J Chem Ecol (2007) 33:1207–1216
DOI 10.1007/s10886-007-9289-y

Chemotaxis of the Pinewood Nematode, *Bursaphelenchus xylophilus*, to Volatiles Associated with Host Pine, *Pinus massoniana*, and its Vector *Monochamus alternatus*

Li Lin Zhao · Wei Wei · Le Kang · Jiang Hua Sun

Received: 20 November 2006 / Revised: 5 February 2007 / Accepted: 13 March 2007 /
Published online: 20 April 2007

Abstract The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, the most important invasive species in pine forests of Asia, is transported to new pine hosts by beetles of the genus *Monochamus*. Third-stage dispersal juveniles (J_{III}) aggregate in pupal chambers around the vector as it matures. We demonstrated that the ratio of three terpenes (α-pinene, β-pinene, and longifolene at 1:2.7:1.1) released by larval *Monochamus alternatus* strongly attract J_{III}, whereas the different ratio (1:0.1:0.01) of these three terpenes found in healthy xylem of *Pinus massoniana* attracts only the propagative stage (J_n) of the nematode. We suggest that the volatiles produced by the host plants could be the basis of a chemoecological relationship between plant parasitic nematodes and their vector insects. Capture of J_{III} with terpene-baited trap tubes deployed for 2 hr in the field was demonstrated. This technique may lead to the development of rapid sampling methodologies for use at either ports-of-entry or in the field.

Keywords Aggregation · *Bursaphelenchus xylophilus* · Chemo-sensation · Location · *Monochamus alternatus* · Terpenes

Introduction

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*(Steiner and Buhrer) Nickle (Tylenchida: Aphenlenchoididae), the causal agent of the destructive pine wilt disease, is native to North America but was introduced to Japan, South Korea, and China, where it has caused irreparable damage to forested ecosystems (Kiyohara and Tokushige 1971; Sun 1982, 2005). It is the most important invasive species in the pine forests of Asia and is

L. L. Zhao · W. Wei · L. Kang · J. H. Sun (✉)
State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, The Chinese Academy of Sciences, Beijing 100080, People's Republic of China
e-mail: sunjh@ioz.ac.cn

L. L. Zhao
Graduate University of Chinese Academy of Sciences, Beijing 100080, People's Republic of China

listed as a quarantine pest in more than 40 countries (Rautapaa 1986; Dwinell 1997; Mota et al. 1999; Schrader and Unger 2003; Yang et al. 2003).

There is no cure for pine wilt disease once a susceptible tree becomes infested with the pinewood nematode. Management of the disease is primarily limited to preventing the introduction and spread of the nematode. The PWN is dispersed to uninfested host pines by its vectors, longhorned beetles in the genus *Monochamus* (Coleoptera: Cerambycidae) (Kobayashi et al. 1984; Linit 1988; Necibi and Linit 1998; Yang et al. 2003; Sun 2005). In Japan and China, *Monochamus alternatus* Hope is the most important vector of *B. xylophilus* (Kobayashi et al. 1984; Sun 2005). Mortality of pine trees caused by the propagative stage (J_n) provides oviposition resources for *Monochamus* (Kobayashi et al. 1984; Linit 1988). As an infested pine dies, the availability and quality of both food and moisture decline, and PWN third-stage juveniles (J_{III}), adapted for dispersal, are produced. These J_{III} aggregate in *Monochamus* pupal chambers as the chambers are formed in the outer xylem by mature *Monochamus* larvae (Mamiya 1972; Linit 1988). In synchrony with beetle eclosion, PWN (J_{III}) molt to the dauer larval stage (J_{IV}) and enter the tracheae of the beetle to be carried to other pine trees (Linit 1988; Necibi and Linit 1998; Yang et al. 2003). The species of *Pinus* attacked, as well as the *Monochamus* spp. utilized as vectors by PWN across its native and invasive ranges, differ (Dwinell 1997; Sousa et al. 2001; Yang et al. 2003). To date, the chemical ecology of *B. xylophilus* in the areas it has invaded has not been studied in detail.

