Journal of Nematology 48(4):241–247. 2016.

Distribution and Longevity of *Pratylenchus penetrans* in the Red Raspberry Production System

Duncan R. Kroese,[1] Jerry E. Weiland,[2] and Inga A. Zasada[2]

Abstract: One of the major constraints on the production of red raspberries in the Pacific Northwest is the presence of the root-lesion nematode *Pratylenchus penetrans*. Current management of this nematode relies heavily on preplant soil fumigation; however, regulations have made the practice more difficult and expensive. Additional issues with soil fumigation include lack of efficacy at deeper soil depths and potential inability to penetrate raspberry root material that remains in the field during fumigation which may harbor *P. penetrans*. To address these issues, two field experiments were conducted in northwestern Washington. In the first experiment, the residency time of *P. penetrans* in root material from the previous raspberry crop, which was terminated with or without the use of herbicides, was monitored over time. *Pratylenchus penetrans* was found in root material from 6 to 8 mon after the crop was terminated, and herbicide application did not reduce *P. penetrans* residency time compared to untreated root material. In a second experiment, the vertical distribution of *P. penetrans* at three different times during the field establishment process (pre- and postfumigation, and at planting) was determined at two locations. Both locations had detectable prefumigation *P. penetrans* populations at all depths. However, postfumigation populations showed a different distribution pattern between locations. The location with coarser soil had populations located mainly at shallower depths with a maximum of 44 *P. penetrans*/100 g soil at 16 to 30 cm deep, whereas the location with finer soil had populations located mainly at deeper depths with a maximum of 8 *P. penetrans*/100 g soil at 76 to 90 cm deep. At planting, distribution tended to equilibrate among depths at both locations, but the overall population pattern across depth at each location was similar to that observed at postfumigation. Understanding more about the residency time and distribution of this nematode may provide growers with information that can be used to more effectively target *P. penetrans*.

Key words: fumigation, root-lesion nematode, *Rubus*, soil type, survival.

The Pacific Northwest produces a majority of the processed red raspberries (*Rubus idaeus*) in the United States. Within this region, Washington produces 95% of the country's total with an estimated value of over $65 million in 2015 (USDA, 2016). One of the most important factors limiting production of raspberry in this region is the presence of the plant-parasitic nematode *Pratylenchus penetrans* (McElroy, 1991; Gigot et al., 2013a).

Pratylenchus spp., root-lesion nematodes, are the third most economically important genus of plant-parasitic nematodes to crop productivity worldwide following cyst nematodes (*Heterodera* spp. and *Globodera* spp.) and root-knot nematodes (*Meloidogyne* spp.) (Davis and MacGuidwin, 2005). Of the *Pratylenchus* species, *P. penetrans* is the most important in red raspberry production (Bélair and Khanizadeh, 1994; McElroy, 1977). *Pratylenchus penetrans*, a migratory endoparasite, has over 400 hosts that include commercial crops, cover crops, and weed species (Davis and MacGuidwin, 2005). Both feeding and migration of *P. penetrans* within a root can damage the plant. Although brief feeding does not typically cause extensive damage, longer periods of feeding may result in cell death. Migration of *P. penetrans* within the root is accomplished by the breakdown of cell walls, causing death of the cells along the migration route (Zunke, 1990). Feeding and migration by *P. penetrans* typically occurs within small diameter fine roots (Eissenstat, 1992) and symptoms appear as necrotic spots or lesions (McElroy, 1992). These necrotic areas can collapse, causing a reduction in fine root abundance and leading to aboveground symptoms of water and nutrient deficiency, such as chlorotic foliage and reduced growth, as uptake is reduced due to root damage (Wilder and Righetti, 1991; Davis and MacGuidwin, 2005). Past field surveys have shown that *P. penetrans* is widespread in red raspberry fields in Canada, Scotland, and northwest Washington (McElroy, 1977; Trudgill and Brown, 1978; Gigot et al., 2013b). It has also been demonstrated that plants infested with *P. penetrans* have reduced establishment, growth, and yield (McElroy, 1977; Trudgill and Brown, 1978; Zasada et al., 2015). Due, in part, to the presence of *P. penetrans*, the productive lifespan of raspberry fields in northwest Washington have decreased from over 10 yr to 5 to 7 yr (McElroy, 1992; Wilcox et al., 1993).

Currently, management of this nematode revolves around the use of preplant soil fumigation with 1-3-dichloropropene and chloropicrin and, to a lesser extent, the use of postplant nematicides. However, recent changes in fumigation regulations may make it increasingly difficult for raspberry growers to rely on soil fumigation as a management practice (USEPA, 2012). Along with new restrictions, there are other concerns regarding the efficacy of soil fumigation. One potential concern is the depth at which fumigation occurs. Fumigants are typically injected into the soil with shanks at an approximate depth of 45 cm. Unlike methyl bromide, currently registered fumigants do not move downward in the soil profile (McGovern et al., 1998; Martin, 2003). Any nematodes that reside below the depth of injection may not come in contact with applied fumigants, and could thus be a potential source of

