penetrans*. This observation is in accordance with much of the work reported on intercropping with *Tagetes* (Tarjan 1960, Miller and Aherns 1969, Hackney and Dickerson 1975, Motsinger et al. 1977.). The potential competition effect caused by *T. patula* together with it's poor performance at reducing *P. penetrans* populations suggest that it is of little value as an intercrop.

The effectiveness of *T. patula* as a cover crop for *P. penetrans* reduction between tree removal and replanting is unsubstantiated by the results of the noncropped experiment. However, lack of repitition of this trial and the considerable success of *P. penetrans* control by *T. patula* reported in the literature supports further investigation of *T. patula* for *P. penetrans* control.

From a practical basis, *T. patula* proved difficult to establish and competed poorly with weeds. These are serious limitations for a cover crop and were recognized by Winoto-Suatmadji (1969) as being two of the primary restrictions to *Tagetes* use. If the few weeds that proliferated in the plots were the source of the *P. penetrans* found in the *T. patula* plots in the noncropped experiment, then the development of a herbicide program for *T. patula* culture is critical. Another major obstacle with using *T. patula* in an orchard rotation for *P. penetrans* control is that it requires the orchard to be out of production for an additional growing season, which is an unacceptable cost to many producers.

5.3 PLASTIC MULCHES

Solarization with clear polyethylene mulch maintained low nematode populations by mid-season in both field experiments (Tables 4.1 & 4.4). The duration of the solarization at mid-season was longer than that used by many other workers (Katan 1984, Stapleton and DeVay 1983), nevertheless the plots were maintained to allow comparisons with other treatments. The

rebounding of the population at the final sampling date may have resulted from the following phenomena. In the noncropped experiment weeds were effectively controlled by the solarization effect until the beginning of September. After this time weeds began to proliferate under the plastic, presumably due to the lower soil temperatures accompanying the cooler weather. Any surviving nematodes not killed by solarization may have then reproduced on the weeds. Moreover, the low mid-season count may have been a result of the nematodes moving deeper into the soil in response to increased soil temperatures and resurfacing when these extreme temperatures subsided later in the season.

A significantly lower *P. penetrans* population density was observed in the roots of apple trees under the clear plastic mulch than in the weeded control plots at the mid-season (Table 4.4). However, there was no corresponding reduction in soil population densities of *P. penetrans* (Table 4.3); this discrepancy and the extreme variability observed in all plots suggest that significant differences in root counts in this situation may have been an artifact of the data. This mid-season trend did not show at the final sampling date. Weed control was a continuous problem under the clear plastic in the orchard as the plastic could not be sealed and heat build-up at the base of the tree was most likely limited.

Sub-optimal soil temperatures, 30° C to 40° C, have been demonstrated to reduce root and shoot growth of apple trees (Gur et al. 1972). Therefore, any positive effect resulting from decreased *P. penetrans* root densities following solarization might well be offset by a corresponding reduction in growth from excessive heating.

Considerable technical problems were encountered with the Omnidata Easylogger and complete temperature data sets were not obtained. However, temperatures in early August under the clear plastic reached 49° C at 5 cm and 32° C at 20 cm. Mamiya (1971) reported that the development of *P. penetrans* was inhibited in conifer seedlings grown at a constant soil temperature of 33° C.

The temperatures recorded in this experiment are comparable to temperatures reported by others in successful solarizations (Stapleton and DeVay 1986) and it indicates that solarization has potential at locations in the interior of British Columbia.

Use of solarization in apple replant situations is somewhat limited by seasonal considerations. To be most effective in the Okanagan solarization should be implemented during the hottest months of July and August, and replanting should be done in the spring to enable tree establishment by fall. The incompatibility of simultaneous solarization and establishment of young trees would require that the land be left uncropped in the year of treatment. Additionally, in the summer of treatment further management would be required to control weeds following solarization to discourage a rebuilding of *P. penetrans* populations. This might be achieved through the use of residual herbicides or a competitive cover crop that is a poor host of *P. penetrans*.

The root count of *P. penetrans* under the black polyethylene mulch (Table 4.4) at the orchard site was greater than 300 *P. penetrans* per gram of root compared to less than 150 *P. penetrans* per gram of root for each of the other three treatments. Significantly different ($P < 0.05$) than counts from the weeded control or the *Tagetes* treatment, this result suggests that the black plastic mulch may have created an environment in the roots advantageous to the development of the *P. penetrans* populations perhaps by increasing soil temperatures closer to 30° C which has been determined to be advantageous to development of *P. penetrans* (Mamiya 1971, Mai et al. 1977). This result contradicts the work of Miller and Waggoner (1963) and Colbran (1979) who observed significant reductions in *P. penetrans* populations in the soil under young apple trees mulched with black plastic.

