JOURNAL OF NEMATOLOGY

VOLUME 49 DECEMBER 2017 NUMBER 4

Journal of Nematology 49(4):341–347. 2017.

Chemical Signals of Vector Beetle Facilitate the Prevalence of a Native Fungus and the Invasive Pinewood Nematode

Bin Zhang,[1,4] Wei Zhang,[1,4] Min Lu,[1] Faheem Ahmad,[1,2] Haokai Tian,[1] Jing Ning,[1] Xiaolong Liu,[1] Lilin Zhao,[1,3] and Jianghua Sun[1]

Abstract: In China, the invasive *Bursaphelenchus xylophilus*, the vector *Monochamus alternatus* beetle, and associated fungi exhibit a symbiotic relationship causing serious losses to pine forests. Although this complex system has been intensively investigated, the role of vector beetles on the development of associated fungi and their indirect contribution to the prevalence of pinewood nematode (PWN) is yet unknown. Here, three of the highly prevalent fungal species, viz., *Sporothrix* sp. 1, *Ophiostoma ips*, and *Sporothrix* sp. 2 were isolated from beetle chambers in diseased trees in Guangdong province, southeast China. Pairwise cultivation of isolated fungi demonstrated the dominance of *Sporothrix* sp. 1 over *O. ips* and *Sporothrix* sp. 2. On the other hand, two fatty acid ethyl esters (FAEE), ethyl palmitate (EP) and ethyl linoleate (EL), isolated from the body surface of the vector beetle enhanced the growth of *Sporothrix* sp. 1. When PWN were cultured on *Sporothrix* sp. 1, the fecundity and the body length were increased significantly as compared with when cultured on *O. ips* and *Sporothrix* sp. 1. Our results suggest that the vector beetles promote *Sporothrix* sp. 1 to occupy more niches by rapid growth and spread, which in turn better support PWN population, hence facilitate PWN pathogenicity in the invasive regions.

Key words: blue-stain fungi, invasive species, *Monochamus*, pinewood nematode, *Pinus massoniana*.

Bursaphelenchus xylophilus (Steiner and Buhrer) Nickle (Tylenchida: Aphenlenchoididae), commonly known as PWN is a destructive pathogen causing the pine wilt disease. It has caused significant damage to forest ecosystems since its introduction to Asia and Europe (Kiyohara and Tokushige, 1971; Rautapaa, 1986; Mota et al., 1999; Sun, 2005). The severity of PWN pathogenicity can be attributed to its complicated ecological interactions with other associated organisms involved in the disease cycle. Several cerambycid beetles from the genus *Monochamus* serve as disease vectors by disseminating PWN (Mamiya and Enda, 1972; Morimoto and Iwasaki, 1972; Nickle et al., 1980; Linit, 1988). Every autumn, the third-stage dispersal juveniles (J_{III}) of PWN aggregate around the pupal chambers of vector beetles in diseased pine trees for their dispersal to healthy pine trees along with the eclosing beetles (Kobayashi et al., 1984; Togashi, 1985; Dwinell, 1997). The wood in the wilt-killed pine trees, especially around the pupal chambers of the beetles, are usually stained in blue because of the establishment of blue-stain fungi of the genus *Ophiostoma* (Wingfield, 1987; Zhao et al., 2013a). Thus, the three aforementioned groups of species coexisting in the beetle chamber develop a symbiotic association with one another (Maehara and Futai, 1997; Zhao et al., 2013a, 2014).

Two different regions, the Jiangsu, Zhejiang, and Anhui provinces in eastern China and Guangdong province in southern China, have been invaded initially by PWN from different countries (Pan et al., 2009). The subsequent spread of the disease in Chinese forests is, however, facilitated by a native sawyer beetle species, *M. alternatus*. The disease spreads quickly in Guangdong province because the vector beetles in this region were observed to have two generations in a year (Pan et al., 2009), hence, doubling the chance of the PWN dispersal as compared with that in the eastern China where the beetles have only one generation per year. The rapid disease spread in the southern region of China (Guangdong province) may also be attributed to the symbiotic interactions of PWN with native ophiostomatoid fungal complex. Such interactions have already been reported to prevail in the eastern regions of China (Zhao et al., 2013a).

In the symbiotic relationship among PWN, vector beetles, and the ophiostomatoid fungi, *Monochamus* beetles play a vital role in escalating PWN dispersal (Linit, 1988; Necibi and Linit, 1998; Yang et al., 2003; Sun, 2005; Akbulut and Stamps, 2012), using FAEE and ascarosides as the chemical signals to synchronizing their life cycle to the PWN development (Zhao et al., 2013b, 2016). Whereas on the other hand, diacetone alcohol (DAA) from the native ophiostomatoid fungi, *Sporothrix* sp. 1, facilitates the development of PWN and

Received for publication January 26, 2017.

[1]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China.

[2]Department of Biosciences, COMSATS Institute of Information Technology, Islamabad, Pakistan.

