[Original Article]

Control of the dagger nematode *Xiphinema brevicolle* (Dorylaimida: Longidoridae) in wrapped root balls of the Japanese holly *Ilex crenata* (Celastrales: Aquifoliaceae) by drenching in a fenitrothion or benomyl solution

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We evaluated fosthiazate, fenitrothion, and benomyl for the management of dagger nematodes (DNs, primarily *Xiphinema brevicolle*), in the root-ball soil of Japanese holly (*Ilex crenata* Thumb.). When root balls were drenched in diluted solutions of these agrochemicals, fenitrothion and benomyl were effective against DN populations but fosthiazate was not. Benomyl suppressed DN population recovery completely from 7 days to at least 60 days after treatment. Although fenitrothion suppressed DN populations completely for seven days after treatment, this duration of the suppressive effect was much shorter than that of benomyl, possibly because of the hatching of surviving eggs. The hatching of eggs that survived the fenitrothion treatment was hindered by post-treatment preservation at 5°C. Nematol. Res. 45(1), 27-33 (2015).

Key words: Exportation, Japanese traditional ornamental tree

INTRODUCTION

Xiphinema americanum sensu lato has about 51 species (OEPP/EPPO, 2009). Some of these are natural vectors of several nepoviruses (Hewitt *et al.*, 1958; McGuire, 1964; Teliz *et al.*, 1966), which cause serious, economically important damage to crops. Despite several morphological and molecular studies, arguments persist over the classification of this taxonomic group (Luc *et al.*, 1998; Lazarova *et al.*, 2006). For this reason, the distribution of all *X. americanum sensu lato* species or all *Xiphinema* species is subject to quarantine measures in some countries (OEPP/EPPO, 2009; Department of Agriculture, Fisheries and Forestry, Australian Government, 2013).

In Japan, dagger nematodes (DNs, *Xiphinema* species) are frequently detected in commercial ornamental plant nurseries of the Japanese holly *Ilex crenata* Thunb. (Takeda *et al.*, 2013). Japanese holly is widely distributed worldwide as a traditional ornamental tree for bonsai or ground planting. One of the main nematode species detected on *I. crenata* is *Xiphinema*

brevicolle Lordello et Costa, which belongs to *X. americanum sensu lato* (Sakai *et al.*, 2011). There is a risk that detection of *X. brevicolle* can result in temporarily halted transportation, and thus hinder the distribution, of Japanese holly. To avoid this problem, therefore, we need to develop reliable strategies for eliminating *X. brevicolle*.

Before being shipped, Japanese holly trees are dug up at the nursery and the root balls are wrapped in linen. The root ball is then drenched in fosthiazate solution, mainly to control *Pratylenchus penetrans*. The efficacy of fosthiazate for treating nematodes on Japanese holly is recognized only for *P. penetrans*; this chemical is approved in Japan (unpublished data) for this use only, and no studies have evaluated its efficacy against DN. In red raspberry (*Rubus idaeus* L.), fosthiazate has been confirmed efficacious by soil drench against *P. penetrans* (Zasada *et al.*, 2010), but not against *Xiphinema bakeri* (Walters *et al.*, 2009; McElroy, 1992).

Some post-planting nematicide applications are effective against *Xiphinema* species, mainly in fruit trees. These include oxamyl and carbofuran for *X. americanum* and *X. rivesi* in apple (Thompson *et al.*, 1983), fenamiphos, aldicarb, and oxamyl for *X. index* in grape (Hafez *et al.*, 1981), oxamyl for *X. bakeri* in raspberry (Walters *et al.*, 2009), and benomyl for *X. americanum* in

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cucumber seeds (McGuire and Goode, 1970). Currently, however, carbofuran and fenamiphos are not registered for use on agricultural crops in Japan, and only the granule formulation of oxamyl is registered for use.

