

# Seasonal variations in the incidence of pine wilt and infestation by its vector, *Monochamus alternatus*, near the northern limit of the disease in Japan

Kazumasa Ohta · Kazuhiko Hoshizaki · Katsunori Nakamura ·  
Akihiko Nagaki · Yoichi Ozawa · Aoi Nikkeshi · Akifumi Makita ·  
Kazumi Kobayashi · Osamu Nakakita

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**Abstract** During the last few decades, pine wilt disease has spread to cool-climate regions in Japan and, more recently, the potential risk of it spreading into the European midwest has also become a concern. In a coastal pine stand (84.7 ha) in Akita, near the northern limit of pine wilt disease in Japan, we investigated seasonal variations in the incidence of damage caused by the disease to trees and oviposition by the disease's insect vector, *Monochamus alternatus*, during a two-year period. Foliage discoloration occurred throughout each year, and its seasonal variation showed a bimodal pattern in *Pinus thunbergii* (a higher peak in May–June and a smaller peak in October) and a clear peak in June in *P. densiflora*, which differed from the patterns in seasonal variation seen for warm-climate

regions. Oviposition scars by *M. alternatus* were found in 40–45% of the trees damaged each year. The percentage of trees that had oviposition scars was higher in *P. thunbergii* than in *P. densiflora*. This appeared to reflect the difference in seasonal discoloration pattern between the two species. Analysis of the oviposition risk showed that trees that exhibited discoloration starting between July and October had a significantly higher risk or significantly higher oviposition scar densities, particularly for those that became discolored between August and September (2.5–14.6-fold higher risk than during other months). Oviposition scar densities per damaged tree were similar within the period of higher oviposition risk. Considering both oviposition risks and scar densities, we concluded that trees with discoloration that become apparent between July and October are important targets for preventing the spread of pine wilt disease in Akita.

K. Ohta · K. Hoshizaki (✉) · A. Nikkeshi ·  
A. Makita · K. Kobayashi  
Department of Biological Environment,  
Akita Prefectural University, Shimoshinjo-Nakano,  
Akita 010-0195, Japan  
e-mail: khoshiz@akita-pu.ac.jp

## Present Address:

K. Ohta  
Seibu Landscape Co., Ltd, Tokorozawa Office,  
Tokorozawa, Saitama 359-0037, Japan

K. Nakamura · O. Nakakita  
Tohoku Research Center, Forestry and Forest Products  
Research Institute, Morioka 020-0123, Japan

A. Nagaki  
Institute of Forestry, Akita Prefectural Agriculture,  
Forestry and Fisheries Research Center, Akita 019-2611, Japan

Y. Ozawa  
Iwate Prefectural Forestry Technology Center,  
Yahaba, Iwate 028-3623, Japan

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## Introduction

During the last few decades, pine wilt disease, caused by *Bursaphelenchus xylophilus* (Steiner et Buhner) Nickle (Kiyohara and Tokushige 1971), which is mainly vectored by *Monochamus alternatus* Hope (Mamiya and Enda 1972; Morimoto and Iwasaki 1972), has become widespread in eastern Asia, including Japan, Korea, and China. Marked damage in Japan has been recorded in cool-climate regions, such as the Tohoku district and at high altitudes in central Honshu. Since its spread into Portugal in 1999, the potential risk of it spreading into the cooler European

midwest has also become a concern (Mota et al. 1999; Braasch and Enzian 2003; Schonfeld et al. 2008). In Japan, for the last 25 years, the northern limit of its damage has occurred within Akita Prefecture, and in 2008 it approached the border with Aomori Prefecture. In Akita Prefecture, damage has increased mainly along the Sea of Japan coast after the disease was initially confirmed in 1982. Many coastal pine stands were severely damaged in Honjo in the southern region of the prefecture over the Oga Peninsula (Kobayashi 2005). Studies of these advancing disease-damaged regions are required in order to devise a control system for the regional state of damage as a countermeasure to prevent the spread of this disease (Kobayashi 2004, 2005; Hoshizaki et al. 2005).