Chemotaxis plays an important role in host location by nematodes during certain phases of their life cycles (Zuckerman and Jansson 1984; Perry 1996). The primary mechanism involves response to chemotactic factors emanating from the host (Zuckerman and Jansson 1984; Grewal et al. 1994; Perry 1996; Zhao et al. 2000). Futai (1980) reported that the J_n of PWN were attracted by the sap of healthy host pines, but they did not identify the chemical basis of the attraction. Chemotaxis by predatory nematodes can occur across a tri-trophic level, through orientation to metabolites given off by plants fed upon by host insects (van Tol et al. 2001; Boff et al. 2002). Maize roots damaged by weevil larvae, *Diabrotica virgifera virgifera* LeConte, release (*E*)-β-caryophyllene, which attracts the animal-parasitic nematode *Heterorhabditis megidis*, thereby bringing the nematodes in close proximity to their prey weevil larvae (Rasmann et al. 2005). In a similar fashion, PWN is transported to new host pines by exploiting chemicals from vector beetles (Miyazaki et al. 1977; Bolla et al. 1989; Necibi and Linit 1998; Stamps and Linit 2001). Effective location of *Monochamus* pupal chambers by third-stage dispersal juveniles (J_{III}) is of primary importance in maintaining the host–vector relationship and allowing exploitation of new host pines (Miyazaki et al. 1977; Bolla et al. 1989). However, species-specific volatile attractants of J_{III} have not yet been identified (Mamiya 1972; Miyazaki et al. 1977; Linit 1988; Bolla et al. 1989; Stamps and Linit 2001).

The objectives of this study were to identify volatiles released by larvae of the vector *M. alternatus* and xylem of the host pine *Pinus massoniana* Lambert, and to determine the effects of these compounds on the chemotaxis of J_{III} and J_n PWN.

Methods and Materials

Insects, Nematodes, and Host Materials In April 2005, fifth instars of *M. alternatus* were obtained from Anhui Province of China. PWNs were obtained from Zhejiang, China. J_{III} were extracted from *M. alternatus* pupal chambers. J_n were cultured with the fungus *Diplodia* sp.

Wood samples of *P. massoniana* were obtained from Anhui, China. Xylem from the walls of *M. alternatus* pupal chambers was collected by drilling to a depth of 10 mm around pupal chambers. Xylem samples containing unidentified species of blue-stain fungi were also collected from regions of dead logs lacking *Monochamus* pupal chambers. Healthy xylem samples were collected from uninfected trees at roughly the same depth as the pupal chambers.

Artificial pupal chambers containing J_{III} were constructed and used for bioassays (Aikawa and Togashi 1997; Jikumaru and Togashi 2003). A hole (4 cm deep and 1-cm diameter) was drilled in the center of the cut ends of healthy *P. massoniana* bolts (5 cm long, 2.5-cm mean diam). The bolts were autoclaved at 121°C for 30 min, then inoculated with *Diplodia* sp. Two weeks later, 3,000 J_n were inoculated into each chamber and the bolts kept at 25°C in the dark for 2 mo.

Chemicals The chemicals used in gas chromatography-mass spectroscopy (GC-MS) analyses and bioassays included various alkanes (Beijing Chemical Plant, >95%), α-pinene (Acros Organics, 98%), β-pinene (Fluka Chemie AG, 80%), longifolene (Acros Organics, 99%), butylated hydroxytoluene (Acros Organics, 99%), and camphor (Acros Organics, 97%). Brilliant Blue R (2%) used to stain the nematode secretions was obtained from Sigma-Aldrich.

Volatile Collection and Analyses To investigate the chemotaxis of J_{III} PWN, we collected volatile samples from both fifth instars of *M. alternatus* and empty *M. alternatus* pupal chambers, as well as from blue-stained and healthy xylem samples of *P. massoniana*, by the Super Q method. Individual *M. alternatus* larvae used for the collection were removed from wood over 2 d. Fresh *P. massoniana* wood samples were harvested immediately before aeration. Samples were aerated in glass vacuum traps (9-cm diam., 100 cm long). A glass pipette containing Super Q (Alltech Associates Inc., Deerfield, IL, USA) was attached to the outlet of the trap. Air, filtered through activated charcoal, was drawn through the apparatus with a QC-1 gas sampler (Beijing Municipal Institute of Labor Protection, Beijing, China) at a rate of 500 ml/min. Samples were aerated for 3 hr. The Super Q cartridges were eluted with *n*-hexane (Fisher Chemicals, 99%), and the resulting extracts stored at −20°C until needed (Lacey et al. 2004).

Volatile collections from either 10 insects or 10 pine samples were combined, dehydrated and concentrated to 250 μl. One microliter of each extract was analyzed by GC-MS with an Agilent 6890N GC coupled to a 5973 mass selective detector. The GC was fitted with a DB5-MS capillary column (0.25 mm i.d.×60 m; Agilent Technologies Inc. Palo Alto, CA, USA), and the column temperature was programmed from an initial temperature of 50°C for 2 min, then increased by 5°C/min to 220°C. Injections were run in the splitless mode. Components of the extracts were identified by comparing retention times and mass spectra with those of authentic standards. Quantification was performed using an internal standard (*cis*-9-hexadecenal) added to each sample (Lacey et al. 2004).