If black plastic mulch increases nematode reproduction in the roots of apple trees then this drawback must be weighed against the reported benefits of weed control, growth response

to soil warming (Mage 1982), and a positive growth response, comparable to that of biocides when used in some replant situations (Jensen and Buszard 1988).

5.4 CEREALS

Neither the oat variety "Cascade" nor the sorghum - sudangrass hybrid "Pioneer 988" appeared to be an unfavorable host of *P. penetrans* (Table 4.1 and Table 4.2). The conflicting reports in the literature regarding these species suggest that the level of suceptibility may vary greatly between cultivars within a species (MacDonald and Mai 1963, Bird 1968, Marks et al 1972, Marks and Townshend 1973, Colbran 1979). It is interesting to note that the final root count in sorghum-sudangrass treatment was 94 *P. penetrans* per g of root, which is much lower than the mid-season average of 424 *P. penetrans* per gram of root (Table 4.1). Correspondingly the soil count from the sorghum-sudangrass hybrid plots increased over the same period from 126 to 494 *P. penetrans* per 50 ml of soil (Table 4.2). This suggests there may have been a movement from the sorghum roots to the soil later in the season. The soil counts of *P. penetrans* for both the oats and the sorghum-sudangrass hybrid were higher than for the solarization, *T. patula* or weeded treatments, suggesting that these cultivars of oats and sorghum-sudangrass would be unsuitable rotation crops for *P. penetrans* reduction.

The advantages of easy management, soil conservation, addition of organic matter to the soil and economic return through the sale of sillage, hay or grain make cereal crops attractive to the grower. On the basis of the results from this study, further investigation of annual grains as cover crops for *P. penetrans* control, particularly those cultivars reported to support low *P. penetrans* numbers, such as the oat cultivar "Saia" (Colbran 1979, Townshend 1989) and the sudangrass cultivar "Piper" (MacDonald and Mai 1963), is considered necessary.

6. CONCLUSIONS

Each of the three experiments reported upon was done without repitition. Consequently, these "conclusions" should be considered in that context and with the realization that further experiments are necessary.

None of the cultural practices evaluated in this study was more effective at reducing *P. penetrans* populations, either in rotation or intercropped with apple trees, than that which was achieved by clear cultivation.

Results from the *T. patula* and solarization treatments in the absence of apple trees are inconclusive. The greenhouse experiment offers some evidence that intercropping with *T. patula* is ineffective at reducing root densities of *P. penetrans* in apple seedlings. Intercropping of *T. patula* in the greenhouse resulted in a relative reduction in dry weight of the apple seedlings which may be attributable to either competition or allelopathy. Further study is necessary in order to obtain conclusive results.

Both the oat cultivar "Cascade" and the sorghum-sudangrass hybrid "Pioneer 988" were good hosts of *P. penetrans*. Investigation of other sorghum-sudangrass and oat cultivars reported in the literature are warranted.

Based upon the literature reviewed and the observed effects of *P. penetrans* on apple seedling growth in the greenhouse trial it is my opinion that *P. penetrans* can contribute extensively to damaging apple trees. The degree to which *P. penetrans* damages apple trees replanted in the field, the factors contributing and interacting to cause that damage, and the overall importance of *P. penetrans* in tree fruit production in British Columbia, are unclear. Both short and long term field studies are necessary to establish the precise role of this pathogen in apple replant problems. Vrain and Yorston (1987) clearly established that *P. penetrans* is often found in British Columbian orchards. They identified soil population densities of *P. penetrans* in the root

zone of established orchards comparable to the damaging pre-plant thresholds determined by Hoestra and Oostenbrink (1962). However, the relationships among pre-plant soil densities, established root densities and damage are unclear.

Perhaps the most cost effective approach to determining the importance of *P. penetrans* in orchards is combined horticultural/pest management studies similar to the one reported upon here. Essential elements needed in such a study would include: 1) continuous sampling throughout the season to determine its population dynamics of *P. penetrans* in an orchard; 2) a study on the distribution of *P. penetrans* in roots and soil within orchards to calibrate sampling for advisory purposes; 3) determination of a damage threshold under B.C. growing conditions using different cover crops and fumigants to establish different *P. penetrans* pre-plant soil population densities; 4) determination of the relationship among pre-plant *P. penetrans* densities, tree damage and population densities of *P. penetrans* after damage has been incurred, as a diagnostic tool. A suitable time frame for a study of this type would be three to five years to account for seasonal variations and enable sufficient tree growth and crop yield to accurately measure damage.