Our objective was to evaluate root ball drenching with fosthiazate and some additional agrochemicals for the control of *X. brevicolle* in Japanese holly and to develop effective application strategies. After considering the results of the above-mentioned studies and the current usage registrations in Japan, we selected two additional agrochemicals for study, namely benomyl and fenitrothion. Fenitrothion was selected because, like fenamiphos, it is an organophosphorus pesticide available in Japan.

MATERIALS AND METHODS

Application of agrochemicals and nematode survey:

We used Japanese holly packed for transportation. The trees were dug up from fields infested with DN (mainly *X. brevicolle*), and the root balls were then wrapped in linen. The field soil was an Andosol. The root ball diameter was 60 cm, and the tree height was approximately 1.5 m.

The agrochemicals tested were fosthiazate 30% solution (Nemabuster, ISK Biosciences K.K., Tokyo, Japan), fenitrothion 80% emulsion (Sumipain, SumikaGreen Co., Ltd., Tokyo, Japan), and benomyl 50% wettable powder (Benlate, SumikaGreen Co., Ltd., Tokyo, Japan). Fosthiazate, fenitrothion, and benomyl were diluted with water to final concentrations of 0.03, 0.16, and 0.1%, respectively, according to the manufacturers' instructions. Root balls were drenched in these agrochemical solutions for 30 min. After treatment, the trees were potted and in principle kept outdoors, under shade, on ground covered with weed-prevention sheeting. As controls, root balls were drenched in water.

Soil samples were collected from 3 to 6 places per root ball by using a tubular spatula (diameter 19 mm) and combined into one composite sample per tree. DN populations were extracted by using Cobb's wet-sieving technique from 100 g of fresh soil. Each sample, collected with a 75- μ m-mesh sieve, was placed in a Baermann funnel. The nematodes were collected after 24 h at 25°C.

Evaluation of agrochemicals for control of DN populations:

In Trial 1, effective agrochemicals were selected by comparing the temporal effectiveness of each chemical against a control in suppressing DN populations. Applications were made in December 2010 (1 tree per

pot, with 5 replications). Soils were sampled twice from the same pot, namely, before and 30 days after treatment (DAT).

In Trial 2, we evaluated the duration of effectiveness for the selected agrochemicals. The population density of DN was estimated by repeated sampling, namely, before treatment and at 7, 14, 32, and 62 DAT. Applications were made in May 2012 to 1 tree per pot, with 4 replications. If significant recovery of the population was observed, the proportion of first-stage juveniles (J1) was compared with that from a control. More than 50 nematodes were randomly selected and their stylet lengths measured to determine each juvenile stage. Individuals with stylets shorter than 83 μ m were regarded as J1 (Luc *et al.*, 1998).

In Trial 3, we evaluated the effectiveness of low-temperature preservation in suppressing DN population recovery after agrochemical application. Experimental plots were established by using a factorial combination of agrochemical treatments (each agrochemical or no agrochemical) and preservation after treatment (low-temperature or outdoors). Applications were conducted in May 2011 at 1 tree per pot with 2 to 5 replications. Trees tested for low-temperature preservation were placed in a coolroom (5°C and darkness) for about half a day after agrochemical treatment and were moved outdoors after 60 DAT. Soils were sampled 7 times from the same pot, namely, before treatment and at 3, 7, 14, 29, 60, and 91 DAT.

Statistical analysis:

To evaluate population changes in the same root ball between before and after treatment, the number of nematodes per 100 g of wet soil was $\log_e(x + 1)$ transformed and analyzed by using repeated measures of analysis of variance (ANOVA), with agrochemical treatments or preservation as factors and sampling timing as the repeated measures factor. For repeated measures, sphericity was checked by using the Mauchly test, and Greenhouse-Geisser correction was applied when necessary. When significant interactions with the sampling timing were found, a simple main effect test and *post hoc* comparisons were performed on each sampling time separately. To analyze the instar proportion, a chi-squared test was performed. In all statistical analyses, significant differences were set at $P < 0.05$ and adjusted by the Bonferroni correction when necessary. Statistical analyses were performed with R ver. 2.15.2 (R Core Team 2012) with the package "car" (Fox and Weisberg, 2011) and the package "multcomp" (Hothorn *et al.*, 2008).

