

Nematode-vector Beetle Relation and the Regulatory Mechanism of Vector's Life History in Pine Wilt Disease

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ABSTRACT

Cerambycid beetles of the genus *Monochamus* transmit the pinewood nematode, *Bursaphelenchus xylophilus*. Nematode-infected trees of susceptible pine species succumb to pine wilt disease. *Monochamus* beetles oviposit in nematode-killed trees. A clumped distribution of diseased trees in a pine stand can be explained by a negative relation between the ability of a beetle to kill a pine tree and its reproduction rate through the number of nematodes carried, and response of sexually mature beetles to diseased trees. *Bursaphelenchus xylophilus* exhibits horizontal transmission between vector beetles and sometimes enter the spermathecae of female beetles. The various transmission pathways lead to the persistence of nematode populations within beetle populations. On the other hand, there are two different lengths of larval development time in each of the two *M. alternatus* subspecies due to diapause induction, aversion, and termination. The difference in larval period leads to the persistence of beetle population in a pine forest. Such traits of nematodes and beetles are considered to be evolved responses to ephemeral and unpredictable resources.

Key Words: *Bursaphelenchus xylophilus*, life history, *Monochamus alternatus*, nematode-vector relation, pine wilt disease

Introduction

Longhorned beetle adults of the genus *Monochamus* transmit pine wilt disease from dead to healthy pine trees (Mamiya, 1983). When pine trees are susceptible, they are killed soon after the infection (Mamiya, 1983). Pine wilt disease occurs in East Asia, Europe, and North America (Mamiya, 1983; Robertson *et al.*, 2011). Primary vectors are different in the different continents; *Monochamus alternatus* in East Asia, *M. galloprovincialis* in Europe, and *M. caloricinensis* in North America (Mamiya and Enda, 1972; Morimoto and Iwasaki, 1972; Wingfield and Blanchette, 1983; Naves *et al.*, 2007). The vector beetles excavate holes with the mandibles on the bark of recently dead pine trees, insert the ovipositors into the inner bark through the holes, and deposit the eggs under the bark (Anbutsu and Togashi, 2000) (Fig. 1). The larvae feed on the inner bark and bore tunnels in xylem. They pupate in the pupal chambers formed at the end of tunnels in most cases. Newly eclosed adults stay in pupal chambers for several days to make the integument harden and then emerge from dead trees. Adults feed on twig bark of healthy pine trees. In Japan, females of *M. alternatus* require *ca* 3 weeks to mature reproductively after leaving dead trees (Enda and Nobuchi, 1970).

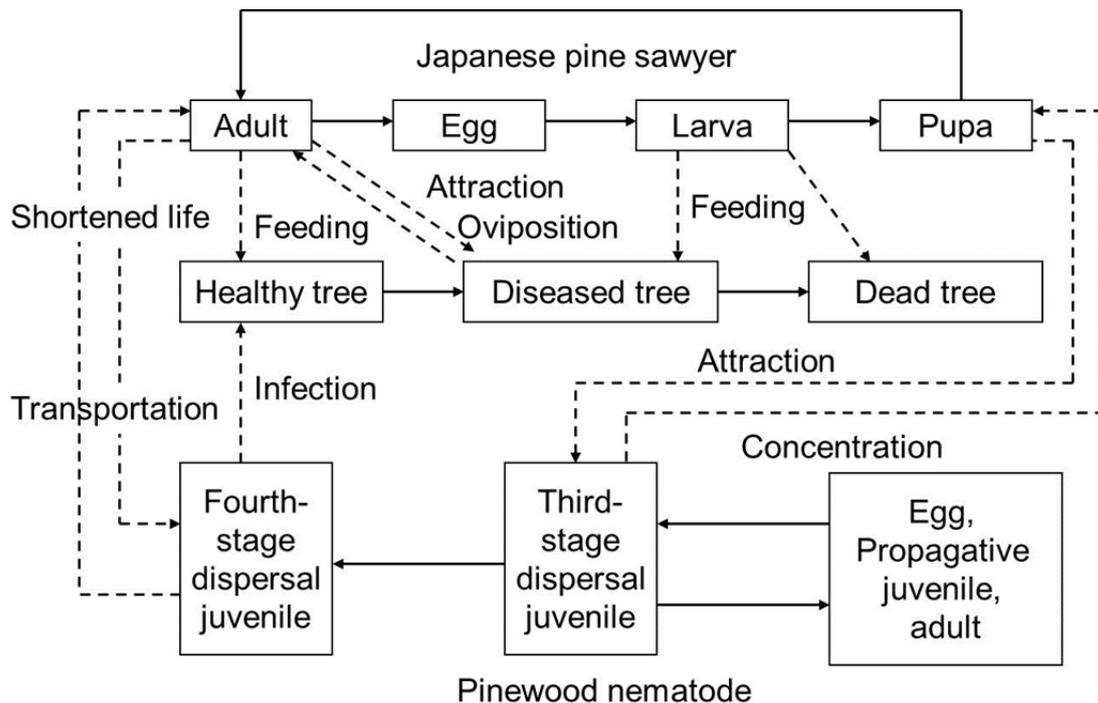


Fig. 1. Relationships between the pinewood nematode (*Bursaphelenchus xylophilus*), the Japanese pine sawyer (*Monochamus alternatus*), and trees of the genus *Pinus* in pine wilt disease system. Solid and broken arrows represent the change of stages or states and the action, respectively (modified from Togashi, 1989e).

Pine wilt disease is caused by the infection of the pinewood nematode, *Bursaphelenchus xylophilus* (Mamiya, 1983) (Fig. 1). Within disease-killed trees, the pathogenic nematodes enter the tracheal system of newly eclosed vector adults as the fourth-stage dispersal juveniles (JIVs), the special stage for transportation (Mamiya, 1983). Their heads are doomed and do not have mouth. After the beetles emerge from dead trees, the JIVs leave the beetle bodies. Exit behavior of the JIVs from the beetle tracheal system is considered to be controlled by the content of neutral storage lipid in nematode bodies (intrinsic cue) and one of pine volatiles, β -myrcene (extrinsic cue) (Ishikawa *et al.*, 1986; Aikawa and Togashi, 1998; Stamps and Linit, 1998, 2001). The JIVs enter the healthy pine trees via the feeding wounds that vector beetles make on twig bark. Lack of ability of oleoresin exudation is one of early symptoms of pine wilt disease. At the stage of disease development, the nematode population starts to increase rapidly through a life cycle including propagative juveniles exclusively (Mamiya, 1983). As the population of nematodes increases, the third-stage dispersal juveniles (JIIs) appear. As the JIIs are attracted and arrested by unsaturated fatty acids such as palmitoleic, oleic and linoleic acids that are included in the excrement of beetle larvae (Miyazaki *et al.*, 1977a, b), they concentrate the pupal chamber. The JIIs molt to the JIVs when the beetle pupae molt to adulthood (Mamiya, 1983).