[3]School of Life Sciences, Anhui University, Hefei 230039, People's Republic of China.

[4]These authors contributed equally to this work.

This work was funded by the National Science Foundation of China (31630013, 31370650, and 31572272), the CAS Key Research Projects of the Frontier Science (QYZDB-SSW-SMC014), and the CAS Knowledge Innovation Key Research Program (KSCX2-EW-J-2).

E-mails: zhaoll@ioz.ac.cn and sunjh@ioz.ac.cn.

This paper was edited by Raquel Campos-Herrera.

TABLE 1. Prevalence of pinewood nematodes (PWN) in the pupal chambers of *Monochamus alternatus*–infected trees at different sampling sites in Guangdong province, China.

Collection sites (location, district)	Latitude, longitude	Average elevation (m)	No. of pupal chambers	Percent of the present PWN
Longtoushan, Huangpu	N 23°10′, E 113°49′	57	29	100
Baiyunshan, Baiyun	N 23°29′, E 113°29′	45	32	100
Datianshan, Huangpu	N 23°13′, E 113°48′	34	28	100
Conghua, Conghua	N 23°55′, E 113°58′	34	27	100

Monochamus beetle within the diseased tree (Zhao et al., 2013a). However, the effect of vector beetle on associated fungi is not well documented.

In a previous study, some of the FAEE, including EP, EL, ethyl oleate (EO), and ethyl stearate (ES), were isolated from the surface of vector beetles during their eclosion, which are known to function as chemical signals for PWN phase transition from reproductive to dispersal stages (Zhao et al., 2013b). These beetle chemicals, thus, may also have potential in influencing the ophiostomatoid fungi.

In this study, we surveyed the presence and distribution of associated ophiostomatoid fungi in Guangdong province, investigated the effect of FAEE from the beetle surface on the growth of the fungi and ultimately the role of fungal prevalence on PWN fitness. To confirm the consistency of these results, the experiments were then repeated with the same organisms isolated from Zhejiang province. This study has its significance in understanding the complex ecological associations of successful invasive species with their native associates (Lu et al., 2010, 2011).

MATERIALS AND METHODS

Identification and diversity of ophiostomatoid fungi: A total of 116 pupal chambers of *M. alternatus* from *Pinus massoniana* trees were collected from four different locations belonging to three districts of Guangdong province (Table 1). Sampling was done from five wilted pine trees (killed because of pine wilt disease within a year) selected at random at least 20 m apart from each other.

To isolate and identify fungus, wood tissues from pupal chambers were chopped and placed directly onto prepared fungal growth medium (20 g Biolab malt extract, 20 g Biolab agar, and 1 liter deionized water, amended with 0.05% cycloheximide and 0.04% streptomycin). The cultures isolated in this study were archived in the culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.

Pure cultures of isolated fungi were sorted into groups based on their morphology. The anamorph structures originated from asexual reproduction were mounted in lactic acid on glass slides and examined microscopically. To induce the production of the fungal fruiting body, perithecia, anamorphic isolates were cultured on 1.5% oatmeal agar (15 g oat powder, 20 g Biolab agar, and 1 liter deionized water) and 2% water-agar (WA) medium (20 g Biolab agar and 1 liter distilled water) with sterilized pine twigs at 25°C in the dark.

To extract and sequence DNA, single hyphal tip cultures were prepared. Each culture was grown in 50 ml malt extract broth (30 g Biolab malt extract and 1 liter distilled water) at 25°C in the dark for 10 d. Mycelium was then harvested and freeze-dried after filtration through Whatman no. 1 filter paper and was ground into a fine powder. DNA was extracted from the resulting powder using PrepMan Ultra Sample reagent (Applied Biosystems, Foster City, CA) following the manufacturer's protocols.

PCR and DNA sequencing reactions were prepared and performed as previous works (Lu et al., 2009). Data sets were compiled in MEGA 5 (Tamura et al., 2007) and the sequences were deposited in GenBank under the accession numbers provided in Table 2. National Center for Biotechnology Information similarity scores were obtained using a BLAST search of sequences previously deposited in GenBank (www.ncbi.nlm.nih.gov).

TABLE 2. Morphological groups of ophiostomatoid isolates from chambers of *Monochamus alternatus* in Guangdong province, China.

