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The effect of feeding behavior of *Monochamus alternatus* (Coleoptera: Cerambycidae) on the departure of pine wood nematode, *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae)

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SUMMARY STATEMENT

The feeding behavior of *Monochamus alternatus* has no effect on the departure of pine wood nematodes, but the respiration of *Monochamus alternatus* may be the cause.

ABSTRACT

In order to study the causes of pine wood nematode (PWN) departure from *Monochamus alternatus*, the effects of the feeding behavior of *M. alternatus* on the start date of departureof PWN were studied. The start date of the departure of PWN carried by the directly fed *M. alternatus* was 5–13 d after beetle emergence, mainly concentrated within 6–10 d, with a mean (±SD) of 8.02 ± 1.96 d. The start date of the departure of PWN carried by the *M. alternatus* fed after starvation was 5–14 d after beetle emergence, mainly concentrated within 6–9 d, with a mean of 7.76 ± 2.28 d. The results show that there was no significant difference in the start departure date of PWN between the two treatments. This show that the feeding behavior, subsequent metabolic changes caused by feeding behavior of *M. alternatus* and pine volatiles were not the triggers for PWN departure. At the same time, it was found that the

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motility of the PWN carried by *M. alternatus* at 8 d after emergence was significantly greater than that of the PWN carried by the newly emerged *M. alternatus*. And the PWN carried by *M. alternatus* at 8 d after emergence more easily extracted than the PWN carried by newly emerged beetles. Greater motility PWN were easier departure from *M. alternatus*.

INTRODUCTION

Pine Wilt Disease (PWD) constitutes one of the most serious conifer diseases worldwide, affecting *Pinus* spp. from the Far East forestlands (Japan, China and Korea) (Cheng et al., 1983; Yi et al., 1989), and from Europe (Portugal and Spain) (Abelleira et al., 2011; Fonseca et al., 2012; Mota et al., 1999; Robertson et al., 2011). This disease causes significant economic and environmental damage to the countries affected, with large annual losses of timber (Mamiya et al., 2004), increased costs in management procedures, including disease and pest control (Mamiya et al., 2004; Yang et al., 2004). This disease is caused by the pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, and PWN transmission is dependent on vector insects, the main vector insect in East Asia is *Monochamus alternatus* Hope (Linit, 1989; Morimoto and Iwasaki, 1972). There are two developmental forms in the life cycle of the PWN, namely the propagative and dispersal forms. Under favorable conditions, PWN molt into their propagative form, and then reproduce rapidly (Mamiya, 1984; Wingfield, 1983). However, under unfavorable conditions, e.g. high population density, starvation, and high temperature, the propagative second-stage juveniles will molt to produce dispersal third-stage juveniles, which will aggregate around the pupal chamber of the vector insects (Linit, 1988; Zhao et al., 2014). The dispersal third-stage juveniles molt to produce fourth-stage juveniles, which enter the tracheal system of the vector as the beetles emergence (Linit, 1988,1990; Necibi and Linit, 1998; Zhao et al., 2014), the fourth-stage dispersal juveniles to be transmitted to the healthy pine trees through wounds caused by the vector (Niu et al., 2012; Stamps and Linit,1988,2001; Zhao et al., 2007).

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The PWN cannot be transmitted immediately after the emergence of *M. alternatus*. A number of studies have investigated the time after *M. alternatus* emergence when the PWN transmission starts. Fourth-stage dispersal juveniles exit the tracheal system of *M. alternatus* at 3–5 d after emergence (Hosoda and Kobayashi, 1977), or at 5 d after emergence (Jikumaru and Togashi, 2000), or at 7 d after emergence (Aikawa, 2008), others observed that *M. alternatus* started to transmit PWN at 7–12 d after emergence (Wang et al. 2019), or at 10 d after emergence (Togashi, 1985), or within 10 d after the emergence of the beetle (Enda, 1972).