RESULTS

Trial 1:

The mean temperatures in December 2010, when the treatments were applied, and the next month were 10.2 and 5.2°C, respectively. Pretreatment DN population densities did not differ significantly among treatments ($F_{3,16} = 1.17$, $P = 0.35$, Fig. 1). Repeated-measures ANOVA revealed significant effects of treatment $\times$ time interaction ($F_{3,16} = 17.08$, $P = 3.04 \times 10^{-5}$). Univariate analysis at 30 DAT showed that DN population densities were significantly lower after fenitrothion and benomyl treatment than in the control (ANOVA, $F_{3,16} = 16.81$, $P = 3.4 \times 10^{-5}$; *post hoc* Dunnett, fenitrothion vs. control: $t = -5.24$, $P < 0.001$, benomyl vs. control: $t = -5.00$, $P < 0.001$), whereas that after fosthiazate treatment was not (*post hoc* Dunnett vs. control: $t = -0.21$, $P < 0.99$) (Fig. 1).

Trial 2

The mean temperature from May 2012, when the treatments were applied, to July changed from 19.2 to 25.7°C. Experiments were conducted with fenitrothion and benomyl according to the results of Trial 1. Before treatment, DN population densities did not differ significantly between treatments (ANOVA, $F_{2,9} = 0.70$, $P = 0.52$). The density of DN populations treated with fenitrothion decreased by 7 DAT, increased again by 14 DAT, and flattened out at a low level thereafter (Fig. 2). In contrast, the density of those treated with benomyl decreased drastically by 7 DAT, declined even further by

14 DAT, and was still very low at 62 DAT (Fig. 2).

Repeated-measures ANOVA showed significant treatment $\times$ time interaction ($F_{8,36} = 14.71$, $P = 2.84 \times 10^{-9}$, Mauchly test for sphericity, $P = 0.25$), indicating that the groups differed in their responses over time. Comparisons of simple main effects with the Bonferroni correction revealed significant differences between agrochemical treatments at each time point after treatment (ANOVA, $F_{2,9} \geq 14.94$, $P \leq 1.4 \times 10^{-3}$). At 7 DAT, the DN population density with the fenitrothion or benomyl treatment was significantly less than that in the control (*post hoc* Dunnett, fenitrothion vs. control: $t = -4.08$, $P < 0.01$, benomyl vs. control: $t = -5.19$, $P < 0.01$). At 14, 32, and 62 DAT, DN population densities with the fenitrothion treatment did not differ significantly from those in the control (*post hoc* Dunnett, $t \geq -1.87$, $P \geq 0.1$), whereas those densities with the benomyl treatment were significantly less than those in the control (*post hoc* Dunnett, $t \leq -5.63$, $P \leq 0.01$).

The proportion of J1 nematodes at 14 DAT with fenitrothion was compared with that in the control because we had observed substantial population recovery by that time with fenitrothion treatment. The J1 proportion after fenitrothion treatment was significantly higher (49 out of 50) than that in the control (19 out of 100) (likelihood ratio chi-squared test, $\chi^2 = 99.58$, $P < 0.0001$).

Trial 3:

The mean temperature from May 2011, when the treatments were applied, to August changed from 18.4

Fig. 1. Changes of DN population densities in root soil before and 30 days after treatment (DAT). Data are presented as means $\pm$ SD.

*: $P < 0.05$ compared with the untreated group (Repeated measures ANOVA after $\log_e(x + 1)$ transformation).

Fig. 2. Changes of DN population densities in root soil before and after from 7 to 60 DAT. Data are presented as means $\pm$ SD. *: $P < 0.01$ compared with the untreated group (Repeated measures ANOVA after $\log_e(x + 1)$ transformation).

