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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)

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Summary – The Japanese pine sawyer, *Monochamus alternatus,* that emerged from blocks inoculated first with *Ophiostoma minus* then with *Trichoderma* sp. (O+T) carried more pinewood nematodes (PWN, *Bursaphelenchus xylophilus)* than those emerging from blocks inoculated with these two fungi simultaneously (O,T together) or inoculated first with *Trichoderma* (T+O). This was because: *i)* PWN populations were much larger in the O+T blocks than in the blocks with the other treatments, and *ii)* the percentage of third-stage dispersal juveniles, dauer juveniles, and PWN which actually transferred to the beetle were much higher in the O+T blocks. By contrast, the numbers of PWN carried by the beetles emerging from blocks inoculated with both *O. minus* and *Verticillium* sp. were much smaller regardless of the inoculation sequence because PWN populations did not build up. We conclude that the species of fungi which are most prevalent in pine wilt-killed trees will help determine the number of PWN carried by the beetles emerging from the wood.

Résumé – ***Effets des interactions fongiques sur le nombre de* Bursaphelenchus xylophilus *(Nematoda: Aphelenchoididae) transportés par* Monochamus alternatus *(Coleoptera: Cerambycidae)*** – *Monochamus alternatus* sortant de blocs de bois préalablement inoculés avec *Ophiostoma minus* et ensuite avec *Trichoderma* sp. (O+T) transporte un plus grand nombre de *Bursaphelenchus xylophilus* (PWN) que ceux sortant de blocs inoculés simultanément avec ces deux champignons (O, T) ou inoculés avec *Trichoderma* sp. puis avec *O. minus* (T+O). La raison en est que : *i*) les populations de PWN sont plus élevées dans les blocs O+T que dans ceux des autres traitements, et que *ii*) le pourcentage de juvéniles de dispersion de 3^{e} stade, les "dauer" juvéniles et les PWN qui passent réellement dans l'insecte sont plus nombreux dans les blocs O+T. En contraste, le nombre de PWN transportés par l'insecte sortant de blocs inoculés avec *O. minus* et *Verticillium* sp. est beaucoup moins élevé quelle que soit la séquence d'inoculum parce que les populations de PWN ne s'y établissent pas. Il est conclu que les espèces de champignons les plus abondantes dans les pins tués par le dessèchement concourent à déterminer le nombre de PWN transportés par l'insecte sortant du bois.

Key-words : *Ophiostoma minus*, pinewood nematode, pine sawyer, *Trichoderma* sp., *Verticillium* sp.

The pinewood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, the cause of pine wilt disease (Kiyohara & Tokushige, 1971), is vectored from wilt-killed to healthy pines by the Japanese pine sawyer, *Monochamus alternatus* Hope in Japan (Mamiya & Enda, 1972; Morimoto & Iwasaki, 1972). The number of PWNs that enter a healthy tree is directly proportional to the number of PWNs carried by a sawyer beetle (Togashi, 1985). In turn, the rate of disease development is directly related to the number of PWNs in the inoculum (Kiyohara *et al.*, 1973; Hashimoto & Sanui, 1974). Thus, to understand the dynamics of pine wilt disease development, it is important to identify the factors affecting the number of PWNs carried by a beetle, which ranges from 0 to over 200 000.

PWNs are transmitted to healthy trees and invade them through maturation feeding wounds caused by vector beetles. After the tree dies, the PWNs feed on and reproduce on various wood-inhabiting fungi such as blue-stain fungi (Kobayashi *et al.*, 1974, 1975; Fukushige, 1991*a*; Kuroda & Ito, 1992). Fungi which are unsuitable for PWN propagation also occur in killed pines. Maehara and Futai (1996) examined the number of PWNs carried by a beetle which emerged from wood blocks inoculated with a fungus either suitable – the blue-stain fungus, *Ophiostoma minus* (Hedgcock) H. & P. Sydow – or unsuitable – two species of *Trichoderma* – for PWN propagation, and showed that the dominant fungus species in the wood affected both population density and developmental stage of the PWN, and thus the number of PWNs car-

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ried by a beetle. Fukushige (1991*b*) found that some fungi coexisting with a blue-stain fungus had inhibiting effects on PWN propagation.