The relation between vector beetles of the genus *Monochamus* and the pathogenic nematode, *B. xylophilus*, is mutualism on the population level, because vectors take the nematodes as new resources and the nematodes produce the oviposition resource of *Monochamus* adults, resulting in much higher reproduction rate of each party when in the presence of the other than when alone.

This article consists of four parts. The first part explains the association between the pathogenic nematode and its vector beetles and a spreading mechanism of pine wilt disease in a pine stand. The second indicates various transmission pathways of *B. xylophilus* from beetle vectors to host trees. The third describes the flexibility of life histories of two *M. alternatus* subspecies. Finally, the biological traits of the two parties are discussed with relation to the features of resources.

Spreading mechanism of pine wilt disease in a pine stand with relation to nematode-vector beetle association

When *M. alternatus* adults emerge from dead trees, there is a huge difference in the initial load of *B. xylophilus* ranging from 0 to more than 100,000 nematodes (Togashi, 1989d). Some factors are related to the initial nematode load. One of

them is the nematode density in xylem (Togashi, 1989d). As the density of pinewood nematodes per gram of dried xylem increases, mean and variance of initial nematode load increase (Togashi, 1989d). The nematode density in xylem is affected by the flora of fungi in xylem (Maehara and Futai, 1996). Water content in xylem also affects the initial nematode load (e.g., Togashi, 1989d). As larval body mass increases, the initial nematode load increases (Aikawa *et al.*, 1997). This is because the nematodes are attracted to carbon dioxide which is released in large amount by respiration at adult molting (Miyazaki *et al.*, 1978) and the decrease in body mass at that time is much greater for large larvae than small larvae (Aikawa *et al.*, 1997). Intriguingly, there is no relation between the mean initial nematode load of beetles and the month when the trees are diseased in the field (Togashi, 1989d).

As fourth-stage dispersal juveniles (JIVs) of *B. xylophilus* are in tracheal system of vector beetles, they may have deleterious effects on the beetles possibly due to reduced gas exchange and thereby they may affect the transmission patterns of nematodes. To determine the effects of initial nematode load on the transmission curves and beetle lifespan, *M. alternatus* adults were reared singly in funnels covered with deep Petri dishes, whose feet were filled with water (Togashi, 1985). They were provided with sections of fresh pine branches and the branch sections were replaced with new ones every 5 days. The nematodes are extracted from washed branch sections and funnels separately to determine the number of nematodes. Soon after beetles were found to be dead, the nematodes were extracted from beetle bodies and counted. The initial nematode load was estimated by summing the numbers of nematodes extracted because the juveniles do not reproduce in beetle body.

The study showed two types of nematodes transmission curves (Togashi, 1985). One is L-shaped and the other is unimodal. Many studies conducted in Japan show that unimodal transmission curves are common. Intriguingly, Kishi (1995) found that mean transmission curve changed from L-shaped to unimodal type as the pine wilt epidemic progressed in a pine stand.

When beetles carry more than 10,000 nematodes initially, they can transmit a mean of 1,500 nematodes to twigs of healthy trees for 5 days (Togashi, 1985) (Fig. 2). Beetles stay on a pine tree for about 2.5 days in pine stands with an open canopy (Togashi, 1990). Thus a mean of 750 nematodes may be transmitted to a healthy tree. Hashimoto and Sanui (1974) reveal that an inoculation of 300 JIVs or more causes 8 year old pine trees to succumb to wilt disease. Consequently, beetles with heavy nematode loads can kill a pine tree. By contrast, beetles that carry a moderate number of nematodes ranging from 1,000 to 9,999 transmit a mean of 370 nematodes at the peak of transmission curves. So, when they aggregate on a tree, they can kill it.

On the other hand, beetles carrying less than 1,000 nematodes cannot kill pine trees even when they concentrate on them.

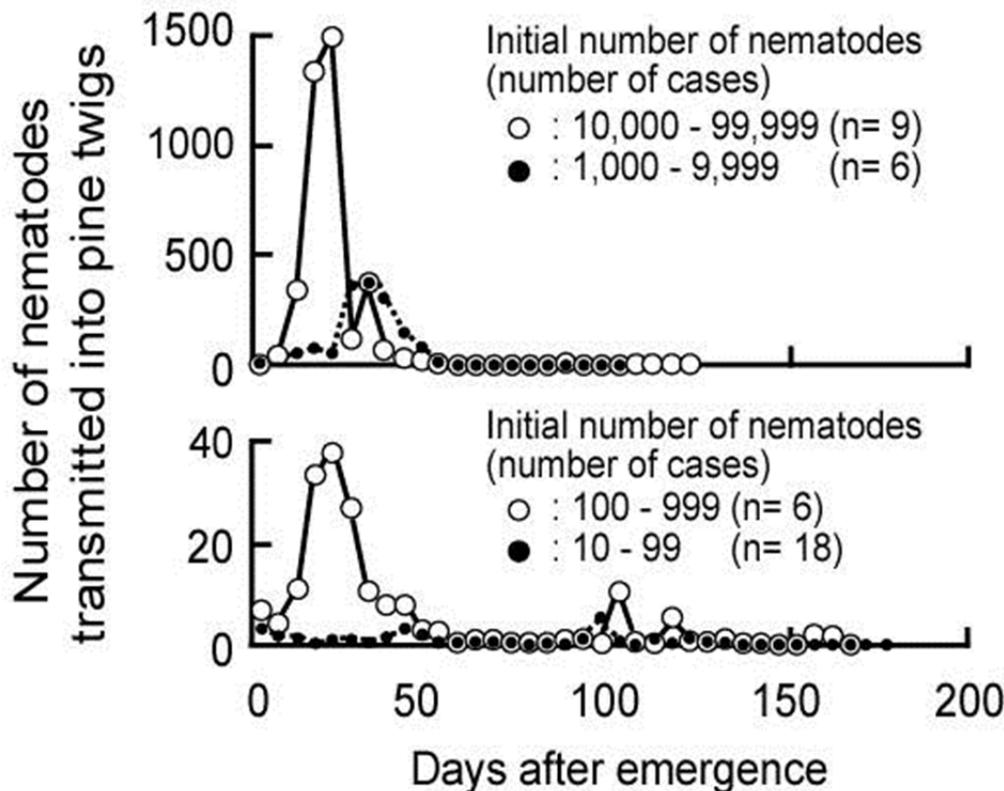


Fig. 2. Transmission curves of *Bursaphelenchus xylophilus* from *Monochamus alternatus* adults to pine branch sections. Adults are grouped by the level of initial number of nematodes carried. (after Togashi, 1985)

Mean lifespan of beetle adults decreases as the initial nematode load increases (Togashi and Sekizuka, 1982) (Fig. 3). Mean lifespan is 32, 51, and 94 days for beetles carrying heavy, moderate, and light nematode loads, respectively. Consequently, mean fecundity decreases with increasing initial nematode load; it is estimated to be 50, 143, and 172 eggs for beetles with heavy, moderate, and light nematode loads, respectively. Considering the relationships between nematode load, nematode transmission curve, and beetle lifespan together, it is suggested that the nematodes carried force an adult beetle population to divide into three functionally different subpopulations, i.e. adults producing food for offspring, those reproducing, and those having intermediate characters. Every vector beetle does not have a mutualistic relation with the nematodes.