Isolate no.	Anamorph in culture	GenBank no.		Closest matching GenBank	Score		Accession no. of match		Species identified
		ITS	β-tubulin		ITS	β-tubulin	ITS	β-tubulin	
30634[a]	*Pesotum*	GU170426	GU170410	*Ophiostoma ips*	99	99	AY172021	FJ012142	*Ophiostoma ips*
30635	*Sporothrix*	GU170430	GU170414	*Ophiostoma stenoceras*	88		AF484458		*Sporothrix* sp. 2
30636	*Sporothrix*	GU170419	GU170403	*Ophiostoma breviusculum*	95	92	AB200423	AB200428	*Sporothrix* sp. 1
30637	*Sporothrix*	GU170431	GU170415	*O. stenoceras*	88		AF484458		*Sporothrix* sp. 2
30638	*Sporothrix*	GU170432	GU170416	*O. stenoceras*	88		AF484458		*Sporothrix* sp. 2
30697	*Sporothrix*	GU170422	GU170406	*O. breviusculum*	95	92	AB200423	AB200428	*Sporothrix* sp. 1

[a] CMW = Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

The species diversity of fungal isolates from different sites was compared. The number of species (*S*), Simpson's index (D = Sum [Pi^2], where Pi is the number of a given species divided by the total number of isolates observed) and Simpson's index of diversity ($1 - D$) were used to evaluate species diversity (Lu et al., 2009).

Interaction of associated ophiostomatoid fungi: To determine the effects of a fungus on another related species, three fungal species, i.e., *Sporothrix* sp. 1, *Sporothrix* sp. 2, and *O. ips* were tested through a modified pairwise cultivation method (Hofstetter et al., 2005). The fungal isolates from both the Zhejiang and Guangdong provinces were used in these experiments. Specifically, a mycelial disc (5 mm diameter) was taken out from the periphery of an actively growing 10-d-old culture on 2% malt extract agar (MEA: 20 g malt extract, 20 g agar, and 1 liter deionized water) and was placed on a 90 × 20 mm petri dish containing 20 ml of 2% MEA. The two different inoculates were placed on opposed edges of the petri dishes and incubated at 25°C in the dark. Ten replicates for each treatment, including the controls (the same species of fungi on two sides of the plate), were performed. Radial growth for each species was measured along the line (ca. 5 cm

TABLE 3. No. of isolates of the different fungal species obtained from the pupal chambers of *Monochamus alternatus* in Guangdong province, China.

Collection sites	*Sporothrix* sp. 1	*Sporothrix* sp. 2	*Ophiostoma ips*	Total no. of isolates
Longtoushan	7	5	6	18
Baiyunshan	6	5	4	15
Datianshan	6	7	4	17
Conghua	5	6	4	15
Total (percentage frequency)	24 (36.9)	23 (35.4)	18 (27.7)	65