Received for publication May 13, 2016.
[1]Department of Botany and Plant Pathology, Oregon State University, Cordley Hall, Corvallis, OR 97331.
[2]USDA-ARS Horticultural Crops Research Laboratory, 3420 Northwest Orchard Avenue, Corvallis, OR 97330.
We thank Tom Walters, Walters Ag Research, for assistance with this research.
Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.
E-mail: inga.zasada@ars.usda.gov.
This paper was edited by Jim Lamondia.

inoculum for the next planting. *Pratylenchus penetrans* can be found at soil depths of 25 to 30 cm in red raspberry (Forge et al., 1998) but *Pratylenchus* spp. have also been detected 60 to 70 cm deep in maize fields and up to 80 cm deep in apple orchards (Pudasaini et al., 2006). Therefore, it is likely that *P. penetrans* may occur deeper in red raspberry field soils than the 45-cm fumigation depth.

Another concern with traditional fumigation is the presence of infected root material that remains in the field at the time of fumigation. Typical preparation of a raspberry field for fumigation occurs very rapidly. After a harvest, trellises are removed and plants are either treated or not treated with herbicide, based on grower preference, and mowed. The field is then deep ripped and 1 to 2 mon after the termination of the previous planting, fumigation takes place. This process does not involve removal of old plant or root material from the field prior to fumigation or replanting the following spring. If old roots are infected with *P. penetrans*, they may have the potential to provide protection from fumigation (Zasada et al., 2010) and create an inoculum source for the following planting.

The goals of this study were to (i) determine the longevity of *P. penetrans* within infected raspberry roots over time in a buried root assay under field conditions and (ii) determine the vertical distribution of *P. penetrans* in soils during the raspberry establishment timeline. Knowing more about the biology and distribution, *P. penetrans* will provide raspberry growers with more information to improve current fumigation management practices.

Materials and Methods

Survival of P. penetrans *in raspberry root material:* To evaluate the longevity of *P. penetrans* in roots over time, bags containing nematode-infested roots and autoclaved field soil were prepared for a buried root assay at two field sites. In September 2013, roots and soil were collected from a 'Meeker' raspberry field known to be infested with *P. penetrans* in Lynden, WA. At this time, the field was in the beginning stages of being replanted. The trellises had been removed and the old canes mowed, leaving the roots and crowns of the plants in the field. Two different groups of roots were collected: roots from plants treated with herbicides prior to mowing (herbicide-treated) and roots from plants that were not treated with herbicides (nontreated). Herbicide treatment included the application of Roundup (glyphosate; Monsanto Company, St. Louis, MO) and Crossbow (2,4-dichlorophenoxyacetic acid, Triclopyr BEE; Dow AgroSciences, Indianapolis, IN) both at a rate of 4.7 liter/ha. Roots were collected by digging around a plant crown with a shovel and pulling the root system out by hand. Both fine (<1 mm in diam.) and coarse (>1 mm in diam.) root materials were collected from herbicide-treated and nontreated plants. Herbicide-treated and nontreated roots were kept separate, and transported to the laboratory in coolers. Field soil, collected adjacent to herbicide-treated and nontreated plants, respectively, was added to the coolers to cover the corresponding root samples and prevent roots from desiccating.

In the laboratory, roots were shaken free of soil and the soil was reserved for incorporation into the root inoculum bags. Although still keeping treatments separate, roots were then chopped into 2- to 6-cm long pieces and mixed until homogenized. To determine initial population densities of *P. penetrans* in the root material, six 18 g subsamples of roots were collected from both herbicide-treated and nontreated roots and placed under intermittent mist for 7 d (Ingham, 1994). After extraction of nematodes, roots were then placed in a 65°C drying oven and weighed after 5 d to determine dry weights. Extracted juvenile and adult *P. penetrans* were enumerated using a dissecting microscope at ×40 magnification. The soil reserved after processing the roots was autoclaved for 30 min at 121°C and 103 kPa. To prepare root inoculum bags for placement in the field, 18 g of root material collected from the field, a mixture of approximately equal mass of fine and coarse roots, was weighed and placed in a nylon bag (Hanesbrands, Winston-Salem, NC). Approximately 250 cm^3 of autoclaved field soil was then added to the bag. The nylon bag was tied off and a 60 cm piece of fishing line (South Bend, Northbrook, IL) was tied around the knotted end. The type of root material in the nylon bag was then indicated by tying colored flagging to the free end of the fishing line. A total of 240 root inoculum bags were prepared for each treatment.

Two field sites were selected for this experiment. The first location was at the Botany and Plant Pathology Farm in Corvallis, OR, and the second location was in a commercial field in Lynden, WA. At each location, a total area of 2,000 m^2 was designated for the trials. This area was then divided into ten 4.5 × 4.5 m blocks. Within each block, 24 holes were drilled in a 4 × 6 m grid pattern using a two man auger with a 15 cm diam. bit (Ground Hog, San Bernardino, CA) to a depth of 30 cm spaced 0.9 m apart. Then, in October 2013, a single bag of either herbicide-treated or nontreated roots was placed at random into each hole so that there were 12 bags of each treatment per block. Each hole was then filled while making sure that the flagging indicating treatment was visible aboveground.