Diseased trees show a clumped distribution in a pine stand at an early stage of pine wilt disease epidemic, when nematode-killed trees are removed from the stand during a period between the end of flight season in the previous year and its start in

the current year (Togashi, 1991c). The degree of aggregation is higher in June or July than after August. By contrast, diseased trees show a uniform distribution in June or July in years after the peak of disease epidemic, whereas they show a clumped pattern after August.

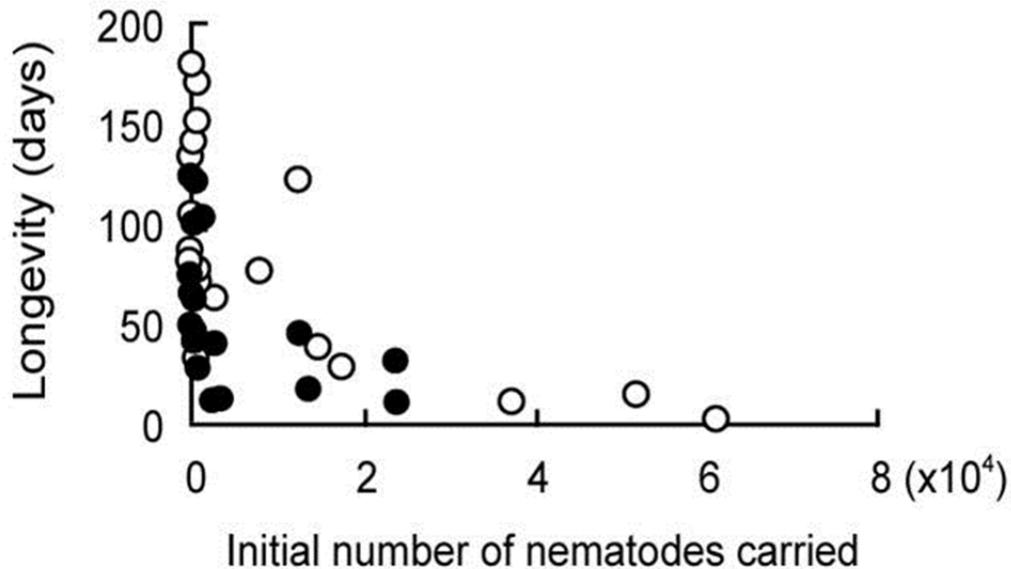


Fig. 3. Relation between the initial number of *Bursaphelenchus xylophilus* carried and longevity of *Monochamus alternatus* adult. Open and solid circles represent females and males, respectively. (after Togashi and Sekizuka, 1982)

Monochamus alternatus adults emerge from dead trees between June and July in central Japan and move to healthy trees to feed on the bark of pine twigs. The newly emerged adults are considered to be densely distributed on healthy trees near the dead trees in the early season due to their limited capacity of locomotion (Togashi, 1990). Thus, the transmission of pinewood nematodes to those healthy trees by newly-emerged adults may result in the occurrence of pine wilt disease in the early season. The adults do not mature reproductively at the time of emergence. It takes 16-30 days for females to mature after emergence (Enda and Nobuchi, 1970) and 5-15 days for males (Nobuchi, 1976). It is considered that the immature adults move around without being affected by the presence of diseased trees (Ikeda, 1981). Therefore, a beetle adult which carries more than 10,000 nematodes at emergence can transmit enough nematodes to induce pine wilt disease during an average residence time of 2-5 days on a healthy tree in the early season (Togashi, 1985, 1990).

Since the reproductively mature adults are attracted to diseased trees, they are considered to aggregate on diseased trees and neighboring healthy trees for mating, oviposition, and feeding (Togashi, 1989e). Though the adults with numerous nematodes are short-lived, those with a moderate number of nematodes are

considerably long-lived (Togashi and Sekizuka, 1982). Some of the aggregated adults carry a moderate number of nematodes at emergence and their transmission rate peaks during the 30th-35th days after emergence (Togashi, 1985). A contagious distribution of such adults contributes to the occurrence of newly-diseased trees near the trees which have already been diseased. Therefore, this process may cause compact clumps of diseased trees in the early season to become larger in the late season. It also may cause a uniform distribution of diseased trees in the early season to change a clumped distribution in the late season. In addition, nematode infection via fused root system between pine trees (Tamura, 1983; Tanaka and Gyokusen, 2004) may be also involved in a mechanism for forming the clumped distribution patterns of diseased trees.

Monochamus carolinensis adults with a heavy initial nematode load have a significantly shorter flight distance and duration than beetles with a moderate or light nematode load (Akbulut and Linit, 1999). A mathematical model indicates that the distribution of the dispersal distance of the beetles critically affects the expansion rate of the pathogenic nematode (Takasu *et al.*, 2000). As the fraction of the beetles that travel over long distance increases from zero, the range expansion accelerates sharply (Fig. 4). However, too frequent long-range dispersal results in a failure of the beetle invasion due to the Allee Effect, which operates on beetle reproduction, and consequently the pathogenic nematode cannot invade a pine stand, once the beetle density decreases below a threshold (Yoshimura *et al.*, 1999). The effect of nematode load on beetle flight has not been evaluated yet in the expansion of disease-infected area.