What causes the departure of PWN from its insect host? A number of studies have tried to investigate the factors: the degradation of neutral storage lipid was correlated with nematode exit (Stamps and Linit, 1988), assumed *β*-myrcene to play an important role in the transmigration of the PWN from the sawyer to the pine tree (Ishkawa et al., 1986), PWN has a trait of spontaneous departure from *M. alternatus*, pine volatiles repress PWN departure from *M. alternatus*(Aikawa and Togashi, 1998), PWN departure behavior possibly endogenous nature factors (Aikawa and Togashi, 1998; Stamps and Linit, 1988), when CO_2 concentration in the trachea reaches a certain critical value, PWN begins to escape from beetle (Wu et al., 2019). The feeding period of *M. alternatus* is an important stage in the life cycle of the beetles as well as a key step in PWN transmission (Yoshimura et al., 1999; Togashi and Shigesada, 2006). Whether the feeding behavior of *M. alternatus* is the factor of PWN departure has not been studied. In order to further study the causes of PWN departure from *M. alternatus*, we investigated the effect of the feeding behavior of beetle on PWN departure, the differences in motility and difficulty level of extracted between the PWN carried by *M. alternatus* at 8 d after emergence and newly emerged.

RESULTS

Effect of beetle feeding behavior on PWN departure from *M. alternatus*

Data from the study into the time after *M. alternatus* emergence required for PWN start departure from beetles are shown in Table 1. It can be seen from the table that the mean start time for PWN departure from directly fed *M. alternatus* was 8.02 ±

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1.96 d after beetle emergence, and there was no significant difference between male and female beetles fed directly (*P*=0.47). The mean start time for PWN departure from *M. alternatus* fed after starvation was 7.76 ± 2.28 d after beetle emergence, with no significant difference between male and female beetles (*P*=0.19). There was no significant difference in the time that PWN started to departure from the beetles under the two feeding regimes (*P*=0.34), with no significant differences in the start time of PWN departure from female (*P*=0.58) or male (*P*=0.12) beetles under the two feeding regimes.

Table 1. The start date of PWN departure from *Monochamus alternatus*

Treatment	Direct feeding		Feeding after starvation	
Sex of insects	♀	♂	♀	♂
Days after emergence of *Monochamus alternatus*	5	6	5	5
	6	6	6	6
	6	6	6	6
	6	6	6	6
	7	6	6	6
	7	6	6	6
	7	7	6	6
	7	7	7	6
	7	7	7	7
	7	8	7	7
	7	9	7	7
	7	9	8	8
	7	9	8	9
	8	10	9	9
	8	10	9	9
	9	10	9	12
	9	11	10	
	9	12	11	

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	10	12	12	
	10		13	
	10		14	
	13			
Average (mean ± SD)	7.82±1.82	8.26±2.13	8.19±2.54	7.19±1.80
	8.02±1.96		7.76±2.28	

The start date of PWN departure from *M. alternatus* fed directly was mainly concentrated in the period 6–10 d after emergence, which thirty-six *M. alternatus* accounted for 87.8% of the total. The start date of departure of PWN from *M. alternatus* fed after a starvation treatment was mainly concentrated in the period 6–9 d after emergence, which twenty-nine *M. alternatus* accounted for 78.4% of the total. There was no significant difference between the two treatments in peak period of PWN start departure from beetles ($P = 0.078$).

These results indicated that the feeding behavior of *M. alternatus* had no significant effect on the departure of PWN. It also suggest that the consequent metabolic changes caused by feeding behavior and volatiles from pine twigs had no effect on the departure of PWN (When the beetles were treated with starvation, they were not stimulated by volatiles.).

Comparisons of nematode motility

The effect of beetle feeding behavior on the motility of the PWN carried by the beetles is shown in supplementary video 1 (the motility of the PWN carried by the newly emerged *M. alternatus*) and video 2 (the motility of the PWN carried by the beetles at 8 d after emergence).