The present study was made to determine how the kinds and sequence of colonization by wood fungi affect the number of PWNs carried by a beetle. Specifically, we examined the transfer of PWNs to aseptically-reared beetles from wood blocks inoculated with both a suitable fungus (*O. minus*) and an unsuitable fungus (*Trichoderma* sp. or *Verticillium* sp.) for PWN propagation.

Materials and methods

Rearing aseptic *Monochamus alternatus*

In June 1994, adults of *M. alternatus* emerging from logs of killed Japanese red pine, *Pinus densiflora* Sieb. & Zucc., or killed Korean pine, *Pinus koraiensis* Sieb. & Zucc., in the Kamigamo Experimental Station of Kyoto University Forest, were captured. In August, they were allowed to oviposit on recently-cut logs of healthy *Pinus thunbergii* Parl. After 4 days, eggs were collected from the logs, dipped in 70 % ethanol for 10 s, then in 0.05 % benzethonium chloride for 5 min, and finally rinsed three times with sterilized distilled water (Kosaka & Ogura, 1990; Kosaka & Enda, 1991). The eggs were then placed on an artificial diet modified from the diet proposed by Kosaka and Ogura (1990) and Kosaka and Enda (1991), *i.e.*, 8 g of 1-year-old *P. densiflora* needles dried at 90°C for 20 h and milled into powder for 2 min, 26.8 g artificial diet for silkworms (SILKMATE 3M, powder, Nippon Nosan Kogyo Co., Japan), 3.2 g dried yeast, and 62 ml of distilled water. About 20 g of the diet was put into each 50 ml Erlenmeyer flask, which was then plugged with a silicon-rubber stopper and autoclaved at 121°C for 20 min. The hatched larvae were reared at 25°C in the dark for 4 months. After the larvae matured, they were incubated at 10°C in the dark for 11 months, then at 25°C in the dark for 7 days.

Transfer of pinewood nematodes to aseptically-reared *Monochamus alternatus*

Wood blocks (2.5 × 2.5 × 5.0 cm) with bark on one side were obtained from two healthy, 10-year-old Japanese red pine trees and a hole (*ca.* 1.7 cm at the major axis × 1.2 cm at the minor axis × 4.0 cm deep, which simulated a *M. alternatus* pupal chamber) was bored into the top of each block. Each block was then put into a 70 ml wide-mouthed bottle plugged with a silicon-rubber stopper (Fig. 1). The bottles were autoclaved at 121°C for 30 min. One or two 7 mm diameter mycelial disks of the test fungi growing on potato dextrose agar (PDA) were placed inside the hole of each block. Three fungi were used: *i*) the blue-stain fungus *Ophiostoma minus* (Hedgcock) H. & P. Sydow

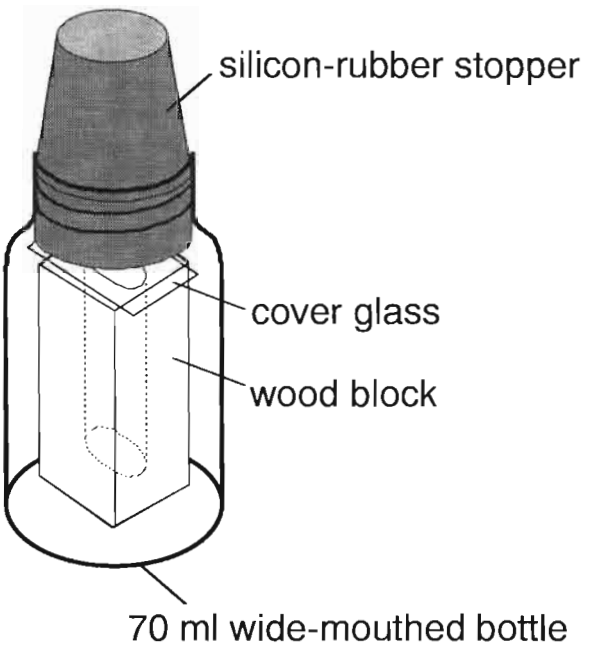

Fig. 1. *Simulated pupal chamber of* Monochamus alternatus.