It has been pointed out that pine wilt disease in cool-climate regions has different features compared with the disease in warm-climate regions in Japan (reviewed in Zinno et al. 1987; Nakamura-Matori 2008). The pattern of occurrence of damaged trees in warmer regions was investigated in detail in Ibaraki (Kishi 1995), Chiba (Mamiya et al. 1973) and Ishikawa (Togashi 1989a, b); the incidence of external symptoms of the disease was found to be concentrated in summer, and most damaged trees were infested by the insect vector *M. alternatus* (Togashi 1989c; Nakamura-Matori 2008). In contrast, in cool-climate regions, the activities of *B. xylophilus* and *M. alternatus* are suppressed because of the low temperature (Rutherford et al. 1990), which delays the development of the disease, resulting in the year-round occurrence of damaged pines (Zinno et al. 1987; Nakamura-Matori 2008). Moreover, the flight season of adult *M. alternatus* is limited due to the low temperatures in cool-climate regions, shortening the oviposition period (Togashi et al. 1993). Based on these findings, it has been proposed that *M. alternatus* only oviposits in a proportion of the damaged trees within a year (Zinno et al. 1987; Hoshizaki et al. 2005; Kobayashi 2005). Considering these characteristics, Akita Prefecture has recently introduced a control strategy, the Akita system, which reliably selects damaged trees for removal with the aim of eradicating the insect vector.

However, unexpectedly, these characteristics of the seasonal incidence of damaged trees and vector infestation in cool-climate regions have not been demonstrated convincingly, and so our understanding of this aspect of the disease remains collective and qualitative (e.g., Zinno et al. 1987). Although some papers have dealt with both damage incidence and vector infestation (Hayasaka et al. 1982; Sakuyama and Chida 1983; Umeda and Komatsu 1986), they often lack surveys performed in summer and autumn, or are based on small sample sizes of damaged trees. Therefore, the accumulation of detailed quantitative data concerning the seasonal incidence of damaged trees and the ratio of trees infested by *M. alternatus* is particularly

important in order to advance our knowledge of practical approaches for controlling pine wilt disease in cool-climate regions.

In this study, we investigated the patterns of occurrence of damaged trees and *M. alternatus* infestation in a coastal pine stand in Akita in order to compare these patterns for cool-climate regions with those for warmer regions. The questions we addressed in the study are as follows. (1) In which month does the incidence of damaged trees peak? (2) Is the pattern in cool-climate regions different from that in warmer regions? (3) What fraction of trees damaged per year is infested by *M. alternatus*? (4) Among the damaged trees that show various onset times for foliage discoloration, which are more likely to be infested by *M. alternatus*?

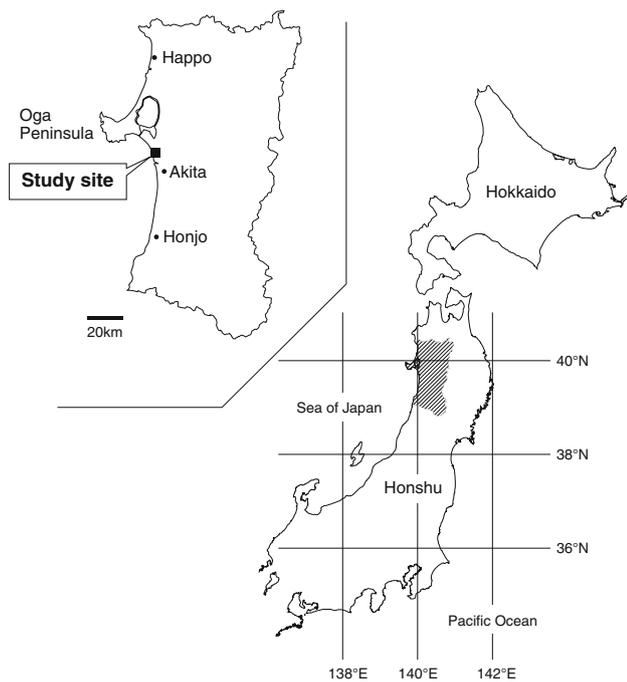
## Materials and methods

### Study site

The study site (Shimoshinjo, Akita City: 39°48'N, 140°02'E) (Fig. 1) was in a coastal pine stand, Yuhi-nomatsubara (total area: about 950 ha), located about 60 km south of the northern limit of pine wilt disease in Japan (Happo Town). The annual mean temperature in Akita City is 11.4°C, with a maximum of 24°C in August. Precipitation is 1,713 mm year<sup>-1</sup> in this region and as snow during December–March. Rainfall is lower from January to June, and July is the rainy season.