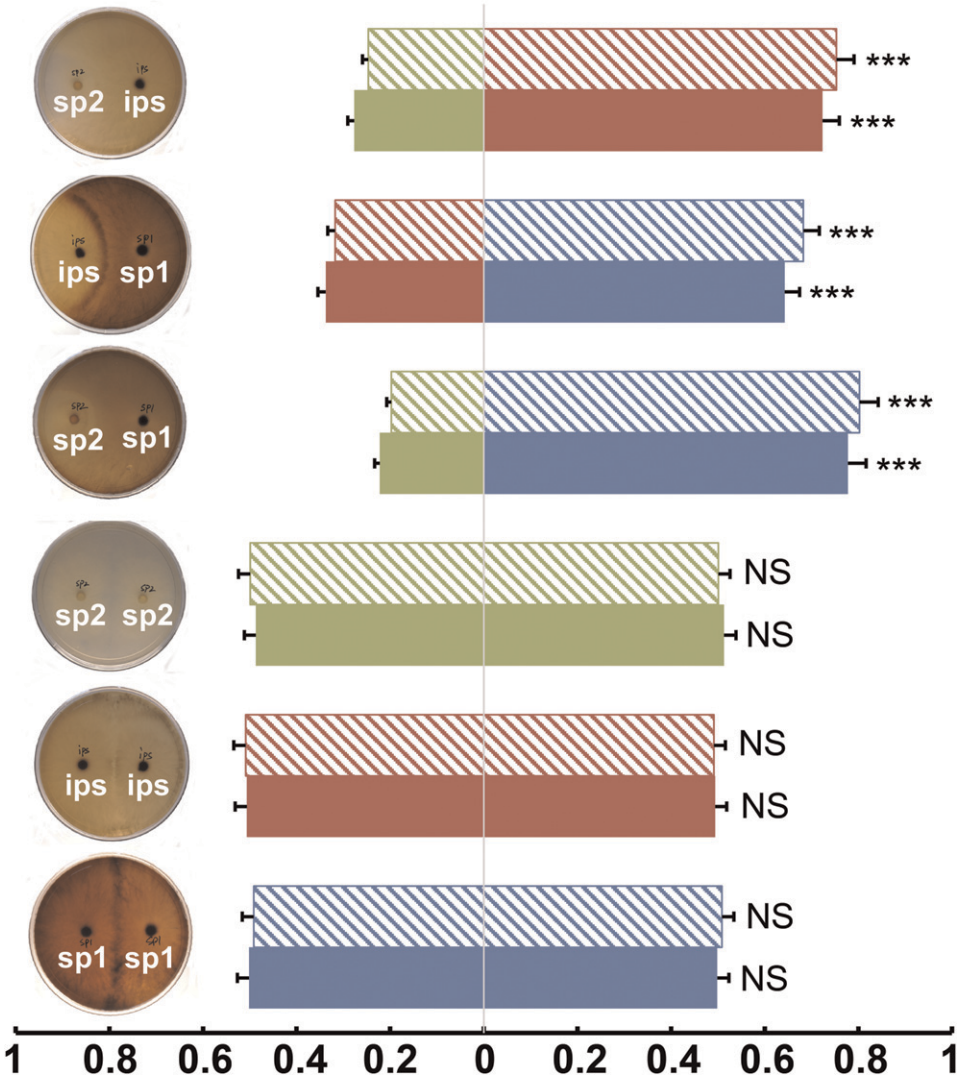

FIG. 1. Competitive development of associated ophiostomatoid fungal species. The proportion of each species accounting for the line between two inoculates were showed for each treatment. The columns with solid fill represent the fungi from Guangdong province, whereas those with backslash fill represent data from Zhejiang province. Blue column and sp1, *Sporothrix* sp. 1; red column and ips, *Ophiostoma ips*; green column and sp2, *Sporothrix* sp. 2. *** indicates $P < 0.001$ using the paired t test with the arcsine-transformed data.

in length) between two inoculates after 6 d, and the proportion of each species accounting for the line was calculated. Arcsine transformation was performed, where required to normalize the data, and paired *t* test was used to test the significance of difference. All statistical tests were conducted using the SPSS Statistics v. 19.0 (SPSS, Inc., Chicago, IL).

Effects of FAEE on mycelial growth: To determine the effects of FAEE on the fungal growth, *Sporothrix* sp. 1, *Sporothrix* sp. 2, and *O. ips* were cultured on the medium as described earlier. The most common FAEE compounds, i.e., EP, EL, ES, and EO were used as test treatments at the concentrations of 32.37, 42.55, 30.33, and 104.28 μg/ml, respectively. These concentrations were consistent with those, the nematodes would experience in a beetle chamber containing a single beetle (Zhao et al., 2013b). For each treatment, 1 ml of each test solution was added to a sterile filter paper (80 mm diameter) that was placed inside the lid of a petri dish and sealed with parafilm (Bemis Company, Neenah, WI). The treated plates were incubated at 25°C in the dark. Ten petri dish cultures for each treatment including two controls (filter paper with 1 ml ethanol and filter paper only) were used. Radial growth was measured in four directions (0°, 90°, 180°, and 270°) from the center of cultures every 24 hr for *Sporothrix* sp. 1 and 48 hr for the other two fungal species until the fungi reached the edges of the petri dish. The experiments were repeated with fungal cultures isolated from Zhejiang province. The growth rates were used for statistical analysis. One way analysis of variance (ANOVA) was performed for each fungal species, respectively.

Effects of fungi on the fitness of PWN: Five pairs of adult nematodes were inoculated to each of the three fungal species cultivated on 2% MEA (10 plates per fungus) and were incubated at 25°C in the dark for 9 d. After incubation, live nematodes were recovered using Baermann funnel. The number of individual nematodes was counted, and the body length of female, male, and the J_{III} of PWN ($n \geq 50$ nematodes from each fungal species) were measured using an Olympus BX51 microscope with a digital imaging system Olympus DP2-BSW software (Niu et al., 2012). One-way ANOVA was performed for statistical analysis. To confirm the consistency and repeatability of the results, all the experiments were repeated with the fungal species collected from Zhejiang province.

Results

Species diversity of ophiostomatoid fungi: Among all surveyed sites, we found a significant number of pupal chambers in recently killed trees at all of the four surveyed sites. Moreover, all surveyed beetle chamber was infested with PWN (Table 1).

In total, 65 ophiostomatoid fungal isolates were recovered from *M. alternatus* chambers during the course of this study from Guangdong province (Table 3). Small numbers of other fungi including yeasts were isolated, but these were not included in this study.

Based on culture and anamorph morphology, isolates could be divided into three groups. Two groups presented *Sporothrix* anamorph in the cultures, whereas the remaining groups had a *Pesotum*-like anamorph. Comparing with the referenced sequences in GenBank, two new *Sporothrix* species and *O. ips* were identified (Table 2).

More isolates were retrieved from Longtoushang (number of species: $S = 18$ and Simpson's index of diversity: $1 - D = 0.66$) and Datianshan (number of species: $S = 17$ and Simpson's index of diversity: $1 - D = 0.65$), both of which belong to Huangpu district, than those from Baiyunshan (number of species: $S = 15$ and Simpson's index of diversity: $1 - D = 0.66$) and Conghua district (number of species: $S = 15$ and Simpson's index of diversity: $1 - D = 0.66$). For each fungus, the number of isolates ranged from four to seven in different collection sites. However, we sampled more of the two newly found species in China, i.e., *Sporothrix* sp. 1