– called *Ceratocystis* sp. (isolate No. 2) by Fukushige (1991*a*), *ii*) *Trichoderma* sp. (isolate No. 3), and *iii*) *Verticillium* sp., a nematode parasitic fungus. The first two fungi were isolated from PWN-infected Japanese red pine; *Verticillium* sp. was isolated from PWNs in pine wilt-killed Japanese red pine (Fukushige, 1991*a*). We inoculated the wood blocks with *O. minus* and *Trichoderma* sp. or with *O. minus* and *Verticillium* sp., either simultaneously or with a certain delay between the two inoculations. In the case of sequential inoculations, when *O. minus* or *Trichoderma* sp. was inoculated first, the second fungus was introduced 8 days later to allow the first fungus to spread entirely over the blocks. When *Verticillium* sp. was the first fungus, the second fungus was introduced 24 days later. Control blocks were inoculated with one fungus only: either *O. minus*, *Trichoderma* sp., or *Verticillium* sp. All culture bottles were incubated at 25 °C in the dark until the end of the experiment.

Eight days after making the last inoculation with *O. minus* or *Trichoderma* sp., or 24 days after inoculation with *Verticillium* sp., as solitary inoculum or an alternative inoculum, *ca.* 2000 axenic PWNs (in 0.5 ml sterilized water) were inoculated into the hole in each block. This pathogenic isolate (S10) of *B. xylophilus* was donated by the Kansai Branch of Forestry and Forest Products Research Institute and subcultured on the fungus *Botrytis cinerea* Pers. growing on autoclaved barley grains. The nematodes used

for inoculation were separated from the *B. cinerea* hyphae by the method of Iwahori and Futai (1993) where a suspension of nematodes is passed through four thin layers of Japanese typing paper settled on a Baermann funnel, then through Sephadex G-25 (Coarse) and glass beads packed in a column tube. The resulting suspension was then centrifuged five times at 700 *g* for 1 min to remove any remaining *B. cinerea* mycelium with the supernatant.

Eight days after nematode inoculation, one mature *M. alternatus* larva was placed inside the hole in each block. The development of *M. alternatus* was observed daily using a laminar flow bench. When the insect reached the adult stage, a 2.2 cm long × 2.6 cm wide × 0.17-0.25 mm thick cover glass was inserted between each block and silicon-rubber stopper to prevent the callow adults from emerging from the blocks. The number of days required for larvae or pupae to become pupae or adults, or for the adult to emerge from the blocks were recorded. When adults had not emerged 8 days after adult eclosion, we took them out of the blocks. Immediately after emergence or removal, each beetle was ground for 10 s using a mill with *ca.* 40 ml tap water and placed in a Baermann funnel overnight to extract the PWNs, which were then counted using a stereomicroscope. After the fresh weight of each block was measured, the block was cut into pieces with a pair of pruning shears and placed in a Baermann funnel overnight to extract the PWNs. Third-stage dispersal juveniles (JIII), fourth-stage dispersal juveniles (dauer juveniles; JIV), and all other PWN developmental stages (J2-J4 and adults) were counted separately for each sample. The blocks which were used for nematode extraction were dried at room temperature for at least 1 week and then at 105 °C for 3 h to determine their dry weight. The water content of the blocks was calculated from their fresh weight. When the numbers of nematodes were too great, the suspension was diluted so that the nematodes could be counted. The number of samples examined was not equal in all treatments because some larvae or pupae of *M. alternatus* failed to pupate or eclose, or because some blocks were too dry and had to be discarded. One-way analysis of variance (ANOVA) and Tukey-Kramer's multiple comparison test were used to determine the significance of mean differences among fungus treatments. For ANOVA, the numbers of nematodes were log10-transformed and the percentages of each life stage of the PWN were arcsine transformed. Spearman's rank correlation (*rs*) was used to test correlations between any two variables.

Results

For mature larvae of *M. alternatus* put into wood blocks, the average larval and pupal periods were 9 ± 3 (mean ± SD) days and 14 ± 1 days, respectively. Adults emerged from blocks 7 ± 1 (4-8) days after adult eclosion (half of them were taken out of blocks 8 days after adult eclosion). All adults which emerged from blocks were sclerotized.