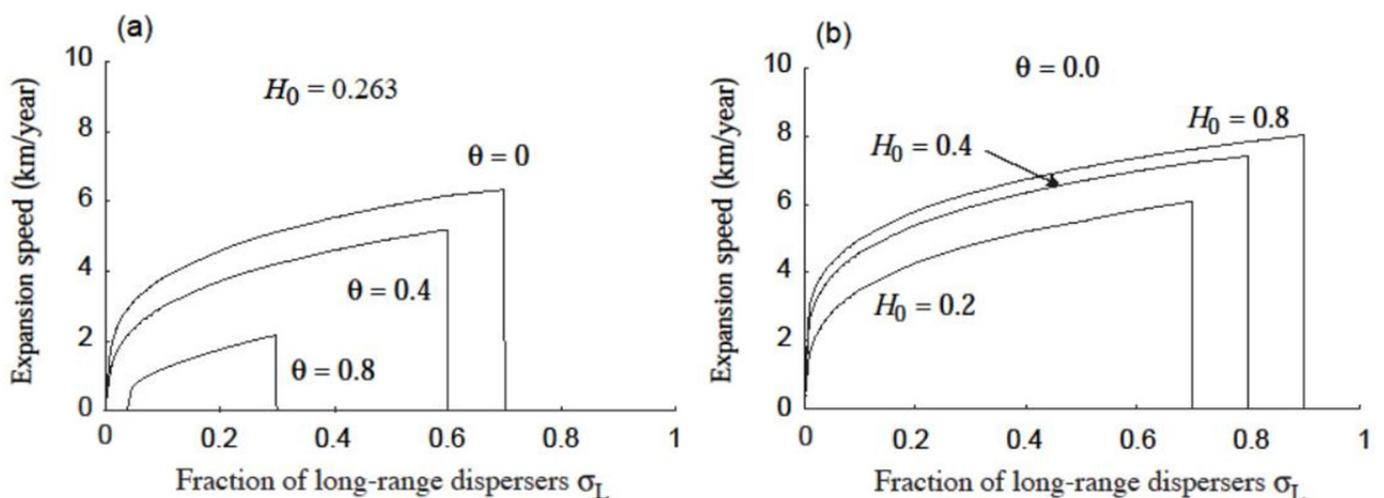


Fig. 4. Dependence of the range expansion rate of the pinewood nematode on the fraction of long-distance dispersers, σ_L . (a) The initial pine density is fixed as $H_0 = 0.263/\text{m}^2$, while the eradication rate, θ , is varied as indicated, (b) the eradication rate is fixed as $\theta = 0$, while H_0 is varied as indicated (after Fig. 7 of Takasu *et al.*, 2000).

Various transmission pathways of *B. xylophilus* from beetle vectors to host trees

Wingfield and Blanchette (1983) and Edwards and Linit (1992) show a transmission of *B. xylophilus* from beetle females to recently dead trees through the oviposition wounds.

In North America, pine wilt epidemics have never occurred in native pine forests because resistant pine species are distributed in warm areas and susceptible pine species in cool-summer areas (Rutherford and Webster, 1987). Therefore, the transmission from vectors to healthy trees via the feeding wounds is ineffective for the persistence of nematode populations in pine forests of North America. Only the transmission via oviposition wounds is effective. As *B. xylophilus* is inferred to be native to North America (Rutherford and Webster, 1987), it is considered that *B. xylophilus* has an ability to distinguish vector females from males. However, there is no difference in the initial nematode load between beetle females and males, suggesting multiple pathways of nematode transmission.

It is revealed that the nematodes are transmitted from vector males to host trees directly (Arakawa and Togashi, 2002). Nematode-infected males, with mandibles fixed experimentally to prevent feeding, were placed for 48 hours with pine bolts containing oviposition wounds that had been made by nematode-free females. After removal of the nematode-infected males, the pine bolts were held at 25 °C for 1 month and then examined for the presence of nematodes. Reproducing nematode populations were recovered from pine bolts that were exposed to male beetles carrying a high number of nematodes. Fourth-stage dispersal *B. xylophilus* juveniles were collected from beetle body surfaces and then were inoculated on pine bolt bark 0, 5, 10, and 15 cm away from a single artificial, small hole. The juveniles successfully entered some bolts. The results indicated that *B. xylophilus* can move a significant distance to oviposition wounds along the bark surface and enter a tree via the wounds (Arakawa and Togashi, 2002).

When nematode-infected beetles of one sex and nematode-free beetles of the opposite sex were paired in containers for 48 or 72 hours, it was shown that nematode-free beetles carried the nematodes (Togashi and Arakawa, 2003). There was no difference in transmission efficacy between male-to-female transmission and female-to-male transmission. Detailed investigation showed that nematode-free beetles acquired the nematodes by the end of the first sexual mounting. A female beetle that received 13 nematodes from a male transmitted one nematode to a *Pinus densiflora* bolt via an oviposition wound (Togashi and Arakawa, 2003). Intriguingly,

the JIVs of *B. xylophilus* are found in the spermathecae, the tubular structure for storing sperms, of *M. alternatus* nematode-free females that are paired with nematode-infected males (Arakawa and Togashi, 2004).

Two or more *M. alternatus* beetles may transmit *B. xylophilus* to healthy pine trees prior to disease development. Reproductively mature beetles are concentrated on diseased trees to oviposit and copulate, and feed on twig bark of surrounding healthy trees (Togashi, 1989e). Therefore, multiple infections occur on individual host trees. Theoretical studies show that multiple infections by two or more strains of parasites induce an increased mean virulence level and an extended variance in virulence by rapid competitive exclusion or coexistence between strains within individual hosts (Nowak and May, 1994; May and Nowak, 1995). Two horizontal *B. xylophilus* transmission pathways (male-to-female and female-to-male beetle) and the invasion of beetle reproductive organs by horizontally transmitted nematodes suggest increased opportunities of multiple infections of different strains of nematodes on a given diseased tree. This should be taken into account in developing control plans for pine wilt by reforestation using resistant trees against the disease because the *B. xylophilus* transmission system may include the mechanism of the evolution toward the higher virulence.

Flexibility of life history of *Monochamus alternatus*

Monochamus alternatus is separated into two subspecies; *M. alternatus alternatus* in China and Taiwan, and *M. alternatus endai* in Japan and South Korea (Makihara, 2004). *Monochamus alternatus alternatus* is bivoltine in southern China (Song *et al.*, 1991) and univoltine in Taiwan (Hwang *et al.*, 2000), whereas *M. alternatus endai* takes one or two years to complete the development in Japan.

In central Japan, *M. alternatus* adults emerge from dead pine trees between May and July. Their flight season is between May and September. The developmental time varies depending on when the eggs are deposited (Togashi, 1989a, c) (Fig. 5). To determine the effects of the time of oviposition on development, *M. alternatus* adults were allowed to deposit the eggs on fresh pine logs each week during three years in field cages and the egg-containing logs were placed in pine stands. The logs were harvested at different occasions and were dissected to determine the developmental stages. This study showed that when the insects were born as an egg in early flight season, they emerged as an adult in the following year. On the other hand, when the insects were born in the late season, they required two years to complete the development. In addition, when larvae overwintered at the third and

fourth instars, they developed to adulthood in the following year of oviposition. By contrast, when they overwintered at the first and second instars, they required two years to complete the development. As Japanese *M. alternatus* larvae are known to enter the diapause at the end of fourth instar before winter, we can divide the larvae into three groups immediately before winter; diapause larvae, pre-diapause third and fourth larvae, and first and second larvae.