7. LITERATURE CITED

Adams, R. W. 1987. Handbook for applicators and dispensers, fifth edition. Ministry of Environment and Parks, Province of British Columbia, Victoria, B.C. 248 pp.

Arneson, P. A. and Mai, W. F. 1976. Root Diseases of Fruit Trees in New York State VII. Costs and returns of Prerplant Soil Fumigation in a Replanted Apple Orchard. Plant Dis. Rep. **60**:1054-1060.

Barker, K. R. 1985. Sampling Nematode Communities. *In* An Advanced Treatise on *Meloidogyne* Volume II: Methodology. Barker, K.R., Carter, C.C. and J. N. Sasser [eds.] Raleigh, N.C., U.S.A. Department of Plant Pathology, North Carolina State University. pp. 3-17

Barker, K. R. and Olthof, T. H. A. 1976. Relationship between nematode population densities and crop responses. Annu. Rev. Phytopathol. **14**:327-353.

Bird, G. W. 1968. Influence of six cover crops on population densities of *Pratylenchus penetrans*. Can. Plant Dis. Surv. **48**:113.

Bird, G. W. 1980. Nematology-Status and Prospects: The Role of Nematology in Integrated Pest Management. J. Nematol. **12**: 170-176.

Brodie, B. B. 1976. Vertical Distribution of Three Nematode Species in Relation to Certain Soil Properties. J. Nematol. **8**:243-247.

Brown, E. B. 1982. Cultural and biological control methods. *In* Plant Nematology. Southey, J. F. [ed.] Her Majesty's Stationary Office. pp. 269-282.

Cameron, H. R., Westwood, M. N. and Lombard, P. B. 1986. Soil-borne organisms affecting replanted pears. Hortscience. **21**: 1306-1310.

Colbran, R.C. 1954. Problems in tree replacement II. The effects of certain methods of management on the nematode fauna of an orchard soil. J. Aust. Inst. Agric. Sci. **20**: 234-237.

Colbran, R. C. 1979. Problems in tree replacement. III The effects of cultural practices and soil fumigation on the root-lesion nematodes and on growth of apple replants. Aust. Agri. Res. **30**:113-123.

Daulton, R. C. A. and R. F. Curtis 1963. The effect of *Tagetes* spp. on *Meloidogyne javanica* in southern Rhodesia. Nematologica **9**:357-362.

Dunn, R.A. and W.F. Mai. 1973. Reproduction of *Pratylenchus penetrans* in roots of seven cover cropspecies in greenhouse experiments. Plant Dis. Rep. **57**:728-730.

Edgerton, L. J. and K. G. Parker. 1958. Effect of nematode infestation and rootstock on cold hardiness of Montmorency Cherry trees. Proc. Am. Hortic. Sci. **72**: 134-138.

Egunjobi, O. E. 1968. An Ecological Study of Some Soil Nematodes Associated with Apple Trees in New Zealand Grassed Orchards. N. Z. J. Agric. Res. **11**:386-406.

Elliot, J. M. 1971. Methods for the statistical analysis of benthic invertebrates. Freshwater Biological Association Scientific Publication 25. Windermere England 156pp.

Ferris, H. 1980. Nematology-status and prospects: practical implementation of quantatative approaches to nematology. J. Nematol. **12**:164-170

Ferris, H. 1984. Nematode Damage Functions: The Problems of Experimental and Sampling Error. J. Nematol. **16**:1-9.

Francl, L. J. 1986. Spatial analysis of *Heterodera glycines* Populations in Field Plots. J. Nematol. **18**:183-189.

Gommers, F. J. 1973. Nematicidal Principles of *Compositae*. H. Veeman & Zonen B. V., Wageningen. 71pp.

Gommers, F. J. and Voorin't Holt, D. J. M. 1976. Chemotaxonomy of Compositae related to their host suitability for *Pratylenchus penetrans*. Neth. J. Plant Pathol. **82**:1-8.

Goodell, P. B. and Ferris, H. 1980. Plant-parasitic Nematode Distributions in an Alfalfa Field. J. Nematol. **12**:136-141.

Goodell, P. B. and Ferris, H. 1981. Sample Optimization for Five Plant Parasitic Nematodes in an Alfalfa Field. J. Nematol. **13**: 304-313.