We established an 84.7-ha survey area (altitude: 5–30 m a.s.l.). The survey area is located about 600 m inland from the shoreline of the Sea of Japan. The region comprises 60- to 90-year-old planted trees of mostly *Pinus thunbergii* Parl. and in part *P. densiflora* Sieb. et Zucc. (Hoshizaki et al. 2005). *Robinia pseudoacacia* L. is also dominant in some patches. A university campus is present in the center of the survey area, and the net forested area for our survey was 60.5 ha. The northeast of the survey area is private forest, the east a residential district and orchard, and the west, northwest, and south are prefectural forest. The control method widely adopted in these areas is the eradication of immature *M. alternatus* by charcoal burning the damaged trees after cutting them down, except in the northeastern private forest, where no control activity is organized. Insecticide is also sprayed aerially in the prefectural stand on the west side.

The standing tree density in the survey area is about 800–2000 trees ha<sup>-1</sup>, and the tree height ranges from 8 to 22 m (Hoshizaki et al. 2005). Pine wilt disease initially spread into Yuhi-no-matsubara in 1988 (Akita Prefecture 2004). Damage in and around the study site has remained relatively mild, at least since 1999 (K. Kobayashi, unpublished results; no information available before 1999).



**Fig. 1** Maps showing the location of our study site. *Lower right*: the position of Akita Prefecture (*hatched*), with surrounding areas from Hokkaido through the Tohoku district, northern Honshu, to warmer regions of Honshu. *Upper left*: detailed location of our study site in Akita Prefecture, along with other places mentioned in the text

The annual ratio (i.e., proportion) of the trees damaged at the study site ranges from 0.1 to 2.4% (Ohta et al., unpublished results). *Rhizina* root rot was noted at two sites, and a small number of trees have recently suffered damage. They were excluded from this study.

#### Seasonal incidence of damaged trees

To investigate the seasonal pattern of the incidence of pine wilt-damaged trees, we surveyed the foliage discoloration of pine trees [ $\geq 5$  cm diameter at breast height (DBH)] over the entire study site in each month from late June 2007 to late May 2009, excluding January and February. Rapid systemic foliage discoloration (needle chlorosis) is a typical and conspicuous symptom of pine wilt disease (Mamiya 1983), and thus can be used as an indicator of disease incidence. In trees damaged by pine wilt disease, the color of the crown foliage generally changes to yellow and red after the one-year-old needles (old leaves) discolor, and then the needles fall. In the survey, we tried to find damaged trees as early as possible, before the old-leaf discoloration had progressed to entire foliage discoloration to yellow.

We conspicuously marked trees that showed foliage discoloration (“discolored trees” hereafter), including those with yellow/red-colored foliage and those with only

old-leaf discoloration, and their positions were recorded using a GPS-equipped palmtop computer (Mio P350; Mitac Japan, Tokyo). Species, suppressed condition (canopy or suppressed), and DBH were also recorded. A suppressed status was assigned when the treetop of the discolored tree was not exposed to the sky. We also checked for the absence of oleoresin flow in each discolored tree by making a 2- to 5-cm cut, with a hatchet, in each trunk at breast height. This was to exclude trees that had discolored needles but normal oleoresin flow; because such trees sometimes recover their vigor, they were carefully checked again during the next month. When all needles had already been shed by the start of the survey, the tree was considered an old, dead tree and excluded from the analyses.

#### Infestation of *Monochamus alternatus*

Oviposition scars by *M. alternatus* were surveyed in 2007 and 2008. The oviposition period of *M. alternatus* in Akita Prefecture is reportedly around mid-July through to mid-September (Togashi et al. 1993), so this survey was undertaken in October and November. Because *M. saltuarius* Gebler, another known vector of *B. xylophilus* (Sato et al. 1987), has not been recorded in this region, *M. alternatus* is the sole vector of pine wilt disease.