After dark treatment for 15 minutes (simulate conditions in the body of the beetle), the PWN carried by the newly emerged beetles were less motility than the PWN carried by the beetles at 8 d after emergence ((starvation beetles have the same results). At the same time, it was found that the PWN carried by *M. alternatus* at 8 d

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after emergence were easier to extracted (starvation beetles have the same results) than the PWN carried by the newly emerged beetles (Table 2). A higher proportion of PWN being collected within12 h than from the newly emerged beetles ($P < 0.01$). These suggests greater motility PWN were easier departure from *M. alternatus*.

Table 2 The percentage of PWN in different extracted periods

		The time of PWN collected		
	NO.	12 h	24 h	36 h
Newly emerged beetles	1	29%	50%	21%
	2	35%	53%	12%
	3	26%	49%	25%
	4	38%	49%	13%
	5	25%	61%	14%
	6	29%	46%	25%
	7	41%	40%	19%
	8	27%	51%	22%
	9	33%	51%	16%
	10	31%	55%	14%
Beetles at 8 day after emergence	11	80%	15%	5%
	12	84%	11%	5%
	13	91%	6%	3%
	14	81%	13%	6%
	15	77%	16%	7%
	16	75%	17%	9%
	17	72%	20%	8%
	18	81%	15%	4%
	19	83%	12%	5%
	20	70%	23%	7%

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mean	31%	51%	18%
	79%	15%	6%

DISCUSSION

A number of studies have investigated the cause which affect PWN departure from beetles, although there were differences in the results. Stamps et al. reported that neutral storage (NS) lipid was correlated with nematode exit and may contribute to PWN exit from vectors(Stamps and Linit, 1988). A rolling fulcrum model of the integration of intrinsic (NS lipid) and extrinsic (volatiles) cues is proposed to explain the behavioural ontogeny of fourth-stage dispersal juveniles in relation to the beetle vector (Stamps and Linit, 2001). Neutral storage (NS) lipid content is proposed as a modifier of PWN response to beetle and tree-produced volatiles, with the lowest NS lipid content were attracted to β-myrcene, a pine volatile, while PWN with the highest NS lipid content were attracted to toluene, a beetle cuticular hydrocarbon (Stamps and Linit, 1998, 2001). Ishkawa et al. (1986) examined the attractant effect of volatile components in *Pinus densiflora*, to the PWN (dispersal nematodes that leave *M. alternatus*), and assumed that β-myrcene play an important role in the transmigration of the PWN from the sawyer to the pine tree and for the movement of the PWN inside the pine wood. However, in the present study, we believe that volatiles were not the cause of PWN departure from *M. alternatus*.

Provided autoclaved or fresh pine twigs to the beetles respectively. Consequently, it was suggested that PWN had a trait of spontaneous departure from *M. alternatus* and that the volatiles from fresh *P. densiflora* twigs repressed PWN departure from *M. alternatus* (Aikawa and Togashi, 1998). Stamps and Linit (1988), examined the effects of various pine volatiles on PWN departure, suggested that PWN exit behavior possibly controlled by endogenous nature factors. Volatiles thought to be attractants may actually act more as aggregation stimulants than as attractants, with PWN moving randomly until the desired chemical is reached, whereupon movement decreases or stops (Futai, 1980; Stamps and Linit, 1998). Volatiles may only play a

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role in fourth-stage dispersal juveniles movement from beetles into pine once the nematodes have physically dropped onto the tree surface (Stamps and Linit, 1988, 1998).