Gur, A., Bravdo, B. and Mizrahi, Y. 1972. Physiological responses of apple trees to supraoptimal root temperature. Physiol. Plant. **27**: 130-138.

Hackney, R. W. and Dickerson, O. J. 1975. Marigolds, castor bean, and Chrysanthemum as controls of *Meloidogyne incognita* and *Pratylenchus alleni*. J. Nematol. **7**:84-90.

Hague, N. G. M. and Gowen, S. R. 1987. Chemical control of nematodes. *In* Principles and Practice of Nematode Control in Crops. Brown, R. H. and B. R. Kerry (eds.) Academic Press, New York. pp. 131-178.

Hijink, M. J. and Winoto Suatmadji, R. 1967. Influence of different compositae on population density of *Pratylenchus penetrans* and some other root-infesting nematodes. Neth. J. Plant Pathol. **73**:71-82.

Hoestra, H., and Oostenbrink, M. 1962. Nematodes in relation to plant growth. IV. *Pratylenchus penetrans* on orchard trees. Neth. J. Agric. Sci. **10**:286-296.

Howard, H. W. and Cotten, J. 1982. Nematode resistant crop plants. *In* Plant Nematology. Southey, J. F. [ed.] Her Majesty's Stationary Office.

Hussey R. S. and R. W. Roncadori. 1982. Vesicular-Arbuscular Mycorrhizae may limit nematode activity and improve plant growth. Plant Disease **66**:9-14.

Jaffee, B. A. and W. F. Mai. 1979a. Growth reduction of apple seedlings by *Pratylenchus penetrans* as influenced by seedling age at innoculation. J. Nematol. **2**:161-165.

Jaffee, B. A. and W. F. Mai. 1979b. Effect of water soil potential on growth of apple trees infected with *Pratylenchus penetrans*. J. Nematol. **2**:165-168.

Jaffee, B. A., G. S. Abawi and W. F. Mai. 1982a. Role of soil microflora and *Pratylenchus penetrans* in an apple replant disease. Phytopathology **72**:247-251.

Jaffee, B. A., Abawi, G. W., and Mai, W.F. 1982b. Fungi associated with roots of Apple Seedlings Grown in Soil from an Apple Replant Site. Plant Dis. **66**:942-944.

Jensen, P. and Buszard, D. 1988. The effects of chemical fumigants, nitrogen, plastic mulch and Metalaxyl on the establishment of young apple trees in apple replant disease soil. Can. J. Plant Sci. **68**:255-260.

Johnson, A. W. and G. W. Burton. 1973. Comparison of millet and sorghum-sudangrass hybrids grown in untreated soil and soil treated with two nematicides. J. Nematol. **5**:54-59.

Johnson, A. W., Sumner, D. R., Jaworski, C. A. and Chalfant, R. B. 1977. Effects of management practices on nematode and fungi populations and Okra yield. J. Nematol. **9**:136-142.

Jones, E. M. and Mai, W.F. 1964.[Abs] Migration and survival of various stages of *Pratylenchus penetrans* in relation to soil moisture and aggregate range. Phytopathology **54**:897.

Jones, F. G. W. 1982. The soil-plant environment. *In* Plant Nematology. Southey, J. F. [ed.] Her Majesty's Stationary Office. pp. 46-62.

Karandinos, M. G. 1976. Optimum Sample Size and Comments on some Published Formulae. Bull. Entomol. Soc. Am. **22**:417-421.

Katan, J. 1984. Soil Solarization. *In*: Second International Symposium on Soil Disinfestation. Acta Hortic.,Van Assche, C. [ed] No. 152, Leuven, Belgium: International Society for Horticultural Science. pp 227.

Kimpinski, J. and Dunn, R. A. 1985. Effects of low temperatures in the field and laboratory on survival of *Pratylenchus penetrans*. Plant Dis. **69**: 526-527.

Kleinschmidt G. D. and J. W. Gerdemann. 1972. Stunting of citrus seedlings in fumigated nursery soils related to the absence of Endomychorrhizae. Phytopathol. **62**:1447-1453.

MacDonald D.H. and W.F.Mai. 1963. Suitability of Various Cover Crops as Hosts for the Lesion Nematode, *Pratylenchus penetrans*. Phytopathol. **53**:730-31.

MacGuidwin, A.E. 1989. Distribution of *Pratylenchus scribneri* between root and soil habitats. J Nematol **21**:409-415.

Mage, F. 1982. Black plastic mulching compared to other soil management methods. Sci. Hortic. **16**:131-136.