Behavioral Bioassays Two bioassays were used to test the behavioral responses of J_{III} or J_n to volatile chemicals.

Pupal Chamber Bioassay Individual *M. alternatus* larvae, samples of the volatile blend collected from *M. alternatus* larvae by aeration (natural blend), or a synthetic blend of volatiles with the same ratio of terpenes as that collected from the larval aerations, were assayed in artificial pupal chambers. Ten larval equivalents, in 100-μl *n*-hexane, of the

natural or synthetic volatile blends were pipetted onto rubber septa (Tongzhou Shunyi Rubber Co., Beijing, China). Individual larvae, or septa with the natural or synthetic blend, were inserted into the artificial pupal chambers and held at 25°C in the dark for 1 wk. After this, wood chips were collected from walls of each chamber (to about 0.7 cm depth) and from the exterior of each bolt (to a depth of 0.7 cm), and the nematodes present were extracted by using the Baermann funnel technique. The chips were dried to a constant weight for determination of dry weight. An Aggregation Index (AI) was calculated and a one-way ANOVA (using SPSS 11.0 for Windows) used to analyze differences in the AI across treatments.

$$\text{Aggregation Index (AI)} = \frac{\text{Nematodes per g dry} - \text{chips in the interior of chamber}}{\text{Nematodes per g dry} - \text{chips in the exterior of chamber}}$$

Ethylene Sheet-Agar Bioassay The aggregation responses of J_{III} or J_n to volatile collections and blends of monoterpenes were also assayed in Petri dishes (Miyazaki et al. 1977). A volume of warm agar (0.8%, 10 ml) containing 500 nematodes was spread evenly over each plate (9 cm diam.) and allowed to solidify. Ethylene sheets (0.5-cm diam., Reynolds Metals Ltd., Shanghai, China), treated with either single larval equivalents (in 10 µl *n*-hexane) or 10 µl of *n*-hexane alone (control), were used as odor sources. After the solvent had evaporated, one test and one control sheet were placed at opposite ends of each plate with the treated face of the sheet in contact with the agar. The plates were kept in the dark for 24 hr at 25°C. Nematodes aggregating on and under sheets were counted (Miyazaki et al. 1977; Bolla et al. 1989; Stamps and Linit 2001). Differences in the mean numbers of nematodes between treatment and control were compared by Independent-Samples *t* tests.

Field Assays In the field, 50 *P. massoniana* (10–20 cm in diameter) dying from pine wilt disease were sampled from Zhejiang, China, in April 2006. Sampling holes (2 cm deep and 1-cm diam.) were drilled through the entrance holes of *M. alternatus* larvae connected to pupal chambers. Trap tubes were constructed from 2-ml centrifuge tubes (Beijing Zohonice Science & Technology Development Co., Beijing, China) containing a rubber septum inserted into the bottom of the tube. Agar (0.8%) was allowed to solidify around the tube walls and the septa. Volatile blends to be tested were pipetted onto the septa, with control tubes receiving an equivalent volume of *n*-hexane. After the solvent had evaporated, the lids were removed, and the tubes inserted into the sampling holes. Differences in the number of nematodes captured in the treatment and control trap tubes were compared by Independent-Samples *t* test at different times (0.5–48 hr) after commencement of the test.

Effects of Ratios of Three Terpenes on Secretions from J_{III} and J_n The primary chemosensory organs of the nematode are the amphids, which secrete proteins when simulated by molecules of some chemical substances (Perry 1996; Zhao et al. 2000). Secretions of J_{III} and J_n can be detected by staining (Premachandran et al. 1988; Zhao et al. 2000). The respective ratios of the three terpenes found in healthy pines (H3R) and larvae (L3R), were dissolved in dimethyl sulfoxide (DMSO) and distilled water to a concentration of one larval equivalent/10 ul. A 10-µl sample of J_{III} (containing 100 J_{III}) was mixed with 10 µl of 0.2% brilliant blue (in 20% methanol) and 10 µl of one of the terpene solutions or distilled water (control) on top of a microscope slide. The cover slip was sealed with clear nail polish and slides were incubated overnight at ambient temperature in the dark.