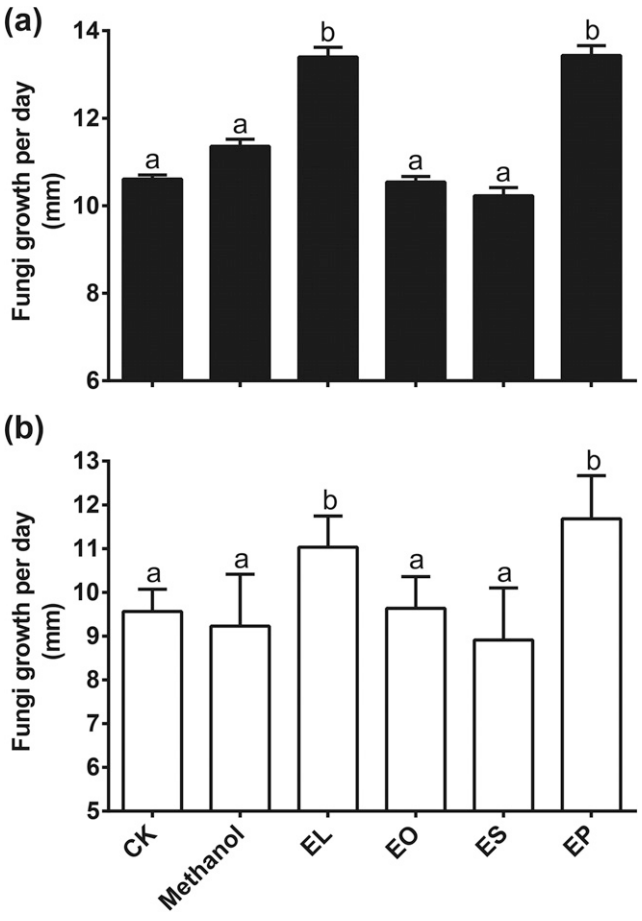

Fig. 2. Effects of fatty acid ethyl esters from *Monochamus alternatus* surface on *Sporothrix* sp. 1. A. Fungi from Guangdong province. B. Fungi from Zhejiang province. EL = ethyl linoleate, EO = ethyl oleate, ES = ethyl stearate, and EP = ethyl palmitate. Bars with the same letters are not significantly different at $P > 0.05$.

(number of species: $S = 24$ and frequency = 36.9) and *Sporothrix* sp. 2 (number of species: $S = 23$ and frequency = 35.4), compared with the common *O. ips* (number of species: $S = 18$ and frequency = 27.7) (Table 3).

Interaction of associated ophiostomatoid fungi: When cultured together, the growth of *Sporothrix* sp. 1 was significantly faster than that of *O. ips* (Guangdong: $t = 9.23$, $P < 0.001$ and Zhejiang: $t = 10.45$, $P < 0.001$) and *Sporothrix* sp. 2 (Guangdong: $t = 42.13$, $P < 0.001$ and Zhejiang: $t = 48.46$, $P < 0.001$) (Fig. 1). At the same time, *O. ips* grows significantly faster than *Sporothrix* sp. 2 (Guangdong: $t = 20.55$, $P < 0.001$ and Zhejiang: $t = 23.46$, $P < 0.001$) (Fig. 1), whereas the two inoculates in all controls (paired cultured with the same species) have no difference ($P > 0.05$ in both Guangdong and Zhejiang) (Fig. 1).

The effect of beetle surface compounds on the fungal growth: The growth bioassays of different fungi using the four main FAEE from the beetle surface showed that EP and EL significantly improved the growth of *Sporothrix* sp. 1 (Fig. 2A,B), but had no effects on the other two species of fungi (Fig. S1). The other two FAEE (EO and ES), however, did not exhibit any activity on the growth of all of the three fungi (Figs. 2,S1).

The effects of fungi on the fitness of PWN: For the organisms from Guangdong, the fecundity of nematode and their body length were both significantly affected by the fungal species they were cultured on. The number of nematodes reared on *Sporothrix* sp. 1 was highest, then *O. ips*, and *Sporothrix* sp. 2 (one-way ANOVA: $F = 33.31$, $P < 0.001$; Fig. 3A). The highest body length of female (one-way ANOVA: $F = 84.05$, $P < 0.001$; Fig. 3B), male (one-way ANOVA: $F = 35.11$, $P < 0.001$; Fig. 3C), and J_{III} (one-way ANOVA: $F = 89.03$, $P < 0.001$; Fig. 3D) were recorded in the nematodes cultured on *Sporothrix* sp. 1, followed by those reared on *O. ips*, whereas significantly smaller lengths of each stage were recorded when they were reared on *Sporothrix* sp. 2. The experiments using the fungi and nematodes from Zhejiang showed the consistent results, except that the body length of J_{III} have no difference when fed on either *Sporothrix* sp. 1 or *O. ips* (Fig. S2).