Starting 2 mon after establishment of the trials, and occurring every 2 mon thereafter until *P. penetrans* was undetectable in the roots on two consecutive sampling dates, one bag of each treatment type was removed at random from each block at each location ($n = 10$). For each bag, a trenching shovel was used to dig down to a sufficient depth so that the bag could be easily

removed by pulling on the fishing line. Bags were placed in a cooler and transported to the laboratory. For processing, the bags were cut open and the roots inside were removed. The roots were washed to remove excess soil and *P. penetrans* was extracted by intermittent mist and roots were dried as described above. Extracted *P. penetrans*, juveniles and adults, were then counted using a dissecting microscope at ×40 magnification. The nematodes were identified as *P. penetrans* based on morphology (Castillo and Volvas, 2007) as well as the presence of males, a diagnostic trait for this species.

Data were $\log_{10}(x+1)$ transformed where x is the number of *P. penetrans*/g dry root to meet normality and equal variance assumptions. Data were then separated by field, collection date, and treatment. Analysis of variance was used to analyze the effects of treatment, sampling date, block, and the interaction of treatment and sampling date, with treatment and sampling date being fixed and block being random effects. Data were then analyzed using Tukey's honest significant difference (HSD) test to adjust for multiple comparisons and to determine if there were any significant differences between treatments and collection dates within a field. All statistical analyses were done using RStudio Version 0.99.491 (RStudio Inc., Boston, MA).

Vertical distribution of P. penetrans *in soil prior to and after fumigation:* In September 2014, two red raspberry fields in northern Washington known to have high population densities of *P. penetrans* were selected for this experiment. The first field was located in Lynden, WA, and had a loamy sand soil texture (70% sand, 20% silt, 10% clay, and 4.2% organic matter; A & L Western Agricultural Laboratories, Portland, OR). The second field was located in Everson, WA, and had a sandy loam soil texture (52% sand, 32% silt, 15% clay, and 2.6% OM). In both fields, raspberry plants had been removed and were being prepared for preplant fumigation. The fields were broadcast fumigated with 35% chloropicrin and 65% 1,3-dichloropropene (Telone C-35; Dow AgroSciences) at a rate of 433 kg/ha using a commercial deep-shank application apparatus with shanks spaced 30 cm apart and with product applied 40 cm below the soil surface (Trident Agricultural Products, Woodland, WA). Following fumigation, both fields were replanted the following spring with the raspberry cultivar Meeker. Population densities of *P. penetrans* in the Lynden and Everson fields in July 2014 were 11,379/g dry root and 4,060/g dry root, respectively. Soil samples, including any associated roots, were collected four times during the raspberry establishment timeline from each location. The first sampling (prefumigation) took place 2 wk before fumigation in early September 2014, the second sampling (postfumigation) took place 4 wk after fumigation (mid-October 2014), the third sampling (at planting) took place 25 wk after fumigation (late-March 2015), and the final sampling (postplant) took place 6 mon after planting (October 2015).

On the first three sampling dates, samples were collected from 10 permanent sampling locations randomly selected within each field, spaced at least 18 m apart. Samples were collected using a 5 cm diam. × 1.2 m long stainless-steel collection tube lined with a 4.5 cm diam. × 1.2 m long removable polyethylene terephthalate plastic collection liner (Giddings Machine, Windsor, CO). The collection tube was driven into the ground to a depth of 90 cm using a demolition hammer (Bosch, Farmington Hills, MI). A high-lift jack was then used to remove the collection tube from the ground. After removal, the plastic collection liner was removed from the collection tube and caps were placed on both ends (Howland et al., 2014). Tubes were then stored in a cooler and transported to the laboratory for processing. In the laboratory, each plastic tube was divided into 15 cm increments using a hacksaw. From each depth increment, roots, if any were present, were picked from the sample, washed free of soil, and then *P. penetrans* was extracted by intermittent mist and dry root weight determined as described above. Also from each depth increment, *P. penetrans* was extracted from soil by placing 50 g of soil on a Baermann funnel for 5 d (Ingham, 1994). In addition, 50 g of soil was placed in a 65°C drying oven and weighed after 5 d to determine soil moisture content. Extracted *P. penetrans* from both roots and soil were enumerated and identified as described above. On the final sampling date (postplanting), root and soil samples were collected from established raspberry plants ($n = 10$). From each location, a 15-cm^3 core was collected from each side of a plant using a square-blade shovel (Walters et al., 2009). Samples were placed in a bag and transported to the laboratory. Roots were separated from soil and nematodes in soil and roots were extracted and dry weights were determined as described above.

Nematode data from roots recovered from cores were not included in the analyses because insufficient data were collected. For each field, nematode soil data were $\log_{10}(x+1)$ transformed where x is the number of *P. penetrans*/100 g dry soil to meet normality and equal variance assumptions. Data were then separated by depth and sampling date. Due to the spatial dependency of nematode populations based on depth within a sampling location and date, no analysis was done comparing populations between depths within the same date. However, analysis was conducted to determine differences between sampling dates within a sampling depth. Analysis of variance adjusted for repeated measure was used to determine the effects of the fixed effects of time, depth, and the interaction of time and depth, and the random effect of block. Tukey's HSD test was used to adjust for multiple comparisons within each sampling depth. All statistical analyses were done using RStudio Version 0.99.491.