Results

In the pupal chamber bioassay, for both the solvent and blank controls (Fig. 1a [S and CK]), more PWN J_{III} were recovered from the wood beyond the walls of the artificial pupal chambers than in the artificial pupal chambers themselves (AI±SE=0.73±0.090). However, the addition of *M. alternatus* larvae to the artificial chamber resulted in the AI increasing to 1.94±0.433 (P=0.048), indicating that nematodes moved into the wood adjacent to the chamber wall. A similar result was also obtained with the volatile extracts from *M. alternatus* larvae (LVE), indicating that the larval volatile extracts contained the components attractive to PWN J_{III} (Fig. 1a). Similarly, in the Petri dish bioassay, the number of J_{III} aggregating on sheets treated with volatile extracts of larvae was significantly higher (P=0.020) than for the control (Fig. 1b). There were no significant differences in the number of J_{III} nematodes on the respective control sheets and on sheets treated with host volatiles collected from healthy, blue-stained, or chamber wood samples of *P. massoniana* (Fig. 1b).

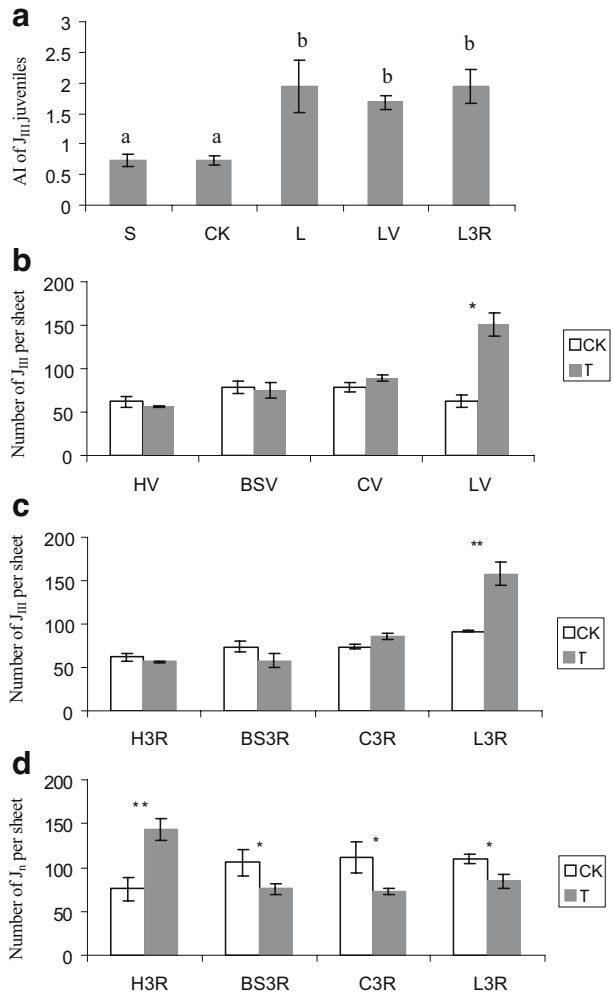

Fig. 1 Aggregation responses of J_{III} and J_n PWN to host and vector volatiles, and blends of terpenes. (**a**) Responses of J_{III} to vector larvae (L), vector larval volatiles (LV) and synthetic terpene blend (L3R) within artificial pupal chambers. AI, Aggregation Index (see text); S, surveyed chambers; CK, control. (**b**) Responses of J_{III} to various volatile collections in Petri dish bioassay. HV, volatiles from healthy pines; BSV, volatiles from blue-stained wood; CV, volatiles from natural pupal chambers. (**c**) Responses of J_{III} to ratios of terpenes in Petri dish bioassay. (**d**) Responses of J_n to ratios of terpenes in Petri dish bioassay. H3R, ratio approximating that of HV=1:0.1:0.01 (1,314 ng/μl); BS3R, ratio approximating that of BSV=1:0.8:0.06 (680 ng/μl); C3R, ratio approximating that of CV=1:0.7:0.08 (242 ng/μl); L3R, the ratio in LV at 1:2.7:1.1 (7.3 ng/μl). Concentrations are expressed for the total amount of the three terpenes. Values indicate the means and standard errors of 10 replicates for each treatment. Bars with the same letters are not significantly different at P>0.05. Asterisks indicate a significant difference from control (*P<0.05; **P<0.01)

The GC-MS traces for the volatile extracts are shown in Fig. 2.Various alkanes (ranging in chain length from C12 to C16) and butylated hydroxytoluene were present only in larval samples, whereas α-pinene, β-pinene, longifolene, camphene, limonene, undecane, and camphor were found in all samples analyzed. Camphor was present in substantially greater amounts in larval samples than in xylem samples. The ratio of α-pinene to β-pinene and longifolene, respectively, varied markedly across the samples. The ratios collected from larvae were lower (1:2.7 and 1:1.1, respectively) than those collected from wood tissue surrounding the pupal chambers or wood from healthy pines (1:0.7 and 1:0.08; 1:0.1 and 1:0.01, respectively).