Discussion

The coexistence of PWN, its vector beetle, and the associated blue stain fungus in diseased trees represents their symbiotic association (Fig. 4) In this association, the influences of the beetles or ophistomatoid fungi on PWN and ophistomatoid fungi on vector beetle have been intensively reported (Linit, 1988; Necibi and Linit, 1998; Yang et al., 2003; Sun, 2005; Akbulut and Stamps,

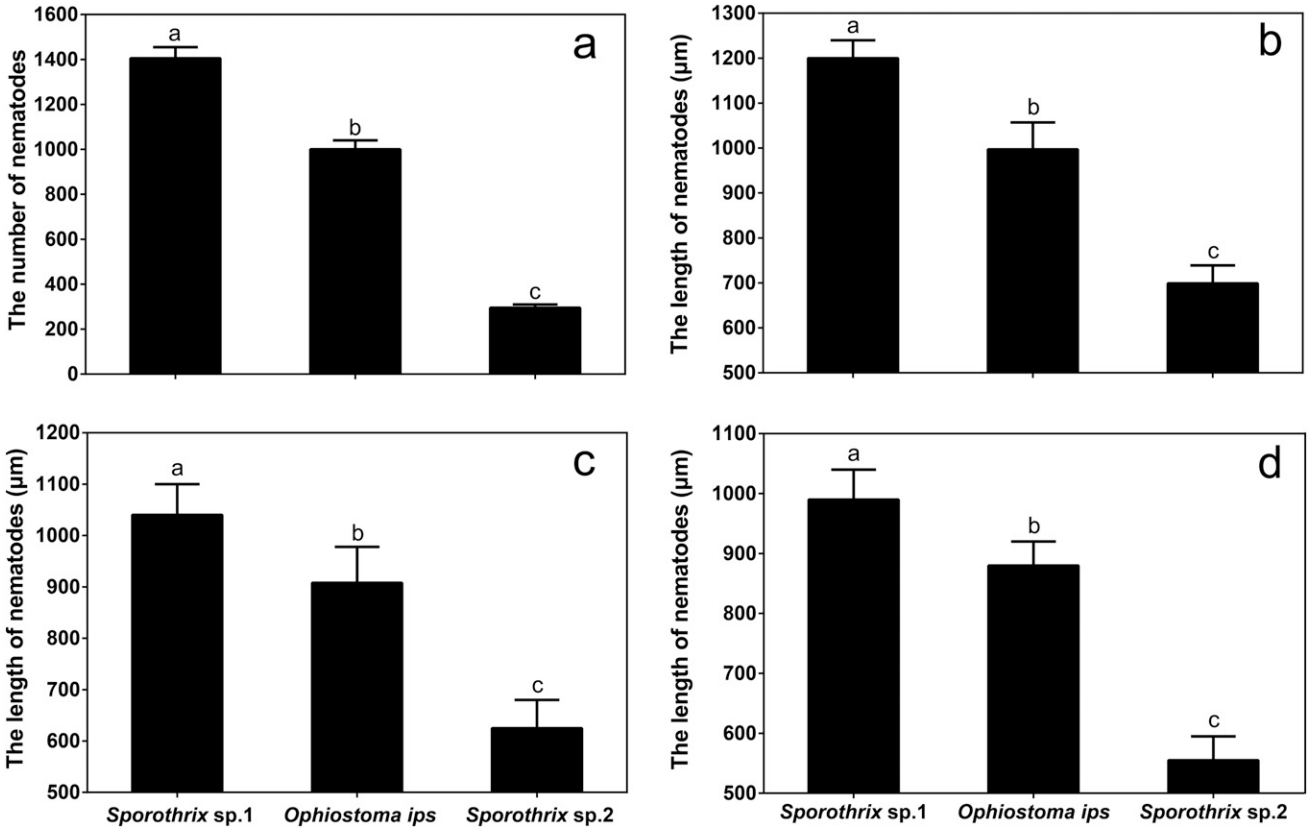

Fig. 3. Effects of fungi on the fitness of pinewood nematode in Guangdong province. The fecundity of nematodes (A), the body length of (B) female, (C) male, and (D) third-stage dispersal juveniles reared on three different fungi was counted and measured. Different letters indicate significant differences.

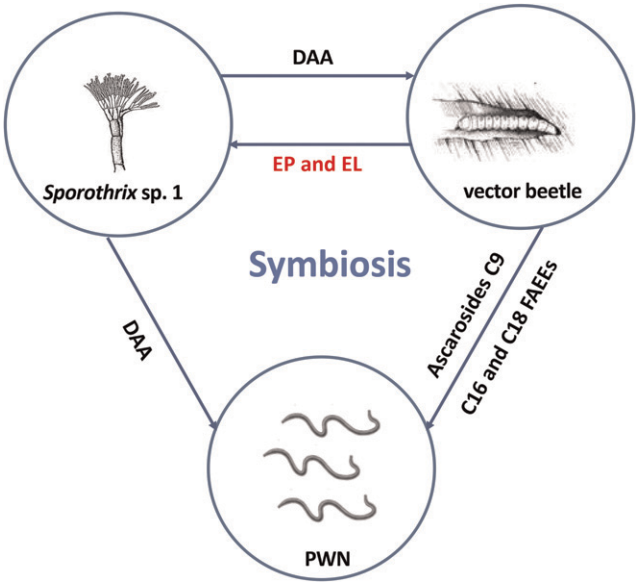

FIG. 4. Schematic presentation of the symbiotic association among *Monochamus alternatus*, *Burcaphelenchus xylophilus*, and associated ophiostomatoid fungi.