In either combination of two fungi, *O. minus-Trichoderma* and *O. minus-Verticillium*, both fungi proliferated in wood blocks, regardless of the inoculation sequence.

The highest numbers of JIVs were carried by beetles which emerged from *O. minus*-inoculated blocks and from the blocks inoculated first with *O. minus* then with *Trichoderma* (Table 1). The mean numbers were 2759 (200-6890) and 2979.2 (350-7920) nematodes, respectively. Conversely, the mean number of PWNs carried by a beetle in the *Trichoderma*-inoculated blocks was small, and no PWNs were found on beetles which emerged from the *Verticillium*-inoculated blocks. In blocks inoculated simultaneously with *O. minus* and *Trichoderma* and blocks inoculated first with *Trichoderma*, the mean numbers of PWNs were also small. The numbers of PWNs were very low (mean less than 2) in blocks inoculated with *O. minus* and *Verticillium*, regardless of the inoculation sequence.

The sum total (= the total number of PWNs) of the number of PWNs carried by a beetle plus those remaining in a wood block was low in all four combinations of fungus-treatments with *Verticillium* regardless of the inoculation sequence (Table 1). The mean total numbers of PWNs in the three fungus-treatment blocks inoculated with *Trichoderma*, except the blocks inoculated first with *O. minus* then with *Trichoderma*, were only a few times higher than that of the inoculum (2000 nematodes). The mean number of PWNs in the blocks inoculated first with *O. minus* then with *Trichoderma* or inoculated with *O. minus* alone increased by 18 and 33 times the initial density, respectively.

The ratio of JIII+JIV to the total of PWNs of all stages was greatest in the blocks receiving first *O. minus* then *Trichoderma*, followed by the ratio in the blocks with only *O. minus*, and the smaller ratios were found in the blocks with all other treatments (Table 2). The ratio of JIV to JIII+JIV, and to the total of PWNs of all stages, and the ratio of PWNs which transferred to a beetle to the total of PWNs of all stages showed the same pattern among the combinations of fungus-treatments as did the ratio of JIII+JIV to the total of PWNs of all stages (Table 2). The total numbers of PWNs in any blocks with *Verticillium* were too small to take into account.

There were no differences in the water content of blocks (36.9 [22.7-49.4] %) among the fungus-treatments ($P = 0.081$). In blocks simultaneously inoculated with *O. minus* and *Trichoderma*, both the number of PWNs carried by a beetle ($rs = 0.853$, $P \leq 0.01$)

Table 1. *Effect of fungi on the number of JIV of* Bursaphelenchus xylophilus *(PWN) carried by the beetle* Monochamus alternatus *and on the total number of PWN of all stages in wood blocks inoculated with ca. 2000 PWN.*

Fungi and inoculation order	Number of observations	Number of PWN carried per beetle	Total number of PWN
Verticillium sp. alone	8	0 *a*	0.3 *a*
Ophiostoma minus then *Verticillium* sp.	5	0 *a*	0.6 *a*
Verticillium sp. then *O. minus*	6	0.3 *a*	0.7 *a*
O. minus and *Verticillium* sp. together	5	1.2 *ab*	3.6 *b*
Trichoderma sp. alone	9	34.9 *bc*	11 914.9 *c*
O. minus and *Trichoderma* sp. together	12	85.8 *c*	16 482.5 *c*
Trichoderma sp. then *O. minus*	9	87.9 *c*	12 172.3 *c*
O. minus alone	10	2759.0 *d*	65 579.0 *d*
O. minus then *Trichoderma* sp.	12	2979.2 *d*	36 187.5 *e*

Means followed by the same letter in a column were not significantly different at $P \leq 0.05$ (Tukey-Kramer's multiple comparison test). Data were log-transformed before analysis and untransformed means are presented.

Table 2. *Effect of fungi on the proportions of different stages of* Bursaphelenchus xylophilus *(PWN) and the proportion of PWN which transferred to* Monochamus alternatus.