In Petri dish bioassays, J_{III} of the PWN were not attracted by alkanes, butylated hydroxytoluene, or camphor (data not shown). Differences in the aggregation of J_{III} were noted with four different ratios of three terpenes, α-pinene, β-pinene, and longifolene. Aggregation of J_{III} to sheets treated with a ratio of these terpenes specific to larvae (L3R, 1:2.7:1.1) was significant (P=0.008), whereas ratios of the terpenes matching healthy pines

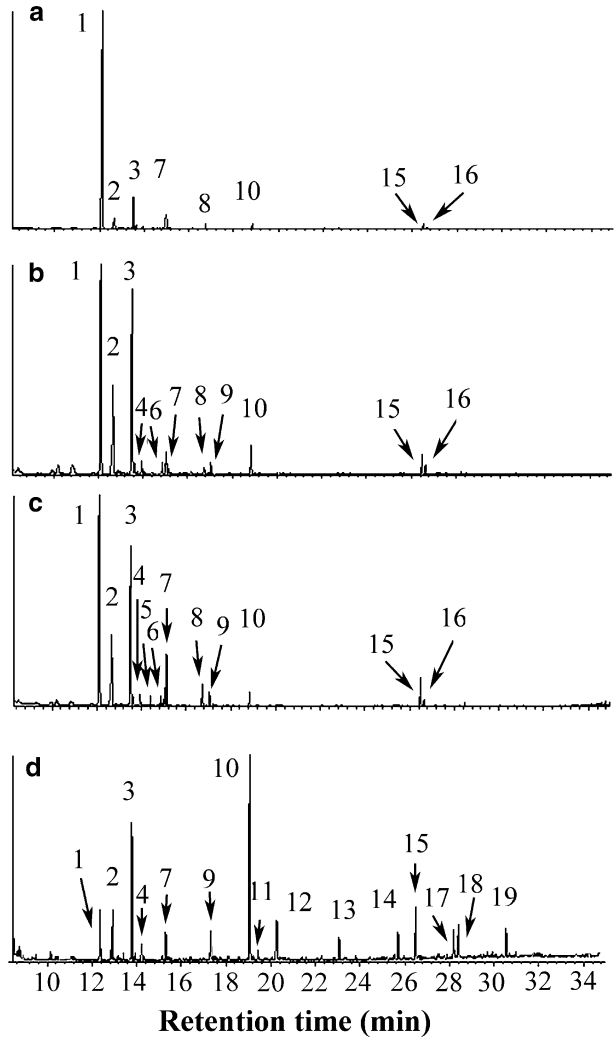

Fig. 2 GC analysis of different volatile collections/treatments. (**a**) Healthy pine samples. (**b**) Blue-stain pine samples. (**c**) Pine chamber samples. (**d**) Larval *M. alternatus*. 1, α-pinene; 2, camphene; 3, β-pinene; 4, decane; 5, 3-carene; 6, *m*-cymene; 7, limonene; 8, β-phellandrene; 9, undecane; 10, camphor; 11, 2-ethylnonane; 12, dodecane; 13, tridecane; 14, tetradecane; 15, longifolene; 16, caryophyliene; 17, pentadecane; 18, butylated hydroxytoluene; 19, hexadecane

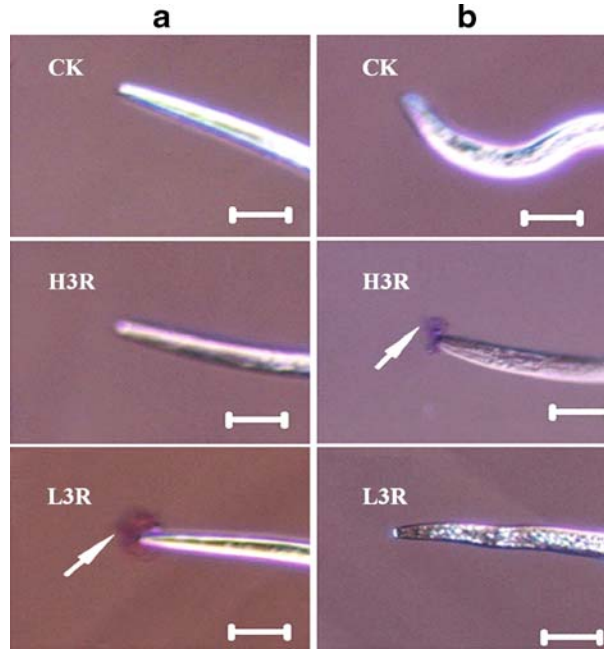

Fig. 3 Stimulation of J_{III} and J_n PWN secretions by different ratios of α-pinene, β-pinene and longifolene. (**a**) J_{III} secretion. (**b**) Stimulation of J_n secretion. Treatments are: H3R, ratio of three terpenes in healthy pine samples at a concentration of 1,314 ng/μl; L3R, ratio of three terpenes released by *M. alternatus* larvae at a concentration of 73 ng/μl. Bar=30 μm. Concentrations are expressed for the total amount of the three terpenes. Arrows point to the stained secretions of nematodes