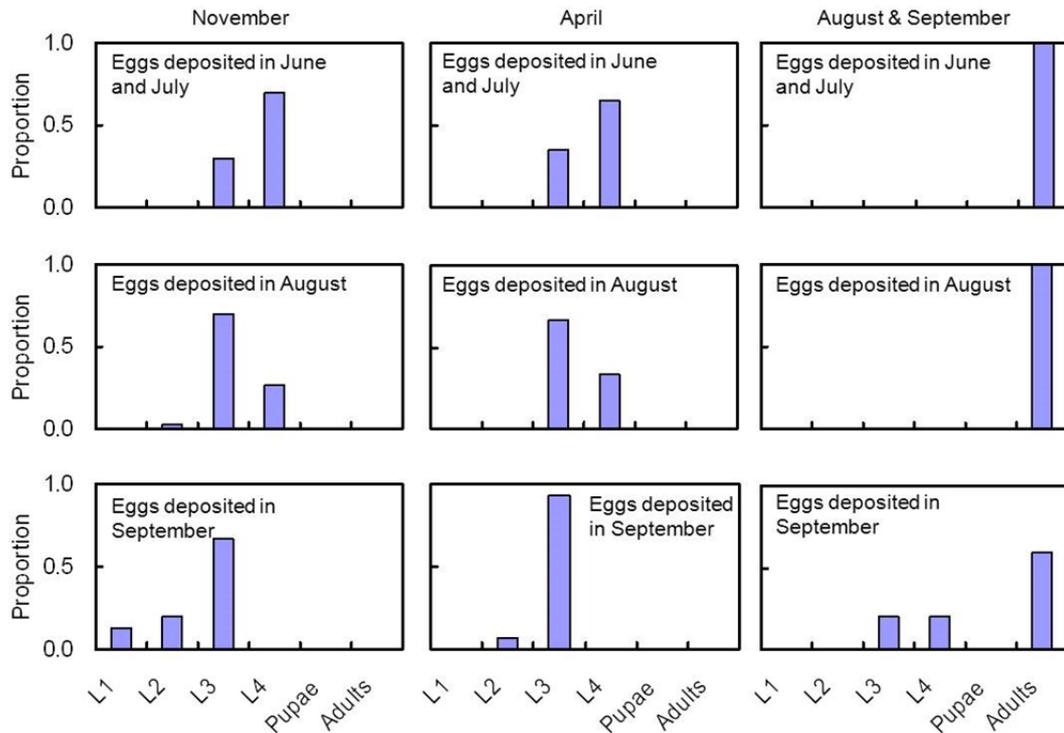


Fig. 5. Development of *Monochamus alternatus* deposited as an egg in different months. Investigation was conducted in November of oviposition year, April and August-September of following year. (after Togashi, 1989a)

Diapause is widely involved in the regulation of insect life cycles in changing environments such as season and unpredictable habitats such as the Arctic and deserts (Tauber *et al.*, 1986; Danks, 1987, 2007). In the case of Japanese subspecies of *M. alternatus*, laboratory experiments show that the larvae enter the diapause at the end of final instar (= fourth instar) at warm temperatures. Diapause larvae are characterized by the yellowish white to yellow body, the lack of fecal materials in the intestine, and no resumption of feeding.

When insects enter the diapause in autumn, cold temperatures often terminate the diapause in the early half of winter and then suppress the development in the late half of winter (Danks, 1987). Thus, when post-diapause insects are transferred to fixed environmental conditions suitable for development from the field at different occasions, they show an identical period of time required to achieve the further stage

of development. In the case of *M. alternatus endai*, diapause larvae were removed to constant conditions of 25 °C and a photoperiodic regime of 16 h photophase and 8 h scotophase (LD 16:8) between December 15 and April 18. This experiment showed that the diapause of *M. alternatus* larvae was terminated in mid-February (Togashi, 1991a). In laboratory, *M. alternatus* is terminated by chilling at 10 °C.

Pre-diapause larvae of Japanese *M. alternatus* resume feeding after overwintering. *Monochamus alternatus* larvae were collected from *P. densiflora* dead trees between 11 and 15 March 1989 and placed them singly into *P. densiflora* fresh bolts (Togashi, 1991b). The larvae were reared under constant conditions of 25 °C and a photoperiod of LD 16:8. The first to pre-diapause fourth instar larvae resumed feeding and then entered the diapause. By contrast, post-diapause fourth instar larvae pupated without resuming feeding. In the field, overwintered larvae cannot feed on fresh inner bark of pine trees because the trees were dead in the previous year.

When pre-diapause larvae of Japanese *M. alternatus* are provided with degraded inner bark of dead pine trees after overwintering, the third and fourth instars resume feeding and pupate, whereas the first and second instars resume feeding and then enter the diapause (Togashi, 1995). In an experiment, the larvae were collected from dead pine trees in December and were placed them singly into three kinds of *P. densiflora* bolts; fresh bolts, fresh and old bolts immersed in hot water of 60 to 70 °C for about 12 hours (Togashi, 1995). Old pine bolts were obtained from *P. densiflora* trees that had been felled *ca* five months ago and enclosed in wire-netting bags. Larva-containing pine bolts were placed in field cages and were monitored daily between May 1 and August 31. In October, the bolts were dissected to determine the developmental stages in xylem. The results showed that a fraction of the third instar larvae had pupated in the three kinds of pine bolts, indicating that they averted the diapause (Fig. 6). Diapause aversion was also confirmed in pre-diapause fourth instar larvae that were inoculated into boiled fresh pine bolts. By contrast, the first and second instars entered the diapause after overwintering.

In conclusion, when larvae of Japanese subspecies of *M. alternatus* are deposited as an egg in the early part of flight season, they enter the diapause before winter and pupate after overwintering because of diapause termination by cold temperatures in winter. The third and pre-diapause fourth instar larvae grow sufficiently after overwintering and pupate without entering the diapause, resulting in one-year life history. When larvae are deposited as an egg in the late part of flight season, they overwinter at the first and second instars. Such larvae develop to the fourth instar and enter the diapause in the following year of oviposition, resulting in two-year life history.