Fungi and inoculation order	Number of observations	(JIII+JIV)/total PWN × 100	JIV/(JIII+JIV) × 100	JIV/total PWN × 100	PWN (JIV) transferred to a beetle/total PWN × 100
Trichoderma sp. alone	9	4.1 *a*	4.3 *a*	0.3 *a*	0.2 *a*
Ophiostoma minus and *Trichoderma* sp. together	12	3.7 *a*	13.3 *ab*	0.5 *a*	0.5 *a*
Trichoderma sp. then *O. minus*	9	5.7 *a*	13.0 *ab*	0.9 *a*	0.8 *a*
O. minus alone	10	14.8 *b*	28.2 *bc*	4.3 *b*	4.0 *b*
O. minus then *Trichoderma* sp.	12	20.5 *c*	41.1 *c*	9.5 *c*	9.3 *c*

Means followed by the same letter in a column were not significantly different at $P \leq 0.05$ (Tukey-Kramer's multiple comparison test). Data were arcsine transformed before analysis and untransformed means are presented

(Fig. 2) and the total number of PWNs ($rs = 0.678$, $P \leq 0.05$) were significantly related to the water content. The water content of the wood blocks was also significantly related to the percentage of JIIIs+JIVs in the blocks receiving first *O. minus* then *Trichoderma* ($rs = 0.706$, $P \leq 0.05$), *Trichoderma* then *O. minus* ($rs = 0.717$, $P \leq 0.05$), and to the ratio of JIVs to the total number of PWNs in the blocks simultaneously inoculated with these fungi ($rs = 0.769$, $P \leq 0.05$) or inoculated first with *O. minus* then with *Trichoderma* ($rs = 0.685$, $P \leq 0.05$).

In the present study, the time from adult eclosion to emergence of a beetle was 4 to 8 days. There was no relation between this time and the number of PWNs carried by a beetle in any fungus-treatment.

Discussion

The number of PWNs carried by a beetle is affected by *i)* reproduction of the PWNs in PWN-killed pines, *ii)* occurrence of the dispersal form (JIII and JIV) of the PWNs, and *iii)* transference of JIV from infested wood to the beetles.

Kobayashi *et al.* (1974, 1975) and Fukushige (1991*a*) compared PWN reproduction on various fungi from healthy and PWN-killed pines, and showed that, while the PWNs fed and multiplied on some of them, others were unsuitable for PWN reproduc-

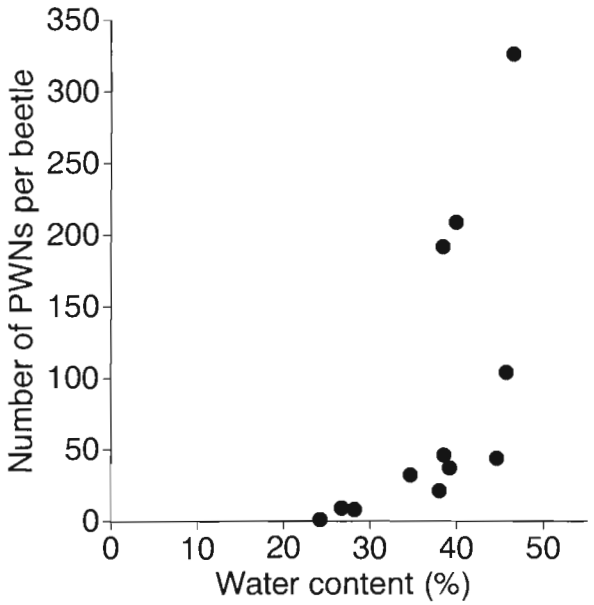

Fig. 2. *Relationship between the percent water content of wood blocks inoculated with* Ophiostoma minus *and* Trichoderma *sp. simultaneously and the number of pinewood nematodes carried by* Monochamus alternatus.

tion. Fukushige (1991*b*) examined PWN reproduction on a suitable fungus host – *O. minus*, which was called *Ceratocystis* sp. (isolate No. 2) by Fukushige (1991*b*) – paired with one of several unsuitable fungi, *i.e.*, *Trichoderma* spp. and individual species of *Penicillium*, *Arthrobotrys*, or *Verticillium*. When *O. minus* and the other fungus were inoculated simultaneously, PWN reproduction was always inhibited by the unsuitable fungi. When *O. minus* was inoculated 1 week before any of the other fungi, nematode reproduction was only inhibited by *Arthrobotrys* sp. and *Verticillium* sp. In our study, the number of PWNs was very small in the *Verticillium*-inoculated wood blocks regardless of the sequence of inoculation because this fungus kills PWNs. PWN reproduction was poor in the blocks inoculated with *Trichoderma* except when *O. minus* was inoculated before *Trichoderma*. This shows that *Trichoderma* suppresses PWN reproduction not by killing PWNs but by outcompeting *O. minus*, the food source of the PWNs.