For trees with discolored canopies, we recorded the presence or absence of oviposition scars on the trunk surface initially through climbing (up to 5.5 m) and then, if necessary, by cutting. We also counted oviposition scars at 1–2 and 4.5–5.5 m, and measured the trunk diameter at a height of 1.5 and 5.0 m. The density of oviposition scars per discolored tree was calculated as the ratio of the numbers of scars at the two height levels relative to the trunk surface areas surveyed for each tree. Cutting was performed only when no oviposition scar was confirmed up to 5.5 m high, and oviposition scars were carefully searched for on the upper trunk and branches. For these surveys, we used canopy trees that showed the onset of discoloration in June through November. Trees in which discoloration occurred in April and May were surveyed only north and east of the campus in 2008. Among the suppressed, discolored trees, all trees that showed the onset of discoloration between June and November were cut, and oviposition scars were similarly searched for.

#### Statistical analyses

We first compared the monthly occurrence patterns of discolored trees during the two-year periods for each *Pinus* species (June 2007–May 2008 vs. June 2008–May 2009) using a  $\chi^2$  test. The patterns for *P. thunbergii* and *P. densiflora* were then also compared using a Kolmogorov–Smirnov two-sample test.

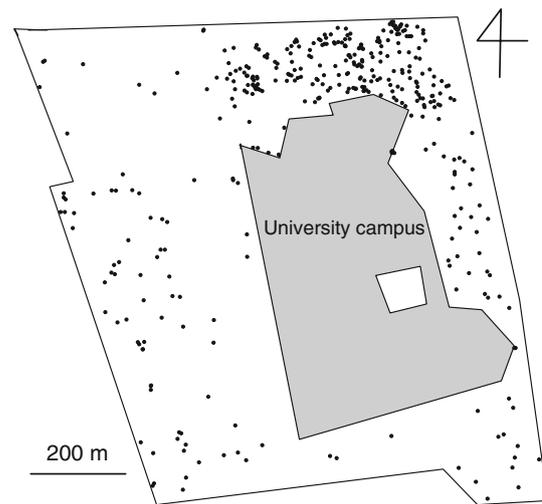
To examine the relationship between oviposition in discolored trees by *M. alternatus* and the onset time of discoloration (i.e., the month when discoloration became apparent), we analyzed seasonal patterns of infestation in two ways: in terms of the proportion of trees with oviposition scars caused by *M. alternatus* (“oviposited trees” hereafter) and the density of oviposition scars per discolored tree. We performed risk analyses among trees with different discoloration onset months using generalized linear models (GLMs). In the first GLM, we analyzed the proportions of oviposited trees in which the presence or absence of oviposition scar(s) for a given tree was the response variable (logit link function) with a binomial error distribution (“logistic model” hereafter). Using this GLM, the risk that a tree which discolored in a given onset month has an oviposition scar relative to this risk in a specific baseline month is computed as the exponential function of the model coefficient [i.e.,  $\exp(\text{coefficient})$ ] for the same month (Hamajima 1993). The baseline month was changed from June to November, and the relative risks of all pairs were calculated. Each coefficient was regarded as significant when its 95% confidence interval did not include 0 (i.e., relative risk = 1).

The second GLM, a *negative binomial model*, was examined in a similar way. In this model, the number of oviposition scars (total counts for each tree) was the response variable (log link function), adopting the surveyed area (trunk surface areas pooled for each tree) as an offset term. A negative binomial distribution was specified as the error structure because marked individual variation was present (variance  $\gg$  mean) in the number of oviposition scars. For both models, data were pooled for 2007 and 2008, and the two *Pinus* species were combined in order to evaluate the stand-level patterns of *M. alternatus* oviposition. This was also done because disease control practices in this region are performed irrespective of the pine tree species involved. Only canopy trees were used in the negative binomial model because very few oviposition scars were found in suppressed trees. R ver. 2.7.1 (R Development Core Team 2008) was used for both model calculations.

## Results

### Seasonal incidence patterns of discolored trees

During the two-year period from June 2007, foliage discoloration occurred in 763 trees in all survey areas (379 trees during the first year from June 2007 and 384 trees during the second year). The discolored trees comprised 405 canopy and 358 suppressed trees, of which 644 were *P. thunbergii* and 119 *P. densiflora* trees. There were many discolored trees in the north of the campus, while discolored trees were much more scarce in the east, west, and