Results

Survival of P. penetrans *in raspberry root material:* At the time of bag burial, October 2013, initial *P. penetrans* population densities were not significantly different with 205 ± 52 and 242 ± 62 *P. penetrans*/g dry root in herbicide-treated and nontreated roots, respectively. Analysis of variance indicated that treatment, time, and the interaction of treatment and time were all significant in determining the number of *P. penetrans* recovered in both fields ($P < 0.05$). At the Lynden, WA field, *P. penetrans* population densities dropped to 1 ± 0.5 and 5 ± 2 *P. penetrans*/g dry root for herbicide-treated and nontreated roots, respectively, 2 mon after burial of bags (December 2013; Fig. 1A). *Pratylenchus penetrans* population densities in herbicide-treated and nontreated roots remained at this same level over the next two sampling dates (February and April 2014). Eight months (June 2014) after burial of bags, there was a marked decrease in *P. penetrans* population densities in nontreated roots to 1 ± 0.5 *P. penetrans*/g dry root. Ten and 12 mon (August and October 2014) after the initiation of the experiment, population densities had dropped to zero *P. penetrans*/g dry root in herbicide-treated and nontreated roots, respectively (Fig. 1). The only sampling date at which a significant difference ($P = 0.003$) in *P. penetrans* population densities in herbicide-treated and nontreated roots was detected was at 6 mon (April 2014; Fig. 1A).

At the Corvallis, OR field, a similar decrease over time of *P. penetrans* population densities in roots was observed. Initially, 2 and 4 mon after bag burial (December 2013 and February 2014), there were fewer *P. penetrans* remaining in herbicide-treated roots compared to nontreated roots ($P \leq 0.007$; Fig. 1B). On average, there were 7 ± 1 *P. penetrans*/g dry nontreated root across these sampling times compared to 2 ± 1 *P. penetrans*/g dry herbicide-treated root. Starting in April 2014, 6 mon after bag burial, population densities of *P. penetrans* were similar in herbicide-treated and nontreated roots, averaging 1 ± 0.2 *P. penetrans*/g dry root. *Pratylenchus penetrans* was undetectable in herbicide-treated roots 8 mon after bag burial, whereas in nontreated roots *P. penetrans* was undetectable 10 mon after bag burial (Fig. 1B).

Vertical distribution of P. penetrans *pre- and postfumigation:* Time, depth, and the interaction of time and depth were significant in determining the number of *P. penetrans* recovered for both fields. At the Lynden, WA field, *P. penetrans* was present at all depths down to 90 cm prior to soil fumigation (Fig. 2). Mean populations ranged from 206 ± 131 *P. penetrans*/100 g dry soil at 16 to 30 cm to 5 ± 3 *P. penetrans*/100 g dry soil at 61 to 75 cm. After fumigation, *P. penetrans* population densities decreased at all soil depths compared to prefumigation densities; however, this decrease was only significant ($P < 0.05$) at 0 to 15 cm, 16 to 30 cm, and 31 to 45 cm. Mean postfumigation populations ranged from 44 ± 21 *P. penetrans*/100 g dry soil at 16 to 30 cm to 0 *P. penetrans*/100 g dry soil at 61 to 75 cm and 76 to 90 cm. At planting, *P. penetrans* population densities in soil decreased at 0 to 15 cm and 31 to 45 cm compared to postfumigation densities ($P < 0.05$). The only depth where an increase in *P. penetrans* population densities was detected was at 61 to 75 cm, where there were 0 *P. penetrans*/100 g dry soil postfumigation and then 2 ± 1 *P. penetrans*/100 g dry soil at planting; however, this increase was not significant. At planting, mean population densities ranged from 2 ± 1 *P. penetrans*/100 g dry soil at 0 to 15 cm, 16 to 30 cm, and 61 to 75 cm to 0 *P. penetrans*/100 g dry soil at 76 to 90 cm. Six months after planting, nematode populations averaged 364 ± 86 *P. penetrans*/100 g dry soil at 0 to 15 cm.