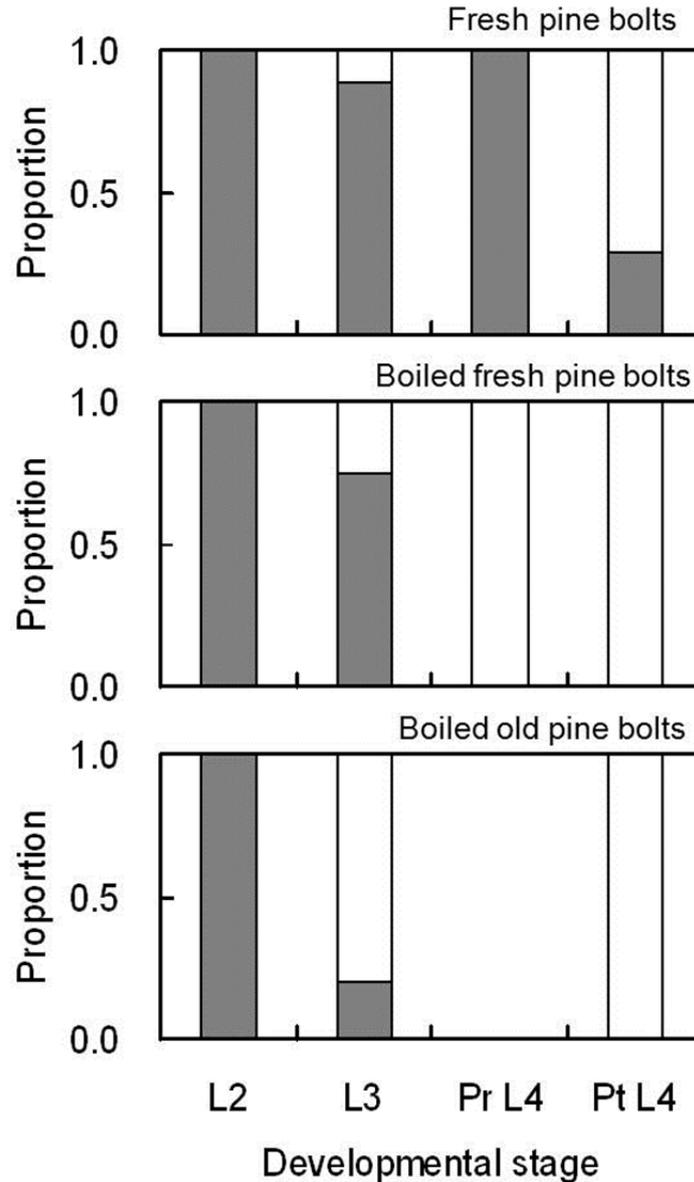


Fig. 6. Developmental responses of different instars of *Monochamus alternatus* overwintering larvae to fresh bolts and boiled fresh and old bolts of *Pinus densiflora*. Horizontal axis indicates the developmental stages of larvae inoculated into pine bolts; L2, L3, Pr L4, and Pt L4 for second, third, pre-diapause fourth, and diapause fourth instars, respectively. Grey and white parts of bars indicate the living diapause larvae and emerging adults in autumn. Dead insects were excluded from the calculation of proportion. (after Togashi, 1995)

The Formosan subspecies of *M. alternatus* has facultative diapause. Rearing the Formosan subspecies on artificial diets shows that part of larvae arrest the development under combined conditions of two air temperatures of 26 °C and 29 °C and three fixed photoperiodic regimes of LD 20:4, 12:12 and 0:24 (Enda and Kitajima,

1990). By contrast, all the larvae arrest the development under constant conditions of 23 °C and LD 12:12. In the case of the European species *Monochamus galloprovincialis*, rearing on artificial diet shows that some larvae arrest the development but the others pupate under four photoperiodic regimes of LD 0:24 (induction of diapause = 0.500), LD 9:15 (0.632), LD 12:12 (0.778) and LD 15:9 (0.500) at 23 °C (Naves *et al.*, 2007).

Diapause induction is related primarily to photoperiod and temperature in most insects (Tauber *et al.*, 1986; Danks, 1987, 2007). In addition, it is affected by other environmental conditions such as food deficiency and population density. To determine the effects of larval density on the diapause induction, one or two newly hatched larvae of the Formosan subspecies were inoculated into *P. densiflora* bolts and were reared under constant conditions of 25 °C and LD 16:8. Pine bolts were monitored daily for 150 days after larval inoculation and then were dissected to determine the developmental stages in xylem. A total of 81 adults emerged from pine bolts and 44 larvae entered the diapause. The proportion of non-diapause larvae was significantly greater at density 2 (0.775) than at density 1 (0.481) (Togashi unpublished). Another experiment showed that the probability of inducing diapause decreased as the area of inner bark supplied decreased (Togashi submitted).

Studies so far conducted show that auxiliary cues such as enhanced population densities and food deficiency, which indicate or suggest environmental deterioration, induce the diapause in insects (Danks, 1987). For examples, the incidence of diapause increases with increasing population density in the moths *Naranga aenescens* (Iwao, 1956), *Plodia interpunctella* (Tsuji, 1959) and *Laspeyresia pomonella* (Brown *et al.*, 1979). It also increases in response to lack of food in the bug *Dysdercus bimaculatus* (Derr, 1980), the Colorado beetle *Leptinotarsa decemlineata* (de Wilde *et al.*, 1959) and the weevil *Hypera postica* (deWitt and Armbrust, 1972). The proportion of reproductively inactive females increases in relation with seasonal decline of host leaf quality in the leaf beetle *Plagioderia versicolora* (Ishihara and Ohgushi, 2006). Such positive relationship between environmental deterioration and diapause induction is not observed in the Formosan subspecies of *M. alternatus*. There is a great difference in feature of food resource between *M. alternatus alternatus* and so far reported insects including phytophagous and stored product insects. Food resources of the latter insects are reproducible or are supplied in the same place multiple times. Therefore it is possible that post-diapause insects acquire the fresh foods. By contrast, food of *M. alternatus* larvae is not reproducible. Thus, an extended period of larval stage would not bring about the great growth but lead to the increased mortality. Diapause aversion may

be an adaptive response of *M. alternatus* larvae to deteriorated environmental conditions.

Discussion

Recently dead trees are ephemeral resources (Lindgren and Raffa, 2013). They are also unpredictable resources because it is difficult to predict when and where they occur in a forest and how many trees decline at a given time. Both the pinewood nematode, *B. xylophilus*, and its vector beetle, *M. carolinensis*, depend on recently dead pine trees in North America.

Takahashi (1977) shows the carry-over of a small fraction of a biological population to the further generations is effective for population persistence under changing environments. Under favorable environments, generation carry-over brings about high reproductive rates of populations that correspond to those without generation carry-over, whereas it inhibits the reproductive rates from reducing to zero unlike population without generation carry-over under unfavorable environments.

There are observed two different lengths in larval developmental time in the Japanese subspecies of *M. alternatus*. It is regulated by diapause induction under favorable conditions and the diapause termination and diapause aversion due to cold temperatures in winter. In the Formosan subspecies of *M. alternatus*, on the other hand, a fraction of members in a population induce the diapause under favorable conditions. This population trait is considered to be effective for population persistence (Takahashi, 1977; Danks, 2006).

In the case of *B. xylophilus*, the JIVs travel between vector beetles. That indicates that the death of a vector beetle does not always mean the deaths of all members of a nematode population that have not entered tree bodies from the beetle yet. All nematodes that transfer to other beetles are not short-lived, because some are in spermathecae of female vectors. During the period of nematode infection in the field, the horizontal transmission of nematodes between vector beetles may increase the persistence of nematode population within a vector population.

On the other hand, nematode populations are likely to survive within pine trees two or more years after the infection. There are some evidences. When field-infected *P. thunbergii* trees display the inability of oleoresin exudation in October, some of them are killed in the following year (Togashi, 1989b). Furthermore, techniques of molecular biology show that some *P. thunbergii* trees harboring the pathogenic nematodes survive for one or more years after the infection

without displaying any wilting symptoms in the field (Takeuchi and Futai, 2007). These processes may have enhanced effects on the persistence of nematode population within a tree population.

It is, therefore, very difficult to eradicate pine wilt disease once it is introduced in new pine forests. That is because the two organisms involved with the disease have depended on ephemeral and unpredictable resources.