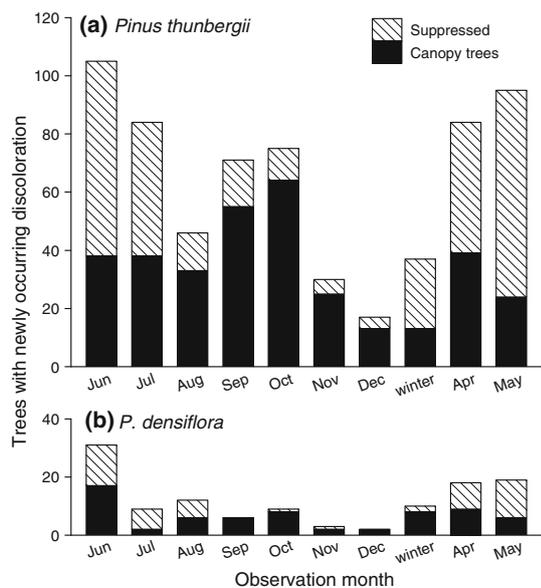
**Fig. 2** Survey area (84.7 ha) and positions of trees that showed foliage discoloration. Trees discolored during the one-year period from June 2007 are presented as dots ( $n = 379$ ). No pine trees grew in the shadowed area on the Akita Prefectural University campus

south districts (Fig. 2). The overall density of discolored trees was  $6.3 \text{ trees ha}^{-1} \text{ year}^{-1}$ .

The discoloration of trees occurred throughout the year. We found no interannual variation in the incidence patterns in either of the two species ( $X^2 = 11.1$ ,  $P = 0.26$  for *P. thunbergii*;  $X^2 = 9.42$ ,  $P = 0.40$  for *P. densiflora*), so we pooled the two-year period in the following analyses. The seasonal pattern of discoloration differed significantly between species (Kolmogorov–Smirnov two-sample test,  $P = 0.01$ ); in *P. thunbergii*, discoloration peaked between May and June and between September and October, whereas in *P. densiflora*, an apparent peak was found only in June (Fig. 3). In May and June, many suppressed trees were included in both species, whereas the ratio of suppressed trees decreased from summer (July–September) to fall (October–November) and winter (December and after) ( $X^2 = 103.7$ ,  $P < 0.001$  for *P. thunbergii*;  $X^2 = 9.53$ ,  $P = 0.02$  for *P. densiflora*) (Fig. 3). When limited to canopy trees, the incidence of discolored trees peaked in October in *P. thunbergii*, whereas in *P. densiflora* discoloration peaked in June (Fig. 3).

### Overall infestation level by *Monochamus alternatus*

Among the trees discolored during the two-year period, oviposition scars by *M. alternatus* were found in 325 trees (235 canopy and 90 suppressed trees). The annual proportion of oviposited trees was 44.9% (170 of all 379 discolored trees) in 2007–2008 and 40.4% (155 of 384) in 2008–2009. When the two years were combined, the proportions of discolored canopy and suppressed trees were

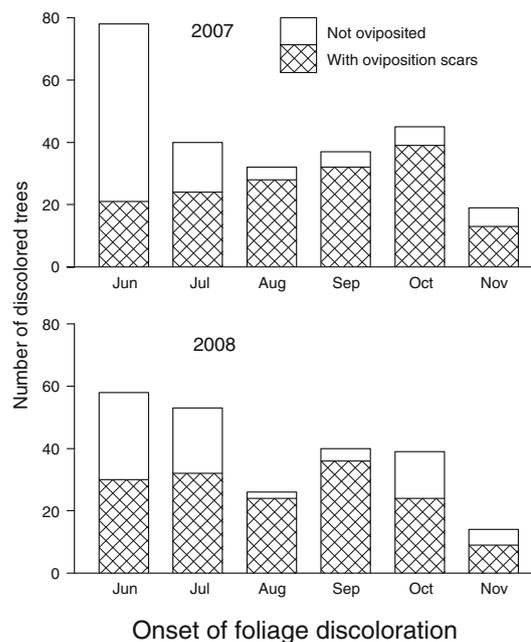


**Fig. 3** The seasonal incidence patterns of discolored trees from June 2007 to May 2009 in the two *Pinus* species. Discoloration in January–March was collectively presented as winter

58.0 and 25.1%, respectively ( $X^2 = 84.3$ ,  $P < 0.001$ ). When classified by species, *P. thunbergii* was more likely to have oviposition scars than *P. densiflora* (44.2 vs. 33.6%;  $X^2 = 4.99$ ,  $P = 0.03$ ).