Fig. 3. Changes of DN population densities in root soil before and after agrochemical and low-temperature preservation treatments. Data are presented as means $\pm$ SD.

*: no interaction between agrochemical and preservation treatment, and $P < 0.008$ compared with fenitrothion and no agrochemical treatment regardless of the preservation treatment (repeated measures ANOVA after $\log_e(x + 1)$ transformation).

†: detected interaction between agrochemical and preservation treatment, and $P < 0.08$ compared with low-temperature and outdoor preservation treatment for fenitrothion treatment, but not for no agrochemical treatment.

to 27.2°C. Experiments were conducted only with fenitrothion. Before treatment, the population densities did not differ significantly between treatments (Fig. 3, ANOVA, $F_{3,11} = 2.07$, $P = 0.16$). Population density in the fenitrothion plus outdoor treatment was lowest at 3 DAT and then increased from 7 to 14 DAT (Fig. 3). Conversely, the graphs of both the no-agrochemical plus low-temperature preservation (LTP) treatment and the no-agrochemical plus outdoor treatment did not change greatly (Fig. 3). The population density with the fenitrothion plus LTP treatment decreased until 7 DAT and remained very low at the end of sampling at 91 DAT, even after the pots had been moved outdoors at 60 DAT (Fig. 3).

Two-way repeated ANOVA revealed a significant agrochemical $\times$ LTP $\times$ time interaction ($F_{6,66} = 9.31$, $P = 2.19 \times 10^{-7}$, Mauchly test for sphericity, $P = 0.20$). Agrochemical $\times$ LTP interactions were detected from 29 DAT onward ($F_{1,11} \geq 19.27$, $P < 0.008$). At 3, 7, and 14 DAT, the population density was significantly lower with fenitrothion treatment than with no-agrochemical treatment ($F_{1,13} \leq 16.32$, $P > 0.08$), whereas LTP had no significant influence on the DN population ($F_{1,13} \geq 2.57$, $P < 0.1$). Simple testing for the main effect at 29, 60, and 91 DAT showed that, after fenitrothion treatment, the

population density was significantly lower with LTP than with outdoor treatment ($F_{1,8} \geq 46.42$, $P < 0.008$). There was no significant difference in population density between the two preservation methods in the absence of fenitrothion ($F_{1,3} \leq 13.76$, $P > 0.008$).

DISCUSSION

Post-plant agrochemical application for nematode management has been developed because it has an advantage over soil fumigation: it can be used for soils containing living plants or seedlings. It becomes even more important for long-lived perennial plant crops, and it is thus likely to be essential for nematode management in Japanese holly.

Trial 1 showed that fenitrothion and benomyl were effective against DN populations when root balls were drenched in the diluted agrochemical solutions, whereas fosthiazate was ineffective. It is interesting that fosthiazate—the only nematicide among registered agrochemicals—had no effect, whereas a pesticide and a fungicide (fenitrothion and benomyl, respectively) effectively suppressed DNs. These results are supported by several other studies in other plants or other *Xiphinema* species, as described below.

Fosthiazate is one of the most generally used non-

fumigant nematicides, and its efficacy has been confirmed for primary plant parasitic nematodes (e.g. *Pratylenchus* species, Sturz and Kimpinski, 1999), *Meloidogyne* species (Ingham *et al.*, 2000), and *Heterodera* species (Hoestra, 1976). In contrast, fosthiazate has shown no effect on *X. bakeri* in red raspberry (McElroy, 1992; Walters *et al.*, 2009).

Benomyl is effective against a wide variety of plant pathogenic fungi (Allam *et al.*, 1969). Some studies have found benomyl to be effective on various free-living and plant parasitic nematodes (Borck and Braymer, 1974; Miller, 1978), but its differential effectiveness depending on the nematode species has also been reported. Benomyl treatment reportedly controlled *X. americanum* on cucumber (McGuire and Goode, 1970), *Aphelenchoides composticola* and *Ditylenchus myceliophagus* on *Agaricus bisporus* (McLeod, 1973), and *Heterodera rostochiensis* on potato (Hoestra, 1976), whereas *Aphelenchoides fragariae* on lily bulbs was not affected (Yamada and Takakura, 1989).