References

- Aikawa T, Togashi K.** 1998. An effect of pine volatiles on departure of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) from *Monochamus alternatus* (Coleoptera: Cerambycidae). *Appl Entomol Zool* 33: 231-237.
- Aikawa T, Maehara N, Futai K, Togashi K.** 1997. A simple method for loading adult *Monochamus alternatus* (Coleoptera: Cerambycidae) with *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae). *Appl Entomol Zool* 32: 341-346.
- Akbulut S, Linit MJ.** 1999. Flight performance of *Monochamus carolinensis* (Coleoptera: Cerambycidae) with respect to nematode phoresis and beetle characteristics. *Environ Entomol* 28: 1014-1020.
- Anbutsu H, Togashi K.** 2000. Deterred oviposition response of *Monochamus alternatus* (Coleoptera: Cerambycidae) to oviposition scars occupied by eggs. *Agr Forest Entomol* 2: 217-223.
- Arakawa Y, Togashi K.** 2002. Newly discovered transmission pathway of *Bursaphelenchus xylophilus* from males of the beetle *Monochamus alternatus* to *Pinus densiflora* trees via oviposition wounds. *J Nematol* 34: 396-404.
- Arakawa Y, Togashi K.** 2004. Presence of the pine wood nematode, *Bursaphelenchus xylophilus*, in the spermatheca of female *Monochamus alternatus*. *Nematology* 6: 157-159.
- Brown GC, Berryman AA, Bogyo TP.** 1979. Density-dependent induction of diapause in the codling moth, *Laspeyresia pomonella* (Lepidoptera: Olethreutidae). *Can Entomol* 111: 431-433.
- Danks HV.** 1987. Insect dormancy: an ecological perspective. Ottawa, Canada: Biological Survey of Canada (Terrestrial Arthropods). 439 pp.
- Danks HV.** 2006. Key themes in the study of seasonal adaptations in insects II.

Life-cycle patterns. *Appl Entomol Zool* 41: 1-13.

Danks HV. 2007. The elements of seasonal adaptations in insects. *Can Entomol* 139: 1-44.

De Wilde J, Dunintjer CS, Mook L. 1959. Physiology of diapause of in the adult Colorado beetle (*Leptinotarsa decemlineata* Say) – I. the photoperiod as a controlling factor. *J Insect Physiol* 3: 75-85.

Derr JA. 1980. Coevolution of the life history of a tropical seed-feeding insect and its food plants. *Ecology* 61: 881-892.

DeWitt JR, Armbrust EJ. 1972. Photoperiodic sensitivity of the alfalfa weevil during larval development. *J Econ Entomol* 65: 1289-1292.

Edwards OR, Linit MJ. 1992. Transmission of *Bursaphelenchus xylophilus* through oviposition wounds of *Monochamus carolinensis* (Coleoptera: Cerambycidae). *J Nematol* 24: 133-139.

Enda N, Kitajima H. 1990. Rearing of adults and larvae of the Taiwanese pine sawyer (*Monochamus alternatus* Hope, Coleoptera, Cerambycidae) on artificial diets. *Trans 101st Annual Meeting Jap Forest Soc*: 503-504. (in Japanese)

Enda N, Nobuchi A. 1970. Studies on the pine bark and wood boring beetles: Maturation of the ovary and its parasitic nematodes. *Trans 81st Annual Meeting Jap Forest Soc*: 274-276. (in Japanese)

Hashimoto H, Sanui T. 1974. The influence of inoculation quantities of *Bursaphelenchus lignicolus* Mamiya et Kiyohara on the wilting disease development in *Pinus thunbergii* Parl. *Trans 85th Annual Meeting Jap Forest Soc*: 251-253. (in Japanese)

Hwang JS, Liu CH, Hsiao CH. 2000. Studies on attractants for the pine sawyer, *Monochamus alternatus* Hope. *Plant Prot Bull (Taiwan)* 42: 115-123. (in Chinese)

Ikeda T. 1981. Host selection and host attraction of *Monochamus alternatus* Hope. *Shokubutsu Boeki (Plant Protection)* 35: 395-400. (in Japanese)

Ishihara M, Ohgushi T. 2006. Reproductive inactivity and prolonged developmental time induced by seasonal decline in host plant quality in the willow leaf beetle *Plagioderma versicolora* (Coleoptera: Chrysomelidae). *Environ Entomol* 35: 524-530.

- Ishikawa M, Shuto Y, Watanabe H.** 1986. β -Myrcene, a potent attractant component of pine wood for the pine wood nematode, *Bursaphelenchus xylophilus*. Agr Biol Chem 50: 1863-1866.
- Iwao S.** 1956. The effect of population density on the induction of diapause in the rice green caterpillar, *Naranga aenescens* Moore (Lepidoptera: Noctuidae). Physiol Ecol Japan 7: 28-38.
- Kishi Y.** 1995. The pine wood nematode and the Japanese pine sawyer. Tokyo: Thomas Company. 302 pp.
- Lindgren BS, Raffa KF.** 2013. Evolution of tree killing in bark beetles (Coleoptera: Curculionidae): trade-offs between the maddening crowds and a sticky situation. Can Entomol 145: 471-495.
- 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 Entomol Zool 31: 443-452.
- Makihara H.** 2004. Two new species and a new subspecies of Japanese Cerambycidae (Coleoptera). Bull Forestry Forest Prod Res Inst 3: 15-24.
- Mamiya Y.** 1983. Pathology of the pine wilt disease caused by *Bursaphelenchus xylophilus*. Annu Rev Phytopathol 21: 201-220.
- Mamiya Y, Enda N.** 1972. Transmission of *Bursaphelenchus lignicolus* (Nematoda: Aphelenchoididae) by *Monochamus alternatus* (Coleoptera: Cerambycidae). Nematologica 18: 159-162.
- May RM, Nowak MA.** 1995. Coinfection and the evolution of parasite virulence. Proc Roy Soc London Biol Sci 261: 209-215.
- Miyazaki M, Oda K, Yamaguchi A.** 1977a. Behaviour of *Bursaphelenchus lignicolus* to unsaturated fatty acids. J Jap Wood Res Soc 23: 255-261. (in Japanese with English summary)
- Miyazaki M, Oda K, Yamaguchi A.** 1977b. Deposit of fatty acids in the wall of pupal chamber made by *Monochamus alternatus*. J Jap Wood Res Soc 23: 307-311. (in Japanese with English summary)
- Miyazaki M, Yamaguchi A, Oda K.** 1978. Behaviour of *Bursaphelenchus lignicolus* in response to carbon dioxide released by respiration of *Monochamus alternatus*