Several studies reported that the percentage of JIII reached a maximum when or after the number of PWNs was at its highest level (Mamiya *et al.*, 1973; Kiyohara & Suzuki, 1975, 1977; Ishibashi & Kondo, 1977; Futai *et al.*, 1986; Tamura, 1986; Fukushige & Futai, 1987; Fukushige, 1991*a*; Forge & Sutherland, 1996), *i.e.*, that a high population density is a prerequisite for the occurrence of JIII. Fukushige (1991*a*), however, suggested that some fungi affected the percentage of JIIIs whether or not the PWNs can propagate on them. In the present study, the percentage of JIII+JIV differed among the fungus treatments, being highest in blocks inoculated first with *O. minus* then with *Trichoderma*, and even higher than in blocks inoculated with *O. minus* only, where PWNs reproduced best. We suggest that this occurs because *Trichoderma*, which is an unsuitable food source for the PWNs, in some way promotes the formation of third-stage dispersal juveniles (JIII). Therefore, the interaction between the two fungi affected the percentage of JIII. There was also a difference among fungus treatments in the ratio of dauer juveniles (JIV) to the total of JIII and JIV. For instance, the mean percentages are 28.2 ± 14.0 % and 4.3 ± 4.1 % in the blocks with *O. minus* alone and *Trichoderma* alone, respectively. This is in contrast with our previous observation (Maehara & Futai, 1996) where there was no difference in the percentage between blocks inoculated with *O. minus* (26.3 ± 13.7 %) and blocks inoculated with *Trichoderma* (isolate No. 3) (23.2 ± 37.4 %). In our previous observation, the percentage in the blocks with *Trichoderma* only varied too much to compare to the present result. The percentage of PWNs which transferred to a beetle was high also in the blocks inoculated with *O. minus* before *Trichoderma* and in those with *O. minus* only. This was undoubtedly attributable to the high percentage of JIV in these blocks.

In the blocks inoculated with *O. minus* only or in those inoculated first with *O. minus* then *Trichoderma*, PWN populations developed very well, and the percentages of JIII, JIV, and PWN that transferred to a beetle were high. Thus, beetles emerging from these blocks carried a large number of PWNs. In contrast, in blocks with either *Verticillium* or *Trichoderma*, the number of PWNs carried by a beetle was small, because of predation in the *Verticillium* blocks and failure in nematode development in the *Trichoderma* blocks.

Previous studies showed that beetles emerging from extremely dry or wet pupal chambers (Morimoto & Iwasaki, 1973) or logs (Terashita, 1975; Kobayashi *et al.*, 1976; Togashi, 1989; Fukushige, 1990) carried fewer PWNs. In wood blocks inoculated simultaneously with *O. minus* and *Trichoderma*, there was a positive relationship between block water content (26.7-46.5 %) and the number of PWNs carried by a beetle, *i.e.*, beetles emerging from extremely dry pupal chambers carried fewer PWNs. In these blocks, there was also a positive relationship between wood water content and the total number of PWNs propagated or the percentage of JIV. PWN numbers also correlated positively with water content in wood chips of eastern white pine (*P. strobus* L.) (Tomminen *et al.*, 1991).

In the present study, the period from adult pine sawyer eclosion to beetle emergence (4 to 8 days) was not related to the number of PWNs carried by a beetle in any fungus treatment. In the previous experiment, however, we indicated that in wood blocks inoculated with *O. minus* only there was a positive correlation between this period and the number of PWNs within 3 days after adult eclosion. However, the 3-days period was too short compared with the 4 to 8 days necessary for the beetle emergence from logs (Ido & Takeda, 1972; Kishi, 1976; Takizawa, 1979; Enda, 1980). The present result strongly suggests that the period would not affect the number of PWNs carried by a beetle in the field.