Fenitrothion is effective against a wide variety of insect pests, but thus far there have been few reports of its effectiveness against plant parasitic nematodes. Yamada and Takakura (1989) reported that *A. fragariae* on lily leaves was suppressed by fenitrothion dipping. Fenamiphos (an organophosphorus pesticide like fenitrothion) was effective against *X. index* on grape (Hafez *et al.*, 1981).

We obtained good control of DN population density by benomyl drench from 14 to at least 62 DAT. Benomyl has an aerobic half-life of 19 h in the soil and is rapidly converted to mainly carbendazim (WHO, 1993), which is a principal fungitoxicant and is stable in the soil for 6 to 12 months (Baude *et al.*, 1974). Additionally, we segregated the agrochemical-treated root balls from the contaminated soil. The long-lasting efficacy of benomyl in suppressing DN populations may have been caused by both the stability of carbendazim and the segregation from soil. Post-planting agrochemical applications to soil have been made by a variety of methods, including sprinkler application (Ingham *et al.*, 2000), trickle irrigation (Funt *et al.*, 1982), granule application (Sturz and Kimpinski, 1999), and soil drench (Zasada *et al.*, 2010). Although drenching can be used only in limited situations (i.e. on seedlings or nursery trees), we consider it to be the most reliable method because the agrochemical components are spread throughout the root ball and segregation avoids recontamination.

Fenitrothion was effective at 3 DAT—earlier than benomyl—but the duration of its effect was inadequate.

Full control by fenitrothion lasted only until 7 DAT, and population recovery was observed by 14 DAT. This population recovery was not caused by contamination from other sources, because it was not observed in benomyl-treated soil. The higher proportion of J1 nematodes in present in the treated soil than in the control soil at 14 DAT suggested that the nematodes had hatched recently (perhaps after the fenitrothion treatment) and were viable because of fenitrothion's short period of efficacy and low ovicidal capacity. Fenitrothion is degraded very fast in the soil, with an initial half-life of less than 7 days (Mikami *et al.*, 1985), and it has been reported to have no ovicidal capacity in some arthropods (e.g. spider mite, Jose and Shah, 1989).

We obtained inconsistent results between Trials 1 and 2. In Trial 1, which was performed in winter, fenitrothion sufficiently suppressed DN populations at about 1 month after treatment, but this was not the case in Trial 2, which was performed in late spring. This inconsistency was thus likely derived from a difference in the temperatures at which treatment was performed and also in the temperatures after treatment. The low winter temperatures probably suppressed or delayed egg hatching. Trial 3 showed that low-temperature preservation inhibited egg hatching, thus supporting this hypothesis. In addition, the population density of Trial 1 was lower than those of Trials 2 and 3. This is thought to be due to the difference in year and season of treatment.

Although we did not examine the total effective temperature or lethal temperature in *X. brevicolle*, our results yielded a finding about the thermal reaction of DN. First, storage at 5°C for 60 days seemed to kill the eggs, because no population recovery was observed at 91 DAT in Trial 3, even after the trees were moved outdoors at 60 DAT.

In conclusion, fenitrothion and benomyl were both effective in controlling DN populations in the root ball of Japanese holly and can be used to provide control before shipping and thus expedite product distribution. Furthermore, the efficacy of these agrochemicals can be assured if they are chosen to suit each situation. For example, when a quick effect is required, fenitrothion should be used. In contrast, when a longer-term effect is required, especially in warm weather, benomyl should be chosen. If trees are to be shipped in refrigerated containers, which can be maintained at low temperatures, then fenitrothion treatment can keep the DN population density sufficiently low for 2 months. These results can support smooth distribution of Japanese holly, and might be applicable for many other trees infested by DN.

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