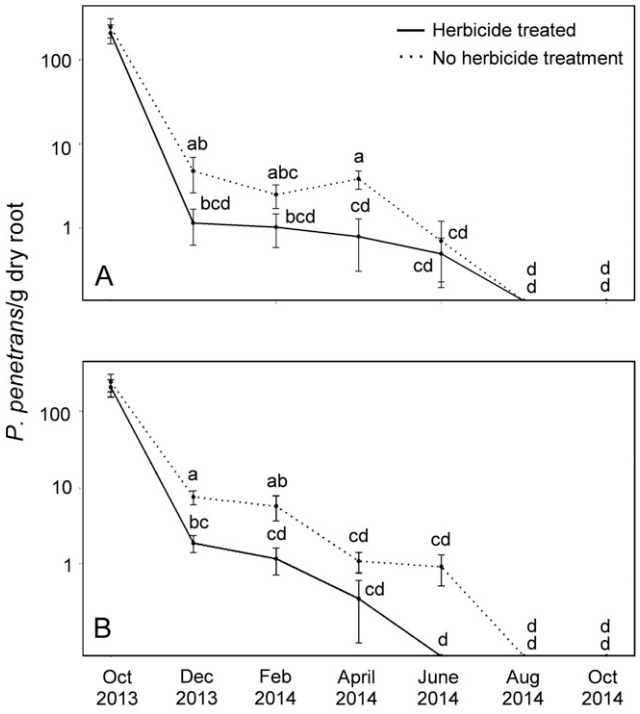

Fig. 1. *Pratylenchus penetrans* population densities in red raspberry (*Rubus idaeus*) 'Meeker' roots collected from plants either treated with herbicide or not treated with herbicide. Roots were buried in bags near A. Lynden, WA, and B. Everson, WA, with initial population densities of 205 *P. penetrans*/g root (herbicide treatment) and 242 *P. penetrans*/g root (no herbicide treatment). Data for each collection date represent the mean of 10 samples for each treatment. Error bars represent standard error. Dates that share a letter are not significantly different ($P \leq 0.05$) based on Tukey's honest significant difference test.

At the Everson, WA field, *P. penetrans* was present at all depths prior to fumigation (Fig. 3). Mean population densities ranged from 46 ± 18 *P. penetrans*/100 g dry soil at 16 to 30 cm to 13 ± 4 *P. penetrans*/100 g dry soil at 46 to 60 cm. Mean postfumigation population densities decreased significantly compared to prefumigation at depths of 0 to 15 cm, 16 to 30 cm, 31 to 45 cm, and 46 to 60 cm with 0 *P. penetrans*/100 g dry soil ($P < 0.05$). *Pratylenchus penetrans* was detectable at 61 to 75 cm and 76 to 90 cm with 6 ± 5 and 8 ± 5 *P. penetrans*/100 g dry soil, respectively, but were not significantly different from

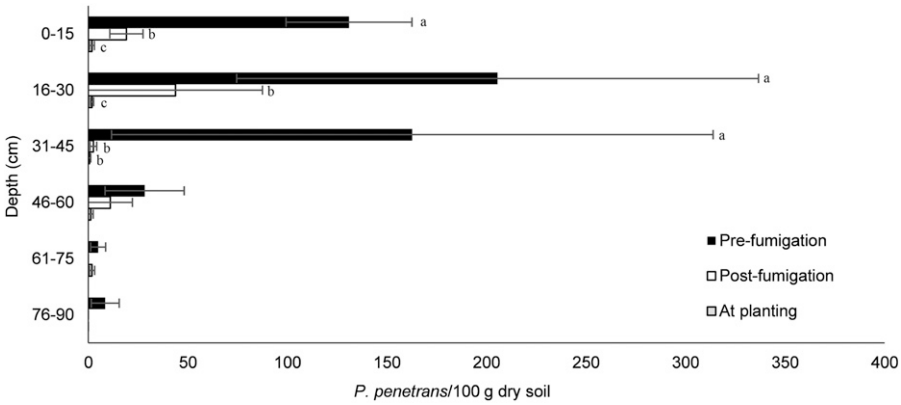

FIG. 2. *Pratylenchus penetrans* population densities at different soil depths within a red raspberry (*Rubus idaeus*) 'Meeker' near Lynden, WA. Samples were collected at three different periods during the replanting process. Data shown for each sampling date and depth are the mean of 10 samples. Error bars represent standard error. Sampling times within the same depth that share the same letter are not significantly different ($P \leq 0.05$) based on Tukey's honest significant difference test. Depths with no letters indicate no significant differences between any of the sampling times.

prefumigation populations at these depths. At planting, *P. penetrans* was detectable at all depths except 16 to 30 cm, which stayed at 0 *P. penetrans*/100 g dry soil. At 0 to 15 cm, 31 to 45 cm, and 46 to 60 cm, there were on average 1 ± 1 *P. penetrans*/100 g dry soil. Deeper in the soil profile, 61 to 75 cm and 76 to 90 cm, there was a decrease in mean *P. penetrans* population densities with 4 ± 3 and 2 ± 1 *P. penetrans*/100 g dry soil, respectively. However, there were no significant differences compared to postfumigation population densities. Six months after planting, nematode populations averaged 88 ± 41 *P. penetrans*/100 g dry soil at 0 to 15 cm.

DISCUSSION

Our data demonstrate that *P. penetrans* is able to survive in roots and soil during the field reestablishment process of the raspberry production system. *Pratylenchus penetrans* was able to survive in old root material up to 8 mon after termination of the prior raspberry planting. In the raspberry production system where rotation is not implemented and planting occurs within 6 mon of removal of the previous crop, roots play a role in allowing nematodes to survive and colonize new plants. In addition to surviving in roots, *P. penetrans* is also able to escape fumigation, and the depth that escape occurs varied between fields with different soil types.