- pupa. J Jap Forest Soc 60: 249-254. (in Japanese with English summary)
- 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. (in Japanese with English summary)
- Naves PM, de Sousa EM, Quartau JA.** 2007. Winter dormancy of the pine sawyer *Monochamus galloprovincialis* (Col., Cerambycidae) in Portugal. J Appl Entomol 131: 669-673.
- Nobuchi A.** 1976. Fertilization and oviposition of *Monochamus alternatus* Hope. Trans 87th Annual Meeting Jap Forest Soc: 247-248. (in Japanese)
- Nowak MA, May RM.** 1994. Superinfection and the evolution of parasite virulence. Proc Roy Soc London Biol Sci 255: 81-89.
- Robertson L, Arcos SC, Escuer M, Merino RS, Esparrago G, Abelleira A, Navas A.** 2011. Incidence of the pinewood nematode *Bursaphelenchus xylophilus* Steiner and Buhner, 1934 (Nickle, 1970) in Spain. Nematology 13: 755-757.
- Rutherford TA, Webster JM.** 1987. Distribution of pine wilt disease with respect to temperature in North America, Japan, and Europe. Can J Forest Res 17: 1050-1059.
- Song SH, Zhang LQ, Huang HH, Cui XM.** 1991. Preliminary study of biology of *Monochamus alternatus* Hope. Forest Sci Technol 6: 9-13.
- Stamps WT, Linit MJ.** 1998. Neutral storage lipid and exit behavior of *Bursaphelenchus xylophilus* fourth-stage dispersal juveniles from their beetle vectors. J Nematol 30: 255-261.
- Stamps WT, Linit MJ.** 2001. Interaction of intrinsic and extrinsic chemical cues in the behaviour of *Bursaphelenchus xylophilus* (Aphelenchida: Aphelenchoididae) in relation to its beetle vectors. Nematology 3: 295-601.
- Takahashi F.** 1977. Generation carryover of a fraction of population members as an animal adaptation to unstable environmental conditions. Res Popul Ecol 18: 235-242.
- Takasu F, Yamamoto N, Kawasaki K, Togashi K, Kishi Y, Shigesada N.** 2000. Modeling the expansion of an introduced tree disease. Biol Invasions 2: 141-150.
- Takeuchi Y, Futai K.** 2007. Asymptomatic carrier trees in pine stands naturally

- infected with *Bursaphelenchus xylophilus*. *Nematology* 9: 243-250.
- Tamura, H.** 1983. Infection of pinewood nematode, *Bursaphelenchus xylophilus*, via fused roots. *Trans 27th Annual Meeting Jap Forest Soc*: 163. (in Japanese)
- Tanaka H, Gyokusen K.** 2004. Infection of pine wilt disease through root grafts among *Pinus thunbergii* trees. *Kyushu J Forest Res* 57: 241-242. (in Japanese)
- Tauber MJ, Tauber CA, Masaki S.** 1986. *Seasonal adaptations of insects*. Oxford, UK: Oxford University Press. 411 pp.
- 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 Zool* 20: 246-251.
- Togashi K.** 1989a. Development of *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) in relation to oviposition time. *Jap J Appl Entomol Zool* 33: 1-8. (in Japanese with English summary)
- Togashi K.** 1989b. Variation in external symptom development of pine wilt disease in field grown *Pinus thunbergii*. *J Jap Forest Soc* 71: 442-448.
- Togashi K.** 1989c. Development of *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) in *Pinus thunbergii* trees weakened at different times. *J Jap Forest Soci* 71: 383-386. (in Japanese with English summary)
- Togashi K.** 1989d. Factors affecting the number of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) carried by newly emerged adults of *Monochamus alternatus* (Coleoptera: Cerambycidae). *Appl Entomol Zool* 24: 379-386.
- Togashi K.** 1989e. Studies on population dynamics of *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) and spread of pine wilt disease caused by *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae). *Bull Ishikawa Forest Exp Sta* 20: 1-142. (in Japanese with English summary)
- Togashi K.** 1990. A field experiment on dispersal of newly emerged adults of *Monochamus alternatus* (Coleoptera: Cerambycidae). *Res Popul Ecol* 32: 1-13.
- Togashi K.** 1991a. Different developments of overwintered larvae of *Monochamus alternatus* (Coleoptera: Cerambycidae) under a constant temperature. *Jap J Entomol* 59: 149-154.
- Togashi K.** 1991b. Larval diapause termination of *Monochamus alternatus* Hope

(Coleoptera: Cerambycidae) under natural conditions. *Appl Entomol Zool* 26: 381-386.

Togashi K. 1991c. Spatial pattern of pine wilt disease caused by *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) within a *Pinus thunbergii* stand. *Res Popul Ecol* 33: 245-256.

Togashi K. 1995. Diapause avoidance for life cycle regulation of *Monochamus alternatus* (Coleoptera: Cerambycidae). International symposium on pine wilt disease caused by pine wood nematode. Chinese Society of Forestry, Beijing, China: 119-127.

Togashi K, Arakawa Y. 2003. Horizontal transmission of *Bursaphelenchus xylophilus* between sexes of *Monochamus alternatus*. *J Nematol* 35: 7-16.

Togashi K, Sekizuka H. 1982. Influence of the pine wood nematode, *Bursaphelenchus lignicolus* (Nematoda: Aphelenchoididae), on longevity of its vector, *Monochamus alternatus* (Coleoptera: Cerambycidae). *Appl Entomol Zool* 17: 160-165.

Tsuji H. 1959. Studies on the diapause of the Indian-meal moth, *Plodia interpunctella* Hübner II. The effect of population density on the induction of diapause. *Jap J Appl Entomol Zool* 3: 34-40.

Wingfield MJ, Blanchette RA. 1983. The pine-wood nematode, *Bursaphelenchus xylophilus*, in Minnesota and Wisconsin: Insect associates and transmission studies. *Can J Forest Res* 13: 1068-1076.

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

松樹萎凋病線蟲及其病媒甲蟲關係和病媒生活史的調節機制

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摘要

長角天牛屬 (*Monochamus*) 的甲蟲會傳播松材線蟲 (*Bursaphelenchus xylophilus*)，感受性的松樹感染線蟲之後得到松樹萎凋病而死亡，長角天牛則將卵產在線蟲致死的松樹上。在松林中病株呈集中分佈狀 (clumped distribution)，這個現象可解釋為由其攜帶的線蟲數量所影響的天牛的致病能力及繁殖能力的負相關性，及性成熟的天牛對病株的反應。松材線蟲可在其媒介天牛間水平傳播，有時可以進入天牛雌蟲的受精囊 (spermathecae) 中。因有各式的傳播途徑，線蟲族群可以持續存在於天牛族群中。另一方面，因滯育的誘發、避開和終止時期的不同，松斑天牛 (*M. alternatus*) 的二個亞種各有二種不同的幼蟲發育時間長度。幼蟲發育期的不同導致天牛族群可以持續存在於松林中。這些線蟲和天牛的特性被認為是對短暫且不可預期的資源所產生的進化反應。

關鍵詞：松材線蟲、生活史、松斑天牛、線蟲及病媒關係、松樹萎凋病