(H3R, 1:0.1:0.01), blue-stain samples (BS3R, 1:0.8:0.06), and empty pupal chambers (C3R, 1:0.7:0.08) elicited no significant aggregation of J_{III} (Fig. 1c). We also tested the responses of J_n to various ratios of the three terpenes (Fig. 1d). J_n were attracted to the ratio found in natural healthy pine volatiles (H3R, 1:0.1:0.01; P=0.003), but were repelled by BS3R, C3R, and L3R.

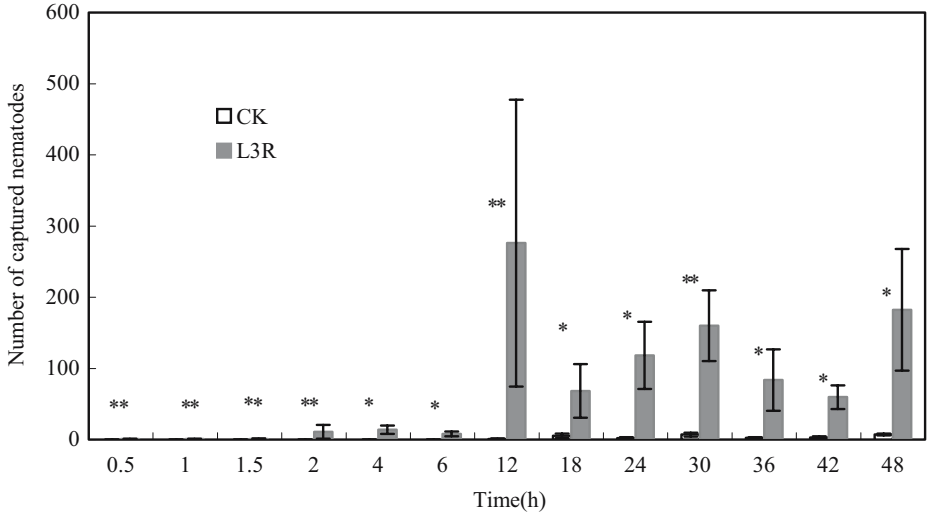

Fig. 4 Catches of PWN in trap tubes, containing different volatile treatments, in the field. Traps were sampled at different times after deployment. L3R, ratio of three terpenes released by *M. alternatus* larvae at a concentration of 7 mg/40 μl. Concentration is for the total amount of the three terpenes. Trial was conducted April 11–15, 2006. Asterisks indicate a significant difference from control (*P<0.05; **P<0.01)

To simulate natural conditions, L3R was also tested in the artificial pupal chamber bioassay. The AI of L3R (1.952±0.277) was significantly (P=0.001) different from that of the control and similar to those of the larvae and larval volatiles (Fig. 1a). This result demonstrates that L3R (1:2.7:1.1) is attractive to J_{III} under natural conditions and that this ratio of the three terpenes is likely the aggregation signal used by the J_{III} juveniles of the PWN.

In the staining assay, for detection of secretions from amphids of PWN, secretions of PWN J_{III} were only detected in response to L3R (Fig. 3a), whereas secretions of J_n were only detected in response to H3R (Fig. 3b). These results demonstrate that the different life stages of PWN distinguish different ratios of terpenes.

In dying *P. massoniana*, significantly greater numbers of nematodes were captured in the L3R-containing traps than in the control traps at all times tested (0.5–48 hr). The greatest number of nematodes was obtained after 12 hr of trapping, with >200 per trap tube baited with L3R (Fig. 4).

Discussion

Animals develop the ability to exploit cues in their habitat during their evolution. We demonstrated that two forms of PWN (reproductive and dispersal) distinguish specific ratios of terpenes produced by their host and vector (Fig. 1; Fig. 3). The reproductive form (J_n) was attracted to the ratio produced by the host tree, but not to the ratio produced by the vector beetle, whereas the dispersal form (J_{III}) was attracted to the ratio produced by the vector, but not to the ratio produced by the host. This attraction demonstrates how J_{III} PWN are able to utilize their vector by moving toward volatile terpenes released by mature *M. alternatus* larvae, allowing the nematodes to aggregate at the site of pupation and adult eclosion of the vector. Subsequent dispersal of the adult beetle allows the nematode access to healthy host pines. Although the specificity and sensitivity of insects to specific ratios of multicomponent pheromones is well established (Monti et al. 1995; Bruce et al. 2005), this is the first report of such a phenomenon in nematodes.