Since *P. penetrans* is an obligate biotroph (Davis and MacGuidwin, 2005), the rapid initial decrease in population densities in roots was likely due to the death and decomposition of living root material which the nematodes feed on into an unusable, dead substrate. This might also explain why *P. penetrans* population densities in general decreased more rapidly in roots treated with a systemic herbicide which resulted in faster root mortality than in roots of plants not treated with herbicide. Despite the large initial decrease, nematodes continued to be collected from root material in both fields after 8 mon, even though no evidence of any living roots was present from either treatment after 4 mon. Although active nematodes cannot survive without living roots, inactive individuals or eggs of *P. penetrans* may continue to survive in dead root material and soil (Mani, 1999). It is important to note that degradation of root fragments

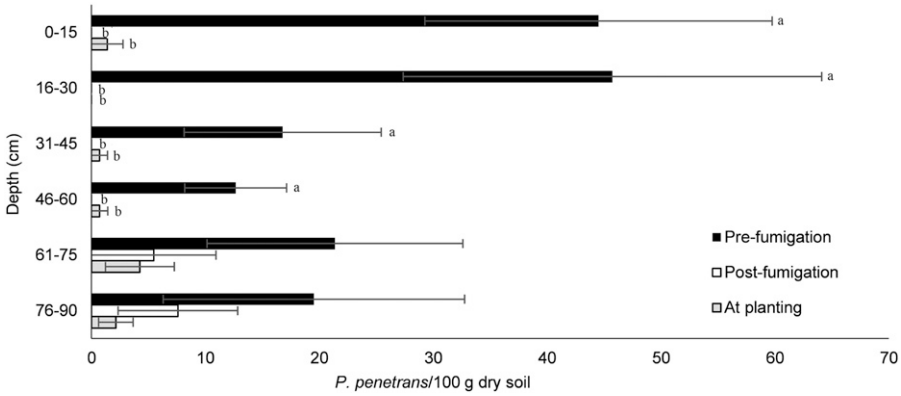

FIG. 3. *Pratylenchus penetrans* populations found at different soil depths within a red raspberry (*Rubus idaeus*) 'Meeker' near Everson, WA, at three different periods during the replanting process. Data shown for each sampling date and depth are the mean of 10 samples. Error bars represent standard error. Sampling times within the same depth that share the same letter are not significantly different ($P \leq 0.05$) based on Tukey's honest significant difference test. Depths with no letters indicate no significant differences between any of the sampling times.

may have been more rapid if bags would have been prepared with nonsterlized soil compared to the sterilized soil used in this study.

Nematode population densities tend to decrease significantly in winter; however, survival stages including both the egg and quiescent stages may allow *P. penetrans* to survive unfavorable conditions (Townsend, 1973; MacGuidwin and Forge, 1991; McSorley, 2003). MacGuidwin and Forge (1991) found that active *Pratylenchus scribneri* populations declined 50% to 63% overwinter when associated with corn and 15% to 84% when associated with potato. Similarly, active *P. penetrans* populations associated with red clover declined 35% to 59% through winter (Kimpinski and Dunn, 1985). Since egg and quiescent stages are not active in the field, it may be possible that they were not detected with the type of extraction method used in this study.

Although this study did not determine if *P. penetrans* was being extracted from fine root material, where nematodes tend to reside (Walters et al., 2009), or coarse root material, it does indicate that nematodes can survive in root material well beyond the typical industry time frame of plant removal to fumigation and replanting. Similarly, it has previously been shown that *Pratylenchus neglectus* can survive in root material after plant removal (Forge et al., 2015). Though little research has been conducted on the survival of nematodes residing in roots after fumigation, it has been shown that fumigation does greatly reduce populations of *Phellinus weirii* in tree stumps, but it does not entirely eliminate the population (Thies and Nelson, 1987). This ability to survive in residual root material may provide protection from the effects of fumigation if nematodes are residing in coarse root material that fumigants may not be able to penetrate.

Though *P. penetrans* populations were relatively low throughout the buried bag study compared to initial populations, in a field setting, these nematodes have the potential to serve as a source of inoculum for the next planting of raspberry. Studies have shown that postfumigation populations are typically undetectable (Stirling et al., 2001; Zasada et al., 2015). However, these studies relied on soil samples to determine nematode population densities, which have been reported to be an unreliable indication of total population densities (Walters et al., 2009) with population densities of *P. penetrans* reaching prefumigation levels within 6 mon after replanting (Zasada et al., 2015; Zasada, unpubl. data). It is unknown how *P. penetrans* populations reestablish so quickly, but it is possible that nematodes residing in root material may provide an inoculum source for the following planting.

Although *P. penetrans* was not detectable at all depths at each sampling date, nematodes were found at at least one depth on all three sampling dates. However, the two fields evaluated in this study showed different dynamics as to where *P. penetrans* were located at each sampling date. Both fields had higher prefumigation nematode population densities at shallower depths. This is expected as the majority of the remaining roots would be in the upper parts of the soil profile (Forge et al., 1998). The distribution dynamics of nematodes in the two fields postfumigation may have to do with the two different soil types.