In the present study, feeding behavior of the beetles had no significant effect on the PWN departure from *M. alternatus*. The results also indicated that volatiles of pine and the metabolic changes of *M. alternatus* caused by feeding had no effect on the departure of PWN. The two main behaviors of *M. alternatus* following emergence are feeding and breathing. Wu et al. reported that the metabolic and respiratory rates of *M. alternatus* after emergence were increased by feeding. This led to a rise in CO_2 concentration in the trachea of *M. alternatus*. When the CO_2 concentration in trachea reached a certain critical level, PWN began to escape from beetle (Wu et al., 2019). In the present study, we observed that the PWN carried by the newly emerged beetles were less motility than the PWN carried by the beetles at 8th d after emergence, and the PWN carried by *M. alternatus* at 8 d after emergence were easier to extracted than the PWN carried by newly emerged beetles, suggest that the departure of PWN from host might be related to their motility. PWN movement requires energy, lipid degradation provides the energy needed for exercise (Stamps and Linit, 1988,1998), and lipid fully degradation to produce large amounts of energy requires sufficient O2. Respiratory behavior leads to a critical level of CO_2 in the trachea of *M. alternatus* (Wu et al., 2019), meanwhile the O_2 concentration in the beetle trachea will also reach a critical level when breathing (the concentration of O2 will increase when inhaling). May be PWN which in the trachea of the beetle receive sustained stimulation of high concentration of O_2, which leads to lipid degradation and expression of motor-related genes, and ultimately leads to increased motility of PWN. The departure may be caused by strong motility of PWN to avoid CO_2 (Wu et al., 2019) or to move toward high-O2 conditions.

Tian et al. (2017) carried out analysis of metabolism related genes and showed that the metabolic level of fourth-stage dispersal juveniles (carried by newly emerged beetles) was much lower than third-stage dispersal juveniles. This was due to the decrease in enzymatic activity in those pathways involving metabolism of glycolysis,

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oxidative phosphorylation, the tricarboxylic acid cycle, gluconeogenesis, etc.(O'Riordan and Burnell, 1989). The expression of sorbitol dehydrogenase gene in the fourth-stage dispersal juveniles (carried by newly emerged beetles) was up-regulated (Tian et al., 2017), this enzyme may be involved in ethanol fermentation under anaerobic conditions (Mcelwee et al., 2006) provides basic energy for PWN. These indicated that the PWN were in a hypoxic environment in the early stage after entering the trachea of *M. alternatus*. In the present study greater motility PWN (carried by *M. alternatus* at 8 d after emergence) were easier departure from *M. alternatus*. Greater motility showed that PWN had a strong metabolism and abundant energy. A large amount of lipids metabolism after a period of emergence of the beetle (Stamps and Linit, 1988), and Oxidative phosphorylation requires sufficient oxygen sustained stimulation. The respiration of the adult beetle may provide sufficient oxygen to PWN. In summary, our view is that may be the respiration of the adult beetle causes the increase in O_2 concentration in the trachea, which causes the degradation of lipid to produce energy which increased the mobility of PWN, eventually triggers the departure of PWN from insect host, with to avoid CO_2 (Bretscher et al., 2008; Wu et al., 2019) or to move toward high-O2 directions. Related molecular mechanisms are under investigation.

MATERIALS AND METHODS

Collection of infected wood and *M. alternatus*

In March 2019, dead specimens *Pinus massoniana* trees, infested by *M. alternatus* larvae and PWN, were collected at Bocun Forest Farm, Huangshan City, Anhui Province in eastern China. The pine trees were cut into logs (1.0–1.2 m in length) and maintained in outdoor insect cages (0.5 m × 0.5 m × 1.2 m), as shown in Fig. 1 A. *Monochamus alternatus* were collected daily (every 6 h) during the period of adult beetles emergence from the logs.

The collected *M. alternatus* were divided into two groups and maintained in tissue culture flasks at 25°C, one of which was fed (with fresh one-year-old pine twigs with the needles removed) immediately after collection (“direct feeding”), whereas

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the second group was fed after a starvation period of 4 d (The mortality rate of the beetle for more than 4 d was very high.). In the present study, 41 *M. alternatus* were fed immediately and 37 were fed after a starvation period, the feeding of the beetles is shown in Fig. 1 B.

Extraction and counting of PWN

The twigs (with the pine needles removed) of *P. massoniana* were replaced daily. The twigs had been fed by the beetles were cut into pieces and the PWN were extracted using Bellman funnel method (Baermann, 1917). Twenty-four hours after extraction, the suspension at the bottom of the funnel was collected with a 10 ml centrifuge tube. The number of PWN was counted with a microscope (Zeiss Axio Lab, A1, Carl Zeiss, Gottingen, GERMANY) under 10×4 field.