PWNs reproduce well in pine wilt-killed trees colonized by blue-stain fungi such as *Ophiostoma*. When *M. alternatus* is present, many dauer juveniles develop and transfer to the vector beetles (Maehara & Futai, 1996). Other fungi, including species of *Trichoderma* and *Verticillium* that are unsuitable for PWN reproduction, often occur in killed trees, in which case PWNs reproduce poorly and only a few dauer juveniles occur and transfer to the vector. Thus, the predominant fungi in wilt-killed trees undoubtedly help to determine the number of PWNs carried by the beetles emerging from such trees.

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References

ENDA, N. (1980). Duration of *Monochamus alternatus* Hope from pupation to emergence. *Trans. 32nd ann. Meet. Kanto Branch Jap. Forest. Soc.*: 91-92.

FORGE, T.A. & SUTHERLAND, J.R. (1996). Population dynamics of the pine wood nematode, *Bursaphelenchus xylophilus*, in excised branch segments of western North American conifers. *Fundam. appl. Nematol.*, 19: 349-356.

FUKUSHIGE, H. (1990). The number of *Bursaphelenchus xylophilus* carried by *Monochamus alternatus* and some possible factors regulating the number. *Jap. J. Nematol.*, 20: 18-24.

FUKUSHIGE, H. (1991*a*). Propagation of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) on fungi growing in pine-shoot segments. *Appl. Ent. Zool.*, 26: 371-376.

FUKUSHIGE, H. (1991*b*). Effects of fungi coexisting with *Ceratocystis* sp. on propagation of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae). *Appl. Ent. Zool.*, 26: 377-380.

FUKUSHIGE, H. & FUTAI, K. (1987). Seasonal changes in *Bursaphelenchus xylophilus* populations and occurrence of fungi in *Pinus thunbergii* trees inoculated with the nematode. *Jap. J. Nematol.*, 17: 8-16.

FUTAI. K., NAKAI, I., FUKIHARU. T. & AKAI, T. (1986). Ecological studies on the infection sources of pine wilt (I). Population dynamics of pine wood nematodes in the withered stems of Japanese red pine. *Bull. Kyoto Univ. Forest*, 57: 1-13.

HASHIMOTO, H. & SANUI, Y. (1974). Behavior of the pine wood nematode in a pine tree in relation with disease development. *Trans. 85th ann. Meet. Jap. Forest. Soc.*: 251-253.

IDO, N. & TAKEDA, J. (1972). Some observations on biology and morphology of the pine sawyer. *Trans. 23rd ann. Meet. Kansai Branch Jap. Forest. Soc.*: 180-182.

ISHIBASHI, N. & KONDO, E. (1977). Occurrence and survival of the dispersal forms of pine wood nematode, *Bursaphelenchus lignicolus* Mamiya and Kiyohara. *Appl. Ent. Zool.*, 12: 293-302.

IWAHORI, H. & FUTAI, K. (1993). Lipid peroxidation and ion exudation of pine callus tissues inoculated with pinewood nematodes. *Jap. J. Nematol.*, 23: 1-11.

KISHI, Y. (1976). Prediction of emergence of the pine sawyer adult through continuous observations of larval growth by the log-cutting method. *Forest Pests*, 25: 96-98.

KIYOHARA, T., DOZONO, Y., HASHIMOTO, H. & ONO, K. (1973). Correlation between number of inoculated nematodes and disease occurrence in pine wilt disease. *Trans. 26th ann. Meet. Kyushu Branch Jap. Forest. Soc.*: 191-192.

KIYOHARA, T. & SUZUKI, K. (1975). Population changes of *Bursaphelenchus lignicolus* in *Pinus thunbergii* after inoculation. *Trans. 86th ann. Meet. Jap. Forest. Soc.*: 296-298.

KIYOHARA, T. & SUZUKI, K. (1977). Population changes of *Bursaphelenchus lignicolus* in *Pinus thunbergii* after inoculation (II). *Trans. 30th ann. Meet. Kyushu Branch Jap. Forest. Soc.*: 243-244.

KIYOHARA, T. & TOKUSHIGE, Y. (1971). Inoculation experiments of a nematode, *Bursaphelenchus* sp., onto pine trees. *J. Jap. Forest. Soc.*, 53: 210-218.