The Lynden, WA field, which has a loamy sand soil type, had higher population densities in the upper 45 cm profile than at deeper depths prefumigation. This trend continued postfumigation, with *P. penetrans* concentrated in the upper 60 cm and undetectable at lower depths. At planting, population densities were more evenly distributed throughout all depths. Coarser soils, such as in the Lynden field, can allow for the escape of fumigants from the upper soil profiles (McKenry and Thomason, 1974; Qin et al., 2013). This coarse soil potentially allowed the fumigant to volatize from the upper depths of the soil before having an effect on *P. penetrans*. The coarser soil can also allow for easier movement of nematodes from lower depths. Larger soil particles provide a pore size that is more suitable for movement of *Pratylenchus* spp. than finer particles (Townsend and Webber, 1971). Nematodes, particularly at depths below the application of fumigants, may have migrated to the shallower depths where more resources are likely to be found.

The Everson, WA field, which has a sandy loam soil type, had more evenly distributed *P. penetrans* populations throughout the soil profiles than the Lynden, WA field, but had slightly higher populations in the upper 30 cm of soil prefumigation. Postfumigation, nematodes were only detected at 61 to 75 cm, with no nematodes detected in the upper 60 cm. At planting, there was a more even distribution of *P. penetrans* across the soil profile, but slightly higher population densities at 61 to 90 cm. Finer soil types, like a sandy loam soil type, may help to keep fumigants in the upper soil profile longer before volatizing into the atmosphere, making fumigation more effective at shallower depths. Qin et al. (2013) demonstrated that fumigant emission is positively correlated to the air-filled porosity of the soil. This indicates that sandy soils, which contain relatively large pore spaces, would be less effective at containing fumigants. The finer soil also makes it more difficult for nematodes from depths below the effective depth of fumigation, to move upward in the soil profile where more resources may be available (Townshend and Webber, 1971).

Although the depth at which *P. penetrans* escaped fumigation may be based on soil type, it is also important to note that there were nematodes present at all sampling dates in both fields. *Pratylenchus penetrans* are escaping fumigation to some extent, possibly through being deeper than the effective depth of fumigation, not coming in contact with toxic concentrations of the fumigant, or through protection within old root material. Nematodes that remain in roots and soil will be a source of inoculum to subsequent plantings, as was demonstrated in this study with *P. penetrans* being found in newly planted raspberry roots 6 mon after planting.

Although fumigation is the primary means by which raspberry growers manage *P. penetrans*, our study not only shows that *P. penetrans* are escaping fumigation, but also provides potential answers to how and where they are surviving. This information will help to educate growers of the threat of not allowing sufficient time to elapse between field renovation and fumigation, and the limitations of soil fumigation. Knowing more about how *P. penetrans* is distributed may allow growers to more effectively target management practices to when and where the nematode is most vulnerable.

Literature Cited

Bélair, G., and Khanizadeh, S. 1994. Distribution of plant-parasitic nematodes in strawberry and raspberry fields in Quebec. Phytoprotection 72:21–25.

Castillo, P., and Vovlas, N. 2007. *Pratylenchus* (Nematoda: Pratylenchidae). Diagnosis, biology, pathogenicity and management. Leiden, the Netherlands: Nematology Monographs & Perspectives.

Davis, E. L., and MacGuidwin, A. E. 2005. Lesion nematode disease. The Plant Health Instructor (June 21, 2015). American Phytopathological Society. DOI: 10.1094/PHI-I-2000-1030-02, http://www.apsnet.org/edcenter/intropp/lessons/Nematodes/Pages/LesionNematode.aspx.

Eissenstat, D. M. 1992. Costs and benefits of constructing roots of small diameter. Journal of Plant Nutrition 15:763–782.

Forge, T. A., DeYoung, R., and Vrain, T. C. 1998. Temporal changes in the vertical distribution of *Pratylenchus penetrans* under raspberry. Journal of Nematology 30:179–183.

Forge, T. A., Larney, F. J., Kawchuk, L. M., Pearson, D. C., Koch, C., and Blackshaw, R. E. 2015. Crop rotation effects on *Pratylenchus neglectus* populations in the root zone of irrigated potatoes in southern Alberta. Canadian Journal of Plant Pathology 37:363–368.

Gigot, J. A., Zasada, I. A., and Walters, T. W. 2013a. Integration of brassicaceous seed meals into red raspberry production systems. Applied Soil Ecology 64:23–31.

Gigot, J. A., Zasada, I. A., and Walters, T. W. 2013b. Impact and occurrence of *Phytophthora rubi* and *Pratylenchus penetrans* in commercial red raspberry (*Rubus idaeus*) fields in northwestern Washington. International Journal of Fruit Science 13:357–372.

Haar, M. J., Fennimore, S. A., Ajwa, H. A., and Winterbottom, C. Q. 2003. Chloropicrin effect on weed seed viability. Crop Protection 22:109–115.

Howland, A. D., Schreiner, R. P., and Zasada, I. A. 2014. Spatial distribution of plant-parasitic nematodes in semi-arid *Vitis vinifera* vineyards in Washington. Journal of Nematology 46:321–330.

Ingham, R. E. 1994. Nematodes. Pp. 459–490 *in* S. H. Michelson, ed. Methods of soil analysis part 2: Microbiological and biochemical properties. Madison, WI: Soil Science Society of America.

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

MacGuidwin, A. E., and Forge, T. A. 1991. Winter survival of *Pratylenchus scribneri*. Journal of Nematology 23:198–204.