2012; Zhao et al., 2013a, 2016). However, the effect of vector beetle on the fungi has not been reported. Here, we have elucidated the underlying chemical basis of the symbiotic association between vector beetle and *Sporothrix* sp. 1. Our findings demonstrate that FAEE (EP and EL) produced on beetles' body surface enhance the growth of *Sporothrix* sp. 1 in a way that the fungus grows faster than its competitive species, *O. ips* and *Sporothrix* sp. 2. This ultimately lead to higher numbers of PWN that develop on *Sporothrix* sp. 1, with increased population number and body size of both adults and the dispersal juveniles (J_{III}). These findings complement to earlier reports stating that nematodes fed on *Sporothrix* sp. 1 have a better reproductive ability because of the DAA produced by this fungal species (Zhao et al., 2013a). Our results suggest that *Sporothrix* sp. 1 gets direct chemical signals from the beetles to expand and exploit a greater niche, ultimately escalating the pathogenicity of nematodes.

Furthermore, isolation of *Sporothrix* sp. 1 from all the sampled pupal chambers depicts a strong association of the fungi with vector beetles. FAEE (C16 and C18) from *Monochamus* beetle are known as chemical signals synchronizing the beetle to PWN life cycle (Zhao et al., 2013b, 2016). We also found the extended scope of these FAEE, especially the EP and EL, which enhanced the growth of *Sporothrix* sp. 1 (Fig. 4). This symbiosis might provide a new aspect of explanation to invasive success of PWN in China.

The successful establishment of the invasive pathogen in the introduced region is often attributed to local associates that enhance their pathogenicity. In China, PWN might have developed a symbiotic association with two of the native fungal species (*Sporothrix* sp. 1 and *Sporothrix* sp. 2), which are absent in North America, the native region of *B. xylophilus*. When developed on *Sporothrix* sp. 1, nematodes increase in body sizes and had higher fecundities, hence, favoring pine wilt disease prevalence in china. These findings are also in accordance with the earlier studies reporting that larger body size in worms produce more offspring (Poulin, 1996).

Besides nematodes, cerambycid beetles are also known to establish mutualistic associations with other microorganisms including mites (Whitney, 1982; Paine et al., 1997; Moser et al., 2005; Hofstetter et al., 2006; Cardoza et al., 2008) that might be responsible for transferring fungi from one cerambycid pupal chamber to the other (Wingfield, 1987). But few studies focus on the role of mites in the cerambycid beetles—nematodes system. Therefore, to complete the picture of pine wilt disease system, future research is necessary to establish the role of mites in the transmission of fungi.

In conclusion, the results present a unique aspect in the symbiotic relationship among vector beetle, associated blue stain fungi, and the PWN. The vector beetle, *M. alternatus*, not only directly disseminates PWN to new hosts but also improves the quality of fungal diet of PWN, hence, indirectly facilitating its prevalence. This information will also contribute to a better understanding of the microecological interactions that facilitate the eruptive population dynamics of important forest pests (Hofstetter et al., 2006).

LITERATURE CITED

Akbulut, S., and Stamps, W. T. 2012. Insect vectors of the pinewood nematode: A review of the biology and ecology of *Monochamus* species. Forest Pathology 42:89–99.

Cardoza, Y. J., Moser, J. C., Klepzig, K. D., and Raffa, K. F. 2008. Multipatrite symbioses among fungi, mites, nematodes, and the spruce beetle, *Dendroctonus rufipennis*. Environment Entomology 37:956–963.

Dwinell, L. D. 1997. The pinewood nematode: Regulation and mitigation. Annual Review of Phytopathology 35:153–166.

Hofstetter, R. W., Cronin, J. T., Klepzig, K. D., Moser, J. C., and Ayres, M. P. 2006. Antagonisms, mutualisms and commensalisms affect outbreak dynamics of the southern pine beetle. Oecologia 147:679–691.

Hofstetter, R. W., Mahfouz, J. B., Klepzig, K. D., and Ayres, M. P. 2005. Effects of tree phytochemistry on the interactions among endophloedic fungi associated with the southern pine beetle. Journal of Chemical Ecology 31:539–560.

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. Annual Review of Entomology 29:115–135.

Linit, M. J. 1988. Nematode-vector relationships in the pine wilt disease system. Journal of Nematology 20:227–235.

Lu, M., Wingfield, M. J., Gillette, N. E., Mori, S. R., and Sun, J.-H. 2010. Complex interactions among host pines and fungi vectored by an invasive bark beetle. New Phytologist 187:859–866.