PWN motility and extraction percentage observations

PWN were extracted from newly emerged *M. alternatus* and at 8 d after emergence (PWN were collected every 4 hours). The PWN were washed with sterile water and treated in the dark for 10 minutes. Then the motility of PWN was observed under a microscope (Zeiss Axio lmager.M2, Carl Zeiss, Gottingen, GERMANY) and videos were taken (Zeiss AxioCam HRc).

Extracted PWN from 10 *M. alternatus* at 8 d after emergence and 10 newly emerged *M. alternatus*, respectively. And calculated the percentage of PWN in different extracted periods (0–12 h, 12–24 h, 24–36 h) accounted for the total extraction.

Statistical analysis

Statistical analysis was performed using SPASS 19.0 (IBM, Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to analyze the differences of the start departure time of PWN between *M. alternatus* feeding after starvation and direct feeding. Mann-Whitney test was performed to analysis the difference of difficulty level of extracted between the PWN carried by *M. alternatus* at 8 d after emergence and newly emerged.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The sponsors had no role in the design, execution, interpretation, or writing of the study

REFERENCES

Abelleira, A., Picoaga, A., Mansilla, J. P., and Aguin, O. (2011). Detection of *Bursaphelenchus Xylophilus*, Causal Agent of Pine Wilt Disease on Pinus pinaster in Northwestern Spain. *Plant Dis.* 95(6), 776.

Aikawa,T. (2008). Transmission biology of *Bursaphelenchus xylophilus* in relation to its insect vector. Pine Wilt Disease, pp 123-138. Springer.

Aikawa, T. and Togashi, K. (1998). An effect of pine volatiles on departure of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) from *Monochamus alternatus* (Coleoptera: Cerambycidae). *Appl. Entomol. Zoolog.* 33(2), 231-237.

Baermann, G. (1917). Eine eifache methode Zur Auffindung von Anklyostomum (Nematoden) larven in Erdproben. *Geneesk. Tydschr. Nederl. Indie*. 57, 131-137.

Bretscher, A. J., Busch, K. E. and De Bono, M. (2008). A carbon dioxide avoidance behavior is integrated with responses to ambient oxygen and food in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U S A.* 105(23), 8044-8049.

Cheng, H., Lin, M., Li, W. and Fang, Z. (1983). The occurrence of a pine wilting disease caused by a nematode found in Nanjing. *For Pest Dis.* 4, 1-5.

Enda, N. (1972). Removing dauer larvae of *Bursaphelenchus lignicolus* from the body of *Monochamus alternatus*. *Kanto. Branch. Jpn. For. Soc.* 32.

Fonseca, L., Cardoso, J., Lopes, A., Pestana, M., Abreu, F., Nunes, N., et al. (2012). The pinewood nematode, *Bursaphelenchus xylophilus*, in Madeira Island. *Helminthologia.* 49(2), 96-103.

Futai, K. (1980). Host Preference of *Bursaphelenchus lignicolus* (Nematoda :

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Aphelenchoididae) and *B. mucronatus* Shown by their aggregation to Pine Saps. *Appl. Entomol. Zoolog*. 15(3), 193-197.

Hosoda, R. and Kobayashi, K. (1977). Drop off procedures of the pine wood nematode from the pine sawyer. *Kanto. Branch. Jpn. For. Soc.* 255-258.

Ishkawa, M., Shuto, Y. and Watanabe, H. (1986). β-Myrcene, a potent attractant component of pine wood for the pine wood nematode, *Bursaphelenchus xylophilus*. *Agric. Bioi. Chem.* 50(7), 1863-1866.

Jikumaru, S. and Togashi, K. (2000). Temperature effects on the transmission of *Bursaphelenchus xylophilus* (Nemata: Aphelenchoididae) by *Monochamus alternatus* (Coleoptera: Cerambycidae). *J. Nematol*. 32(1), 110-116.