With globalization, biological invasions are a significant environmental threat to the maintenance of natural forest ecosystems (Liebhold et al. 1995). An invasive species must adapt to its new environment. For such a species as PWN, whose life cycle involves multi-trophic interactions, adaptation to its environment can be precarious unless it can utilize both new hosts and new vectors. Our work demonstrates how the alien PWN is able to adapt, by utilizing a common chemoecological relationship between both host and vector, in a new environment.

The PWN is listed as a quarantine target or as a dangerous pest in many countries including those in the European Union (Rautapaa 1986; Dwinell 1997). It has recently been detected in Portugal (Mota et al. 1999). Currently, there is no simple, effective and rapid sampling method available for this pest for use in quarantine monitoring. Our demonstration of chemo-attraction of J_{III} PWN to a specific ratio of terpenes, combined with the successful trapping of PWN, suggests that this approach could be developed into an effective detection method, for use at ports-of-entry and in field detection.

Acknowledgments We thank Daniel Miller, David Kulhavy, Marc Linit, and two anonymous reviewers for critical comments on the manuscript, and Prof. Xingzhong Liu for his technical assistance. We are especially grateful to Leland Humble for his review and editing of the revised manuscript. This study was funded by the CAS Knowledge Innovation Program (KSCX2-YW-N-006), National Natural Science Foundation of China

(30621003, 30525009) and a grant (Chinese IPM0502) from the State Key Laboratory of Integrated Management of Pest Insects and Rodents, IOZ. The publication of this work is also supported by the EU ALARM project. All experiments described in this paper were done according to the rules of the ethical board for animal experiments complying with the current laws of China.

References

AIKAWA, T. and TOGASHI, K. 1997. An effect of inoculum quantity of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) on the nematode load of *Monochamus alternatus* (Coleoptera: Cerambycidae) in laboratory. *Japanese Journal of Nematology* 27:14–21.

BOFF, M. I. C., VAN TOL, R. H. W. M., and SMITS, P. H. 2002. Behavioural response of *Heterorhabditis megidis* towards plant roots and insect larvae. *BioControl* 47:67–83.

BOLLA, J. A., BRAMBLE, J., and BOLLA, R. I. 1989. Attraction of *Bursaphelenchus xylophilus*, Pathotype MPS-1, to *Monochamus carolinensis* larvae. *Japanese Journal of Nematology* 19:32–37.

BRUCE, T. J., WADHAMS, L. J., and WOODCOCK, C. M. 2005. Insect host location: a volatile situation. *Trends Plant Sci.* 10:269–274.

DWINELL, L. D. 1997. The pinewood nematode: regulation and mitigation. *Annu. Rev. Phytopathol.* 35:153–166.

FUTAI, K. 1980. Host Preference of *Bursaphelenchus lignicolus* (Nematoda: Aphelenchoididae) and *B. mucronatus* shown by their aggregation to pine saps. *Appl. Entomol Zool.* 15:193–197.

GREWAL, P. S., LEWIS, E. E., GAUGLER, R., and CAMPBELL, J. F. 1994. Host finding behavior as a predictor of foraging strategy in entomopathogenic nematodes. *Parasitology* 108:207–215.

JIKUMARU, S. and TOGASHI, K. 2003. Boarding abilities of *Bursaphelenchus mucronatus* and *B. xylophilus* (Nematoda: Aphelenchoididae) on *Monochamus alternatus* (Coleoptera: Cerambycidae). *Nematology* 5:843–849.

KIYOHARA, T. and TOKUSHIGE, Y. 1971. Inoculation experiments of a nematode, *Bursaphelenchus* sp., onto pine trees. *Journal of Japanese Forestry Society* 53:210–218.

KOBAYASHI, F., YAMANE, A., and IKEDA, T. 1984. The Japanese pine sawyer beetle as the vector of pine wilt disease. *Annu. Rev. Entomol.* 29:115–135.

LACEY, E. S., GINZEL, M. D., MILLAR, J. G., and HANKS, L. M. 2004. Male-produced aggregation pheromone of the cerambycid beetle *Neoclytus acuminatus acuminatus*. *J. Chem. Ecol.* 30:1493–1507.

LINIT, M. J. 1988. Nematode-vector relationships in the pine wilt disease system. *J. Nematol.* 20:227–235.