KOBAYASHI, K., OKUDA, M. & HOSODA, R. (1976). Influence of wood size and humidity on the emergence of Japanese pine sawyer and the number of pine wood nematodes per insect. *Trans. 87th ann. Meet. Jap. Forest. Soc.*: 239-240.

KOBAYASHI, T., SASAKI, K. & MAMIYA, Y. (1974). Fungi associated with *Bursaphelenchus lignicolus*, the pine wood nematode (I). *J. Jap. Forest. Soc.*, 56: 136-145.

KOBAYASHI, T., SASAKI, K. & MAMIYA, Y. (1975). Fungi associated with *Bursaphelenchus lignicolus*, the pine wood nematode (II). *J. Jap. Forest. Soc.*, 57: 184-193.

KOSAKA, H. & ENDA, N. (1991). Simple rearing method of larvae of the Japanese pine sawyer, *Monochamus alternatus* (Coleoptera: Cerambycidae), on artificial diets. *Forest Pests*, 40: 183-187.

KOSAKA, H. & OGURA, N. (1990). Rearing of the Japanese pine sawyer, *Monochamus alternatus* (Coleoptera: Cerambycidae), on artificial diets. *Appl. Ent. Zool.*, 25: 532-534.

KURODA, K. & ITO, S. (1992). Migration speed of pine wood nematodes and activities of other microbes during

the development of pine-wilt disease in *Pinus thunbergii*. *J. Jap. Forest. Soc.*, 74: 383-389.

MAEHARA, N. & FUTAI, K. (1996). Factors affecting both the numbers of the pinewood nematode, *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae), carried by the Japanese pine sawyer, *Monochamus alternatus* (Coleoptera: Cerambycidae), and the nematode's life history. *Appl. Ent. Zool.*, 31: 443-452.

MAMIYA, Y. & ENDA, N. (1972). Transmission of *Bursaphelenchus lignicolus* (Nematoda: Aphelenchoididae) by *Monochamus alternatus* (Coleoptera: Cerambycidae). *Nematologica*, 18: 159-162.

MAMIYA, Y., KOBAYASHI, T., JINNO, Y., ENDA, N. & SASAKI, K. (1973). [Disease development of pine trees naturally infected with *Bursaphelenchus lignicolus*.] *Trans. 84th ann. Meet. Jap. Forest. Soc.*: 332-334.

MORIMOTO, K. & IWASAKI, A. (1972). Role of *Monochamus alternatus* (Coleoptera: Cerambycidae) as a vector of *Bursaphelenchus lignicolus* (Nematoda: Aphelenchoididae). *J. Jap. Forest. Soc.*, 54: 177-183.

MORIMOTO, K. & IWASAKI, A. (1973). Studies on the pine sawyer (IV). Biology of the pine sawyer and the pine wood nematode in the pupal cell. *Trans. 26th ann. Meet. Kyushu Branch Jap. Forest. Soc.*: 199-200.

TAKIZAWA, Y. (1979). Ecology of the pine sawyer in Tohoku District (VIII). Periods of pupal and adult stages in the pupal cell under natural temperature conditions in Morioka City. *Trans. 31st ann. Meet. Tohoku Branch Jap. Forest. Soc.*: 156-157.

TAMURA, H. (1986). Occurrence of dispersal 3rd-stage larvae of *Bursaphelenchus xylophilus* in logs. *Trans. 37th ann. Meet. Kansai Branch Jap. Forest. Soc.*: 201-203.

TERASHITA, T. (1975). Relations between water content of wood, *Bursaphelenchus lignicolus* density in wood and the numbers of the nematode carried by beetles emerged from diseased trees. *Trans. 26th ann. Meet. Kansai Branch Jap. Forest. Soc.*: 279-281.

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. Ent. Zool.*, 20: 246-251.

TOGASHI, K. (1989). Factors affecting the number of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) carried by newly emerged adults of *Monochamus alternatus* (Coleoptera: Cerambycidae). *Appl. Ent. Zool.*, 24: 379-386.

TOMMINEN, J., HALIK, S. & BERGDAHL, B.R. (1991). Incubation temperature and time effects on life stages of *Bursaphelenchus xylophilus* in wood chips. *J. Nematol.*, 23: 477-484.