Linit, M. J. (1988). Nematode-vector relationships in the pine wilt disease system. *J. Nematol*. 20(2), 227-235.

Linit, M. J. (1989). Temporal pattern of pinewood nematode exit from the insect vector *Monochamus carolinensis*. *J. Nematol.* 21(1), 105.

Linit, M. J. (1990). Transmission of pinewood nematode through feeding wounds of *Monochamus carolinensis* (Coleoptera: Cerambycidae). *J. Nematol*. 22(2), 231.

Mamiya, Y., Mota, M. and Vieira, P. (2004). Pine wilt disease in Japan. The pinewood nematode, *Bursaphelenchus xylophilus* an international workshop, University of Évora, Portugal, August.

Mamiya, Y.(1984). The pine wood nematode. *Helminthologia*. 49(2), 96-103.

Mcelwee, J.J., Eugene, S., Eric, B., Janet, T. and David, G. (2006). Diapause-associated metabolic traits reiterated in long-lived daf-2 mutants in the nematode *Caenorhabditis elegans*. *Mech. Ageing. Dev.* 127(5), 458-472.

Mota, M. M., Braasch, H., Bravo, M. A. and Penas, A.C., Burgermeister, W., Metge K., et al. (1999). First report of *Bursaphelenchus xylophilus* in Portugal and in Europe. *Nematology*. 1(1), 727-734.

Morimoto, K., Iwasaki, A. (1972). Role of *Monochamus alternatus* (Coleoptera : Cerambycidae) as a vector of *Bursaphelenchus lignicolus* (Nematoda : Aphelenchoididae). *J. Jpn. For. Soc.* 54(6), 177-183.

Necibi, S., Linit, M. J. (1988). Effect of *Monochamus carolinensis* on

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Bursaphelenchus xylophilus dispersal stage formation. *J. Nematol*. 30(2), 246-254.

Niu, H., Zhao, L., Lu, M., Zhang, S. and Sun J. (2012). The ratio and concentration of two monoterpenes mediate fecundity of the pinewood nematode and growth of its associated fungi. *Plos. One*. 7(2), e31716.

O'Riordan, V. B. and Burnell, A. M. (1989). Intermediary metabolism in the dauer larva of the nematode *Caenorhabditis elegans*-1. Glycolysis, gluconeogenesis, oxidative phosphorylation and the tricarboxylic acid cycle. *Comp. Biochem. Physiol. B-Comp. Biol*. 92(2), 233-238.

Robertson, L., Arcos, S. C., Escuer, M., Merino, R. S., Esparrago, G., Abelleira, A., et al. (2011). Incidence of the pinewood nematode *Bursaphelenchus xylophlius* Steiner & Buhrer, 1934 (Nickle, 1970) in Spain. *Nematology*. 13(6), 755-757.

Stamps, W. T. and Linit, M. J. (1998). Chemotactic response of propagative and dispersal forms of the pinewood nematode *Bursaphelenchus xylophilus* to beetle and pine derived compounds. *Fundam. appl. Nematol*. 21(3), 243-250.

Stamps, W. T. and Linit, M. J. (2001). Interaction of intrinsic and extrinsic chemical cues in the behaviour of *Bursaphelenchus xylophilus* (Aphelenchida : Aphelenchoididae) in relation to its beetle vectors. *Nematology*. 3(4), 295-301.

Stamps, W. T. and Linit, M. J. (1988). Neutral storage lipid and exit behavior of *Bursaphelenchus xylophilus* fourth- stage dispersal juveniles from their beetle vectors. *J. Nematol*. 30(2), 255-261.

Tian, H., Zhang, S., Liu, X., Zhang, Z. and Zhao, L. (2017). Comparative analysis of dispersal and propagative larval stages in the pinewood nematode (*Bursaphelenchus xylophilus*) based on a digital gene expression library. *J. Bios*. 26(2), 111-121.

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. *Appl. Entomol. Zoolog*. 20, 246-251.