Lu, M., Wingfield, M. J., Gillette, N. E., and Sun, J.-H. 2011. Do novel genotypes drive the success of an invasive bark beetle-fungus complex? Implications for potential reinvasion. Ecology 92:2013–2019.

Lu, M., Zhou, X. D., De Beer, Z. W., Wingfield, M. J., and Sun, J.-H. 2009. Ophiostomatoid fungi associated with the invasive pine-infesting bark beetle, *Dendroctonus valens*, in China. Fungal Diversity 38:133–145.

Maehara, N., and Futai, K. 1997. Effect of fungal interactions on the numbers of the pinewood nematode, *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae), carried by the Japanese pine sawyer, *Monochamus alternatus* (Coleoptera: Cerambycidae). Fundamental and Applied Nematology 20:611–617.

Mamiya, Y., and Enda, N. 1972. Transmission of *Bursaphelenchus lignicolus* (Nematoda: Aphelenchoididae) by *Monochamus alternatus* (Coleoptera: Cerambycidae). Nematologica 18:159–162.

Morimoto, K., and Iwasaki, A. 1972. Role of *Monochamus alternatus* (Coleoptera: Cerambycidae) as a vector of *Bursaphelenchus lignicolus* (Nematoda: Aphelenchoididae). Journal of the Japanese Forestry Society 54:177–183.

Moser, J. C., Konrad, H., Kiristis, T., and Carta, L. K. 2005. Phoretic mites and nematodes associates of *Scolytus multistriatus pygmaeus* (Coleoptera: Scolytidae) in Austria. Agricultural and Forest Entomology 7:169–177.

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. Journal of Nematology 30:246–254.

Nickle, W. R., Friedman, W., and Spilman, T. J. 1980. *Monochamus scutellatus*: Vector of pine wood nematode on Virginia pine in Maryland. Cooperative Plant Pest Report 5:383.

Niu, H. T., Zhao, L. L., Lu, M., Zhang, S., and Sun, J. H. 2012. The ratio and concentration of two monoterpenes mediate fecundity of the pinewood nematode and growth of its associated fungi. PLoS One 7:e31716.

Paine, T. D., Raffa, K. F., and Harrington, T. C. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annual Review of Entomology 42:179–206.

Pan, H. Y., Ye, J. R., and Wu, X. Q. 2009. Spatial distribution patterns of pine wilt disease in China. Acta Ecologica Sinica 29:4325–4331.

Poulin, R. 1996. The evolution of life history strategies in parasitic animals. Advances in Parasitology 37:107–134.

Rautapaa, J. 1986. Experiences with *Bursaphelenchus* in Finland. Conference on pest and disease problems in European forests. EPPO Bulletin 16:453–456.

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. Thailand: FAO Regional Office for Asia and Pacific.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24:1596–1599.

Togashi, K. 1985. Transmission curves of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) from its vector, *Monochamus alternatus* (Coleoptera: Cerambycidae), to pine trees with reference to population performance. Applied Entomology and Zoology 20:246–251.

Whitney, H. S. 1982. Relationships between bark beetles and symbiotic organisms. Pp. 527 *in* J. B. Mitton, and K. B. Strgeon, eds. Bark beetles in North American conifers: A system for the study of evolutionary biology. Austin: University of Texas Press.

Wingfield, M. J. 1987. Fungi associated with the pine wood nematode, *Bursaphelenchus xylophilus*, and cerambycid beetles in Wisconsin. Mycologia 79:325–328.

Yang, B. J., Pan, H. Y., Tang, J., Wang, Y. Y., Wang, L. F., and Wang, Q. 2003. *Bursaphelenchus xylophilus*. Beijing: Chinese Forestry Press.

Zhao, L. L., Lu, M., Niu, H. T., Fang, G. F., Zhang, S., and Sun, J. H. 2013a. A native fungal symbiont facilitates the prevalence and development of an invasive pathogen-native vector symbiosis. Ecology 94:2817–2826.

Zhao, L. L., Mota, M., Vieira, P., Butcher, R. A., and Sun, J. H. 2014. Interspecific communication between pinewood nematode, its insect vector, and associated microbes. Trends in Parasitology 30:299–308.

Zhao, L. L., Zhang, S., Wei, W., Hao, H. J., Zhang, B., Butcher, R. A., and Sun, J. H. 2013b. Chemical signals synchronize the life cycles of a plant-parasitic nematode and its vector beetle. Current Biology 23:2038–2043.

Zhao, L. L., Zhang, X. X., Wei, Y. N., Zhou, J., Zhang, W., Qin, P. J., Chinta, S., Kong, X. B., Liu, Y. P., Yu, H. Y., Hu, S. N., Zou, Z., Butcher, R. A., and Sun, J. H. 2016. Ascarosides coordinate the dispersal of a plant-parasitic nematode with the metamorphosis of its vector beetle. Nature Communications 7:12341.