#### Seasonal patterns of oviposition risk

Oviposition scars were present in trees that showed discoloration in June–November. Trees with discoloration that started in September (2008) and October (2007) showed the highest numbers of oviposited trees (Fig. 4). The proportion of oviposited trees was particularly high for trees that first showed discoloration in August and September (Table 1), whereas oviposition was less intense for trees that first showed discoloration in late spring to early summer (Fig. 4; Table 1). The seasonal patterns of oviposition did not differ between 2007 and 2008 ( $P = 0.22$ , GLM with binomial distribution; see the Appendix). Among the trees that first showed discoloration in April and May 2008, oviposition scars were found in five of the 21 canopy trees surveyed. An assessment of the infestation of these trees by other secondary insects (*Arhopalus coreanus* Sharp and scolytid beetles), referring to Oda (1970), revealed that *M. alternatus* had left oviposition scars in three trees (discoloration starting in April) during the previous year, and those in two trees (discoloration starting in May) had been made in the current year, 2008. Because the number of oviposition scars was as small as 0.07–0.11  $m^{-2}$  in both groups, these trees were excluded from the analysis thereafter.



**Fig. 4** Infestation pattern of *M. alternatus* in trees that showed the onset of foliage discoloration in different months

The logistic model showed that trees that first showed discoloration between August and September had a particularly high risk of being oviposited in by *M. alternatus* (2.8–14.6-fold higher risk for August and 2.5–12.7-fold higher for September) compared to trees that first showed discoloration in the other four months (Table 1). Conversely, the oviposition risk for trees that first showed discoloration in June was significantly lower than the corresponding risks for trees that first showed discoloration in any other month (relative risk: 0.07–0.41; Table 1). The risks for trees that first showed discoloration in July and November were higher than the risk for trees that first showed discoloration in June, and the risk for trees that first showed discoloration in October was higher than the corresponding risks for trees that first showed discoloration in June and July (Table 1).

Regarding the density of oviposition scars per damaged tree, trees with discoloration that started between July and October only rarely showed many oviposition scars, but such scars were very infrequent in trees that first showed discoloration in June and November (Fig. 5). The negative binomial model confirmed this pattern; the trees that first discolored between July and October had significantly higher scar densities than those that first discolored in the other two months (Table 2). In the trees with higher oviposition scar densities, there was no significant difference in discoloration onset month (Table 2). Trees that first discolored between July and October accounted for 73.5% ( $n = 239$ ) of all oviposited trees.

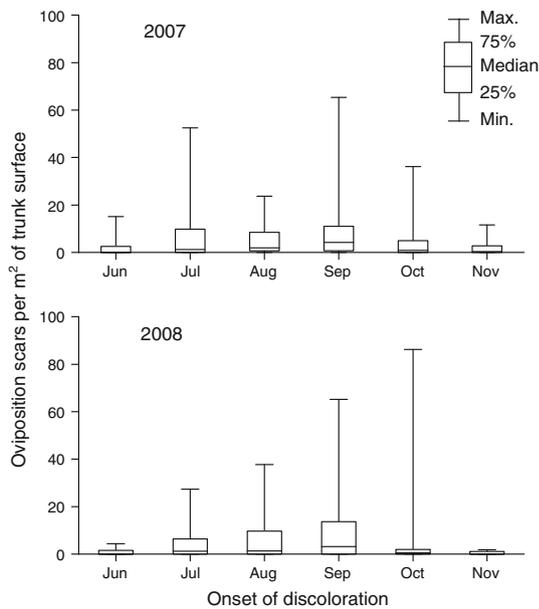
**Table 1** Relative risk, based on the logistic model, that trees which first showed discoloration in a particular month will be oviposited in by *M. alternatus*

Baseline month (% infestation)	Target month					
	June	July	August	September	October	November
June (37.5)	1	2.60***	14.61***	12.74***	5.06***	3.53*
July (60.2)	0.41**	1	5.63***	4.90***	1.95*	1.36
August (89.7)	0.07***	0.18***	1	0.87	0.35*	0.24*
September (88.3)	0.08***	0.20***	1.15	1	0.40*	0.28*
October (75.0)	0.21***	0.51*	2.89*	2.52*	1	0.70
November (66.7)	0.30**	0.74	4.14*	3.61*	1.43	1

The proportions of oviposited trees (% of discolored trees; two years combined) are shown in parentheses