LIEBHOLD, A. M., MACDONALD, W. L., BERGDAH, D., and MASTRO, V. C. 1995. Invasion by exotic forest pests: A threat to forest ecosystems. *For. Sci. Monogr.* 30:1–49.

MAMIYA, Y. 1972. Aggregation of the pinewood nematode around the pupal chamber of the vector beetle. *Trans. Annu. Meet. Kanto Branch Jpn. For. Soc.* 24:30.

MIYAZAKI, M., ODA, K., and YAMAGUCHI, A. 1977. Behavior of *Bursaphelenchus lignicolus* to unsaturated fatty acids. *J. Jpn. Wood. Res. Soc.* 23:254–261.

MONTI, L., LALANNE-CASSOU, B., LUCAS, P., MALOSSE, C., and SILVAIN, J. F. 1995. Differences in sex pheromone communication systems of closely related species: *Spodoptera latifascia* (Walker) and *S. descoinsi* Lalanne-Cassou & Silvain (Lepidoptera: Noctuidae). *J. Chem. Ecol.* 21:641–660.

MOTA, M. M., BRAASCH, H., BRAVO, M. A., PENAS, A. C., BURGERMEISTER, W., METGE, K., and SOUSA, E. 1999. First report of *Bursaphelenchus xylophilus* in Portugal and in Europe. *Nematology* 1:727–734.

NECIBI, S. and LINIT, M. J. 1998. Effect of *Monochamus carolinensis* on *Bursaphelenchus xylophilus* dispersal stage formation. *J. Nematol.* 30:246–254.

PERRY, R. N. 1996. Chemoreception in plant parasitic nematodes. *Annu. Rev. Phytopathol.* 34:181–199.

PREMACHANDRAN, D., VON MENDE, N., HUSSEY, R. S., and MCCLURE, M. A. 1988. A method for staining nematode secretions and structures. *J. Nematol.* 20:70–78.

RASMANN, S., KOLLNER, T. G., DEGENHARDT, J., HILTPOLD, I., TOEPFER, S., KUHLMANN, U., GERSHENZON, J., and TURLINGS, T. C. J. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* 434:732–737.

RAUTAPAA, J. 1986. Experiences with *Bursaphelenchus* in Finland. Conference on Pest and Disease Problems in European Forests. *EPPO Bull.* 16:453–456.

SCHRADER, G. and UNGER, J. G. 2003. Plant quarantine as a measure against invasive alien species: the framework of the international plant protection convention and the plant health regulations in the European Union. *Biological Invasions* 5:357–364.

SOUSA, E., BRAVO, M. A., PIRES, J., NAVES, P., PENAS, A. C., BONIFACIO, L., and MOTA, M. M. 2001. *Bursaphelenchus xylophilus* (Nematoda; Aphelenchoididae) associated with *Monochamus galloprovincialis* (Coleoptera; Cerambycidae) in Portugal. *Nematology* 3:89–91.

SUN, J. H. 2005. Forest invasive species: country report-P.R. China, pp. 80–86, *in* M. Philip, B. Chris, J. H. Sun, and J. Wu (eds.). The Unwelcome Guests, Proceedings of the Asia-Pacific Forest Invasive Species Conference. FAO Regional Office for Asia and Pacific, Thailand.

SUN, Y. C. 1982. *Bursaphelenchus xylophilus* was discovered in Sun Yet-sen's mausoleum in Nanjing. *J. Jiangsu For. Sci. Tech.* 4:47.

STAMPS, W. T. and LINIT, M. J. 2001. Integration of intrinsic and extrinsic chemical cues in the behaviour of *Bursaphelenchus xylophilus* (Aphelenchida: Aphelechoididae) in relation to its beetle vectors. *Nematology* 3:295–301.

VAN TOL, R. W. H. M., VAN der SOMMEN, A. T. C., BOFF, M. I. C., VAN BEZOOIJEN, J., SABELIS, M. W., and SMITS, P. H. 2001. Plants protect their roots by alerting the enemies of grubs. *Ecol. Lett.* 4:292–294.

YANG, B. J., PAN, H. Y., TANG, J., WANG, Y. Y., WANG, L. F., and WANG, Q. 2003. *Bursaphelenchus xylophilus*. Chinese Forestry Press, Beijing.

ZHAO, X., SCHMITT, M., and HAWES, M. C. 2000. Species-dependent effects of border cell and root tip exudates on nematode behavior. *Phytopathology* 90:1239–1245.

ZUCKERMAN, B. M. and JANSSON, H. B. 1984. Nematode chemotaxis and possible mechanisms of host/prey recognition. *Annu. Rev. Phytopathol.* 22:95–113.