Mai, W. F. and Abawi, G. S. 1978. Determining the cause and extent of apple, cherry and pear replant diseases under controlled conditions. Phytopathol. **68**:1540-1544.

Mai, W.F. and G.S. Abawi. 1981. Controlling Replant Diseases of Pome and Stone Fruits in Northeastern United States by Preplant Fumigation. Plant Dis. **65**:859-864.

Mai, W.F., J.R. Bloom and T.A. Chen, 1977. Biology and Ecology of the Plant-Parasitic Nematode *Pratylenchus penetrans*. Bulletin 815, Pennsylvania State University College of Agriculture. Agricultural Experiment Station University Park, Pennsylvania. pp. 64.

Mamiya, Y. 1971. Effects of temperature on the life cycle of *Pratylenchus penetrans* on *Cryptomeria* seedlings and observations on its reproduction. Nematologica **17**: 82-92.

Marks C. F. and J. L. Townshend 1973. Multiplication of the root-lesion nematode *Pratylenchus penetrans* under orchard cover crops. Can. J. Plant Sci. **53**: 187-188.

Marks, C. F., Saidak, W. J. and P. W. Johnson. 1973. Effects of soil management on numbers of the root-lesion nematode *Pratylenchus penetrans* in soils of Ontario peach orchards. Can. J. Plant Sci. **53**: 181-185.

McSorley, R. and Parrado, J. L. 1982. Estimating Relative Error in Nematode Numbers from Single Soil Samples Composed of Multiple Cores. J. Nematol. **14**:522-529.

Merwin, I. A. and Stiles, W. C. 1989. Root-lesion nematode, potassium deficiency, and prior cover crops as factors in apple replant disease. J. Amer. Soc. Hort. Sci. **114**:724-728.

Miller, P. M. 1977 Interaction of Plastic, Hay and Grass Mulches, and Meta-sodium on control of *Pratylenchus penetrans* in Tomatoes. J. Nematol. **9**:350-351.

Miller, P. M., and J. F. Aherns. 1969. Influence of growing marigolds, weeds, two cover crops and fumigation on subsequent populations of parasitic nematodes and plant growth. Plant Dis. Rep. **53**:642-646.

Miller, P.M. and P. E. Waggoner. 1963. Interaction of plastic mulch, pesticides and fungi in the control of soil-borne nematodes. Plant Soil **18**:45-52.

Motsinger, R. E., Moody E. H.and C. M. Gay. 1977. Reaction of certain french marigold (*Tagetes patula*) cultivars to three *Meloidogyne* spp. J. Nematol. **9**:278.

Mountain, W. G. and Patrick, Z. A. (1959) The peach replant problem in Ontario.VII. The pathogenicity of *Pratylenchus penetrans*.(Cobb) Can. J. Bot. **37**:457-470.

Nasbaum, C. J. and Ferris, J. The role of cropping systems in nematode population management. Annu. Rev. Phytopatol. **11**:423-439.

Neter, J., Nasserman, W. and Kutner, M.H. 1990. Applied Linear and Statistical Models. Third Edition. Irwin, Boston. pp. 1181.

Noe, J. P. and Campbell, C. L. 1985. Spatial Pattern Analysis of Plant-parasitic Nematodes. J. Nematol. **17**:86-93.

Olthof, Th. H. A. 1968. Races of *Pratylenchus penetrans*, and their effect on Black Root Rot Resistance of Tobacco. Nematologica **14**: 482-488.

Oostenbrink, M. 1972. Evaluation and integration of nematode control methods. *In* Economic Nematology, ed. J. M. Webster, 497-514. New York: Academic Press. 563 pp.

Parker, K. G.and W. F. Mai. 1974. Root disease of fruit trees in New York State. VI. Plant Dis. Rep. **58**:1007-1011.

Pitcher, R. S., Z.A. Patrick and W.B. Mountain. 1960. Studies on the Host-Parasite Relations of *Pratylenchus penetrans* (Cobb) to Apple Seedlings. Nematologica **5**:309-314.

Powell, N. T., 1971. Interactions between Nematodes and Fungi in Disease Complexes. Annu. Rev. Phytopathol. **9**: 253-274.

Proctor, J. R. and C. F. Marks. 1974. The determination of normalizing transformations for nematode count data from soil samples and of efficient sampling schemes. Nematologica **20**:395-406.

Rackham, R., Radewald, J. D., Hemstreet, C. L. and Shibuya, F. 1975. Lesion nematode [*Pratylenchus*] control in apples. Calif. Agric. **29**: 14-15.