The risk that a foliage-discolored tree that first showed discoloration in a particular month will exhibit oviposition scar(s), relative to the corresponding risk in each baseline month (each regarded as 1), is shown. For example, the risk in July was 2.60, 0.18, 0.20, 0.51, and 0.74 times that in June, August, September, October, and November, respectively (i.e., trees that first showed discoloration in July were more likely to have oviposition scars than those that first showed discoloration in June, less likely than those that first showed discoloration between August to October, and showed a similar risk of oviposition to those that first showed discoloration in November

\*  $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$



**Fig. 5** Density of *M. alternatus* oviposition scars on discolored trees according to the month of onset of foliage discoloration

**Discussion**

Our data show clearly and quantitatively that in Akita, (1) foliage discoloration occurred throughout the year and peaked in June (and also in October in canopy trees), (2) about 40–45% of the trees that discolored annually had oviposition scars caused by *M. alternatus*, and (3) trees with discoloration that started between June and November varied considerably in terms of the risk of being oviposited in by *M. alternatus*.

The distribution of discolored trees was distinctly biased to the north of the university campus. This may reflect the

difference in control activities outside the survey area. Because no control activity has been undertaken in the northeastern private forest, a considerable population of *M. alternatus* may be present there. In contrast, within our survey area, eradications of *M. alternatus* are conducted every year before they emerge. Therefore, the northeastern private stand may be a major source of *B. xylophilus* infection in the northern part of our survey area.

The seasonal pattern for the incidence of discolored trees was distinctly different from those seen in warm-climate regions. So far, comparable data from warmer regions have been made available for Chiba (*P. densiflora*: Mamiya et al. 1973), Ibaraki (*P. densiflora*: Kishi 1995), Gunma (*P. thunbergii*: Yamaguchi and Tanaka 1983, Magarisawa and Yamaguchi 1984, 1985), and Ishikawa (*P. thunbergii*: Togashi 1989a, b), where the annual mean temperatures of these locations (approx. 35°20′–36°50′N) are 2.0–3.2°C higher than that of Akita. In these studies, the patterns for the seasonal occurrence of damaged trees showed an apparent peak in August (Chiba, Gunma) or September (Ibaraki), or a clearly increasing trend from June through summer to fall (Ishikawa). In our data, discoloration peaked in June, which corresponded to the time prior to the peak emergence of adult *M. alternatus* (Hoshizaki et al. 2005); when limited to canopy trees, the peak incidence of discoloration occurred in October in *P. thunbergii* and in June in *P. densiflora* (Fig. 3). Thus, the patterns seen at Akita clearly contrast with those seen in warmer regions, indicating that, compared with warmer regions, the period from disease infection to foliage discoloration is prolonged in Akita due to its lower temperature (Zinno et al. 1987; Nakamura-Matori 2008). In suppressed trees, however, the cause(s) of weakening may not necessarily

**Table 2** Relative densities of oviposition scars caused by *M. alternatus*, based on the negative binomial model, in trees that first showed discoloration in a particular month

Baseline month	Target month					
	June	July	August	September	October	November
June	1	4.22**	3.52**	4.82***	3.06*	0.84
July	0.24**	1	1.20	1.14	0.72	0.20**
August	0.28**	1.20	1	1.37	0.87	0.24**
September	0.21***	0.88	0.73	1	0.63	0.17***
October	0.33*	1.38	1.15	1.58	1	0.27*
November	1.19	5.04**	4.20**	5.76***	3.65*	1

The oviposition scar density for each month of foliage discoloration onset, relative to each baseline density (regarded as 1). Table should be read similarly to Table 1

\*  $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

be related to the disease; other reasons (e.g., shading by neighboring trees) can also cause discoloration.