Mani, A. 1999. Survival of the root-lesion nematode *Pratylenchus jordanensis* Hashum in a fallow field after harvest of alfalfa. Nematology 1:79–84.

Martin, F. N. 2003. Development of alternative strategies for management of soilborne pathogens currently controlled with methyl bromide. Annual Review of Phytopathology 41:325–350.

McElroy, F. D. 1977. Effect of two nematode species on establishment, growth, and yield of raspberry. Plant Disease Reporter 61:277–279.

McElroy, F. D. 1991. Nematode parasites. Pp. 59–62 *in* M. A. Ellis, R. H. Converse, R. N. Williams, and B. Williamson, eds. Compendium of raspberry and blackberry diseases and insects. St. Paul, MN: APS Press.

McElroy, F. D. 1992. A plant health care program for brambles in the Pacific Northwest. Journal of Nematology 24:457–462.

McGovern, R. J., Vavrina, C. S., Noling, J. W., Datnoff, L. A., and Yonce, H. D. 1998. Evaluation of application methods of metam sodium for management of Fusarium crown and root rot in tomato in Southwest Florida. Plant Disease 82:919–923.

McKenry, M. V., and Thomason, I. J. 1974. 1, 3-dichloropropene and 1, 2-dibromoethance compounds: I. Movement and fate as affected by various conditions in several solid; II. Organism-dosage response studies in the laboratory with several nematode species. Hilgardia 42:392–438.

McSorley, R. 2003. Adaptations of nematodes to environmental extremes. Florida Entomologist 86:138–142.

Pudasaini, M. P., Schomaker, C. H., Been, T. H., and Moens, M. 2006. Vertical distribution of plant-parasitic nematode, *Pratylenchus penetrans*, under four field crops. Phytopathology 96:226–233.

Qin, R., Gao, S., and Ajwa, H. 2013. Emission and distribution of fumigants as affected by soil moistures in three different textured soils. Chemosphere 90:66–872.

Ristaino, J. B., and Thomas, W. 1997. Agriculture, methyl bromide, and the ozone hole: Can we fill the gaps. Plant Disease 81:964–977.

Stirling, G. R., Blair, B. L., Pattemore, J. A., Garside, A. L., and Bell, M. J. 2001. Changes in nematode populations on sugarcane following fallow, fumigation and crop rotation, and implications for the role of nematodes in yield decline. Australian Plant Pathology 30:323–335.

Thies, W. G., and Nelson, E. E. 1987. Reduction of *Phellinus weirii* inoculum in Douglas fir stumps by the fumigants chloropicrin, vorlex or methylisothiocyanate. Forest Science 33:316–329.

Thomas, W. B. 1996. Methyl bromide: Effective pest management tool and environmental threat. Journal of Nematology 28:586–589.

Townsend, J. L. 1973. Survival of *Pratylenchus penetrans* and *P. minyus* in two Ontario soils. Nematologica 19:35–42.

Townsend, J. L., and Webber, L. R. 1971. Movement of *Pratylenchus penetrans* and the moisture characteristics of three Ontario soils. Nematologica 17:47–57.

Trudgill, D. L., and Brown, D. J. F. 1978. *Pratylenchus penetrans*: A potential pest of raspberries in Scotland. Plant Pathology 27:101.

United States Department of Agriculture (USDA). 2016. Noncitrus fruits and nuts 2015 summary. http://usda.mannlib.cornell.edu/usda/nass/NoncFruiNu//2010s/2016/NoncFruiNu-07-06-2016.pdf.

United States Environmental Protection Agency (USEPA). 2012. Soil fumigant mitigation factsheet: phase 2 site-specific fumigant management plans and post-application summaries. (October 21, 2015). http://www.epa.gov/sites/production/files/2013-10/documents/sfm-phase2-sitespec-mgmnt-plans-2012.pdf.

Walters, T. W., Pinkerton, J. N., Riga, E., Zasada, I. A., Partika, M., Yoshida, H. A., and Ishida, C. 2009. Managing plant-parasitic nematodes in established red raspberry fields. HortTechnology 19:762–768.

Wilcox, W. F., Scott, P. H., Hamm, P. B., Kennedy, D. M., Duncan, J. M., Brasier, C. M., and Hansen, E. M. 1993. Identity of a *Phytophthora* species attacking raspberry in Europe and North American. Mycological Research 97:817–831.

Wilder, K. L., and Righetti, T. L. 1991. Identifying nutrient deficiency and toxicity in red raspberry (February 2, 2016). Pacific Northwest Extension. https://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/33476/pnw3721991.pdf?sequence=3.

Zasada, I. A., Halbrendt, J. M., Kokalis-Burelle, N., LaMondia, J., McKenry, M. V., and Noling, J. W. 2010. Managing nematodes without methyl bromide. Annual Review of Phytopathology 48:311–328.

Zasada, I. A., Weiland, J. E., Han, Z., Walters, T. W., and Moore, P. 2015. Impact of *Pratylenchus penetrans* on establishment of red raspberry. Plant Disease 99:939–946.

Zunke, U. 1990. Observations on the invasion and endoparasitic behavior of the root lesion nematode *Pratylenchus penetrans*. Journal of Nematology 22:309–320.