Rickard, D. A. and Dupree, A. W. Jr. 1978. [Abs] The effectiveness of ten kinds of marigolds and five other treatments for control of *Meloidogyne* spp. J. Nematol. **10**: 296-297.

Rodriguez-Kabana, R. and Curl, E. A. 1980. Nontarget Effects of Pesticides on Soilborne Pathogens and Disease. Annu. Rev. Phytopathol. 1980. **18**:311-332.

Seinhorst, J. W. 1950. De betekins van de toestand van de grond voor het optreden van aantasting door het stengelaaltje (Ditylenchus dispaci (Kuhn) Filipjev). Tijdschr. PlZiekt. **56**: 289-348.

Seinhorst, J. W. 1965. The Relationship between Nematode Density and Damage to Plants. Nematologica **11**:137-154.

Slykhus, J. T. and Li, T. S. C. 1985. Responses of apple seedlings to biocides and phosphate fertilizers in orchard soils in British Columbia. Can. J. Plant Pathol. **7**: 294-301.

Sokal, R. R. and Rohlf, J.R. 1973. Introduction to biostatistics. W. H. Freeman and Company, San Francisco. pp. 368.

Southwood, T.R.E. 1978. Ecological methods with particular reference to the study of insect populations. New York :Halsted Press. pp. 391.

Stapleton, J.J. and DeVay, J. E. 1983. Response to Phytoparasitic and Free-Living Nematodes to Soil Solarization and 1,3-Dichloropropene in California. Phytopathol. **73**: 1429-1435.

Stapleton, J.J. and DeVay, J. E. 1986. Soil Solarization: a non-chemical approach for management of plant pathogens and pests. Crop Protection 5(3): 190-198.

Tarjan, A. C. 1960. Some effects of African marigold on the citrus burrowing nematode, *Radopholus similis*. [Absract] Phytopathol. **50**: 577.

Townshend, J. L. 1989. Population densities of four species of root-lesion nematodes (*Pratylenchus*) in the oat cultivars, Saia and OAC Woodstock. Can. J. Plant Sci. **69**:903-905.

Townshend, J. L. and T. R. Davidson, 1960. Some weed hosts of *Pratylenchus penetrans* in premier strawberry plantations. Can. J. Bot. **38**:267-273.

Townshend, J. L., Cline R. A., Dirks V. A. and Marks, C. F. 1984. Assesment of turfgrasses for the management of *Pratylenchus penetrans* and *Pratylenchus projectus* in orchards. Can. J. Plant Sci. **64**:355-360.

Traquair, J. A. 1984. Etiology and control of orchard replant problems: a review. Can. J. Plant Pathol. **6**:54-62.

Uhlenbroek, J. H. and Bijloo, J. D. 1958. Investigations on nematicides. 1. Isolation and structure of a nematicidal principe occuring in *Tagetes* roots. Rec. Trav. Chim. Pays-Bas Belg. **77**:1004-1009

Van Berkum, J. A. and Hoestra, H. 1979. Practical aspects of the chemical control of nematodes in soil. *In* soil disinfestation. Mulder, D. [ed] pp. 53-134.

Vrain, T. C. 1988. Fumigants, Fumigation, Facts and Fiction. Proceedings LMHIA 27th Annual Meeting 107-108.

Vrain, T.C. 1987. Effect of *Ditylenchus dipsaci* and *Pratylenchus penetrans* on Verticillium Wilt of Alfalfa. J. Nematol. **19**:379-383.

Vrain, T. C. and Copeman, R. J. 1987. Interactions between *Agrobacterium tumefaciens* and *Pratylenchus penetrans* in the roots of two raspberry cultivars. Can. J. Plant Pathol. **9**: 236-240.

Vrain, T. C. and Yorston, J. M. 1987. Plant-Parasitic Nematodes in Orchards of the Okanagan Valley of British Columbia, Canada. Plant Dis. **71**: 85-87.

Willis, C. B., Kimpinski, J. and Thompson, L.S. 1982. Reeproduction of *Pratylenchus crenatus* and *P. penetrans* on forage legumes and grasses and effect on forage yield. Can. J. Plant Pathol. 4: 169-174.

Winoto Suatmadji, R. 1969. Studies on the effects of *Tagetes* species on plant parasitic nematodes. H. Veenman & Zonen, B. V., Wageningen. 134pp.

Zepp, A. L. and Szczygiel, A. 1987. [Abs] Helminthological Abstracts (Series B) **56**: 96-97.