Togashi, K. and Shigesada, N.(2006). Spread of the pinewood nematode vectored by

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the Japanese pine sawyer: modeling and analytical approaches. *Popul. Ecol.* 48(4), 271-283.

Wang, Y., Chen, F. M., Zhou, Q., Zhou, L. F. and Sun, S. H. (2019). Transmission of the pine wood nematode, *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae), through feeding activity of *Monochamus alternatus* (Coleoptera: Cerambycidae). *J. Nanjing. For. Univ.* 43(6), 1-10.

Wingfield, M. J. (1983). Transmission of pine wood nematode to cut timber and girdled trees. *Plant Dis*. 67(1), 35-37.

Wu, Y. X., Wickham, J. D., Zhao, L. L. and Sun, J. J. (2019). CO2 drives the pine wood nematode off its insect vector. *Curr. Biol.* 29(13), R619-R620.

Yang, B. J., Mota, M. and Vieira, P. (2014).The history, dispersal and potential threat of pine wood nematode in China. The pinewood nematode, *Bursaphelenchus xylophilus* Nematology Monographs and Perspectives, pp. 21-24. The Netherlands: Brill Academica Publishers.

Yi, C. K., Byun, B. H., Park, J. D., Yang, S. I. and Chang, K. H. (1989). First finding of the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle and its insect vector in Korea. *Res. Rep. For. Res. Ins. Seoul.* 38, 141-149.

Yoshimura, A., Kawasaki, K., Takasu, F., Togashi, K., Futai, K. and Shigesada, N. (1999). Modeling the spread of pine wilt disease caused by nematodes with pine sawyer as vector. *Ecology*. 80(5), 1691-1702.

Zhao, L. L., Wei, W., Kang, L. and Sun, J. H. (2007). Chemotaxis of the pinewood nematode, *Bursaphelenchus xylophilus*, to volatiles associated with host pine, Pinus massoniana, and its vector *Monochamus alternatus*. *J. Chem. Ecol.* 33(6), 1207-16.

Zhao, L., Mota, M., Vieira, P., Butcher, R. A. and Sun, J. (2014). Interspecific communication between pinewood nematode, its insect vector, and associated microbes. *Trends Parasitol.* 30(6), 299.

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FIGURE

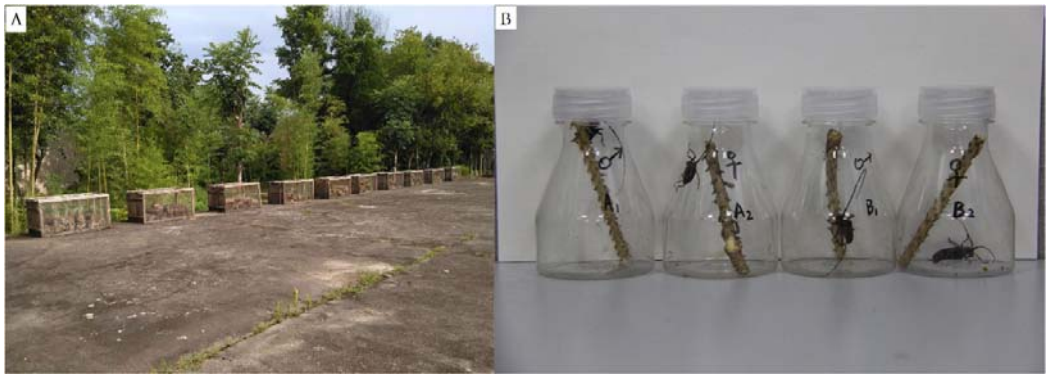

Fig. 1 Collection and feeding of *Monochamus alternatus*

A: The pine trees were cut into logs and maintained in outdoor insect cages. *Monochamus alternatus* were collected daily (every 6 h) during the period of adult beetles emergence. B: The collected *M. alternatus* were divided into two groups and maintained in tissue culture flasks at 25°C, one of which was fed (with fresh one-year-old pine twigs with the needles removed) immediately after collection, whereas the second group was fed after a starvation period of 4 d.