Earlier studies in cool areas have often paid greater attention to the symptom known as over-year wilt: discoloration in the year following infection (Hayasaka et al. 1982; Arihara and Saitoh 1984a, b; Zinno et al. 1987; Ichihara et al. 2006; Ohta et al. 2009). In our study site, adult *M. alternatus* emerge in mid-June through late July (Hoshizaki et al. 2005), and nearly a month is needed at the very least for discoloration to appear after infection by pine wood nematodes (Matsuura et al. 2010). Ten or more days are required, even in warmer regions, for the pine wood nematode to be transmitted from *M. alternatus* after its emergence (Togashi 1985; Aikawa and Togashi 1998). Considering these findings, it is plausible that in Akita, most of the trees that first show discoloration in July or earlier may be presenting over-year symptoms, which would account for at least 64% of all discolored trees. In addition to these trees, the year-round occurrence of discoloration suggests that discolored trees that became apparent in August and thereafter also include over-year wilts in different proportions each month. Therefore, the ratio of current-year to over-year wilts may not parallel the difference in the timing (i.e., the year) of infection. This dichotomy may have implications when determining the trees that should be eradicated in control practices, unless the seasonal occurrence of damaged trees is related to inhabitation by insect vectors of the disease.

The trees that had oviposition scars caused by *M. alternatus* accounted for 40–45% of all discolored trees, and 59% when limited to canopy-damaged trees. As far as we are aware, this is the first quantification of the level of infestation based on all trees that suffered damage during a year in a cool-climate region. Because oviposition scars do not always contain eggs (Togashi and Magira 1981), our investigation may have yielded an overestimated figure for

the true proportion of trees infested by *M. alternatus*. The ratio of oviposited trees was higher in *P. thunbergii* than in *P. densiflora*. This may be due to a difference in the seasonal occurrence patterns of damaged trees, because discoloration of *P. densiflora* peaked in June (i.e., approximately 2–3 months earlier than the oviposition period of *M. alternatus* in Akita; Togashi et al. 1993). Similarly, the lower proportions of suppressed trees that had oviposition scars may reflect the higher abundance of discoloration in June.

The relative importance of *M. alternatus*-oviposited trees as eradication targets depends on the oviposition risk for a damaged tree and on the per-tree abundance of oviposition scars. Our results showed that trees with discoloration that became apparent in August and September had significantly higher oviposition risks and that their oviposition scar densities were similar. Although the oviposition risk for trees with discoloration that became apparent in July was not high compared with August–November (Table 1), the oviposition scar density was high, similar to August–October (Fig. 5; Table 2). Likewise, the risk for trees with discoloration that became apparent in October was lower than in August and September, but similarly high densities of oviposition scars were seen for all of these months (Fig. 5; Table 2). On the contrary, the risk for trees with discoloration that became apparent in November was similar to those in July and October, but oviposition scars were considerably less frequent (Table 2; Fig. 5). Taken together, we suggest that *M. alternatus* mainly infests trees that first show discoloration in July–October, so these trees are of primary importance to the eradication practices implemented in Akita. This high oviposition-risk period, based on the month of discoloration, is longer than the actual oviposition period in this region (July–September: Togashi et al. 1993). This may be due to large individual variations in the time lag of foliage

discoloration after the cessation of oleoresin flow (Arihara 1985; Hoshizaki, unpublished results), which makes the tree suitable for oviposition. In addition, the fact that oviposition scars were found in trees that first showed discoloration in November, when *M. alternatus* adults had already disappeared from Akita, suggests that discoloration is sometimes delayed after the cessation of oleoresin flow, and becomes apparent much later.

Oviposition by *M. alternatus* in trees weakened between June and July or earlier has been reported in southern Tohoku (Hayasaka et al. 1982; Arihara and Saitoh 1984b; Arihara 1985). However, the infestation levels noted in these reports appear to be controversial, because the trees that first showed discoloration in spring and early summer (and August in some papers) were pooled, and rather small sample sizes were employed. The present results from monthly data based analyses of oviposition risk (Tables 1, 2) suggest that the importance of specific trees in relation to the eradication of *M. alternatus* differs substantially with the month of discoloration onset (i.e., June < July). Thus, the month of discoloration onset is a critically important factor in the Akita system, which requires accurate selection of *M. alternatus*-infested trees. Further studies should explore how to maximize the eradication rate of *M. alternatus* in this system.

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**Appendix**

See Table 3.

**Table 3** Comparison of the seasonal patterns of oviposition by *M. alternatus* during the two-year period

Parameter	Coefficient	Standard error	P
Intercept	-507.06	406.47	0.21
Discoloration onset month	0.41	0.07	<0.001
Year	0.25	0.20	0.22

Analysis was based on a generalized linear model, in which the presence or absence of an oviposition scar in each discolored tree was the response variable ( $scar = a + b_1 \times month + b_2 \times year$ ), with a binomial error distribution

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