

Migrations and Multiplications of *Bursaphelenchus xylophilus* and *B. mucronatus* in *Pinus thunbergii* in Relation to Their Pathogenicity

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To evaluate the mechanisms of pathogenicity and non-pathogenicity of *Bursaphelenchus xylophilus* and *B. mucronatus* isolated in South Korea, we used 4-year-old *P. thunbergii* seedlings and 20-cm long one-year-old stem cuttings of 5-year-old *Pinus thunbergii*, and studied distributions and multiplications of pine wood nematodes after inoculation. The distributions of *B. xylophilus* in the 20-cm pine stem cuttings were not significantly different from that of *B. mucronatus*. Conversely, the proliferation rate of *B. xylophilus* on mycelial mats of *Botrytis cinerea* was significantly different from that of *B. mucronatus*. The study using 4-year-old *P. thunbergii* seedlings also showed that *B. mucronatus* can migrate to distal portions of the pine seedlings the same as *B. xylophilus*, but the populations of *B. xylophilus* remaining in the pine seedlings were relatively larger than those of *B. mucronatus*. Therefore, we concluded that the pathogenicity of *B. xylophilus* could be strongly influenced by its ability to multiply.

Keywords : nematode migration, pine wilt disease, population increase, stem cutting

The pine forests all over South Korea are infected by *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle causing pine wilt disease, and the approximate area of damage is 5,123 ha of land in 2011 (Choi et al., 2012). Since 2007, extensive efforts to control pine wilt disease have resulted in the reduction of the damaged area, although there still remain many pine trees affected by this disease (Choi et al., 2012). *B. xylophilus*, the pathogenic pine wood nematode associated with pine wilt disease, has a high virulence that results in 90% to 100% mortality of pine seedlings (Choi and Moon, 1989; Woo et al., 2010; Yoon et al., 2008). A closely related species, *B. mucronatus* Mamiya and Enda, which was initially sighted in 1989 in Jeju Island, South Korea (Choi and Moon, 1989), was classified as being nonpathogenic to pine seedlings based

on experimental testing (Woo et al., 2010). Despite the apparent difference in pathogenicity, the morphology of *B. mucronatus* is highly similar to that of *B. xylophilus* (Braasch, 2001; Choi and Moon, 1989; Mamiya and Enda, 1979). To identify ambiguous nematode species from those of the genus *Bursaphelenchus*, DNA-based analysis was developed and effectively utilized (Han et al., 2008a; Iwahori et al., 1998; Matsunaga and Togashi, 2004; Webster et al., 1990; Zheng et al., 2003). According to the studies by Han et al. (2008a), Braasch et al. (2001) and Togashi et al. (2008), *B. mucronatus* can still be further divided into East Asian and European types. In 2008, both East Asian and European types of *B. mucronatus* were isolated in South Korea from *Pinus thunbergii* Parl. and *P. koraiensis* Seib. et Zucc., respectively (Han et al., 2008a, 2008b).

Migrations of pine wood nematodes within a pine tree vary with the nematode species (Fukuda et al., 1992; Ishida et al., 1993), isolate types of nematodes (Fukuda et al., 1992; Ichihara et al., 2000; Ishida et al., 1993), and resistance levels of the host trees, which can vary among tree species (Kuroda et al., 1991; Oku et al., 1989) and within a species (Matsunaga and Togashi, 2004). According to Fukuda et al. (1992), the distribution range of *B. xylophilus* (S6-1) was larger than *B. mucronatus* in 3-year-old *P. thunbergii* seedlings 1–4 weeks after inoculation. One week after inoculation, *B. xylophilus* (S6-1) and *B. mucronatus* were detected up to a distance of 35 cm and 5 cm from inoculation point, respectively (Fukuda et al., 1992). Similar results were reported by Ishida et al. (1993), in which that the distribution of *B. xylophilus* (S6-1) and *B. mucronatus* in 20-cm stem cuttings of 2-year-old *P. thunbergii* seedlings on 7 days after inoculation were detected up to a distance of 20 cm and 15 cm from the inoculation site, and the number of *B. xylophilus* (S6-1) that traversed the 5-cm stem cuttings were larger than that of *B. mucronatus* at 24 h after inoculation. In these studies, pathogenicity and nonpathogenicity may seem to be related to the migration of pine wood nematodes. However, Togashi and Matsunaga (2003) showed that the number of *B. xylophilus* (S-10) traversing the 5-cm living branch sections of *P. densiflora* Seib. et Zucc. did not significantly differ from that of *B. mucronatus*.

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(Un-1) at 24 h after inoculation.

The reproduction of pine wood nematodes is considered as a key factor in the development of pine wilt disease. Several factors have been shown to influence the population growth of pine wood nematodes, including the resistance level (Futai, 1980; Kuroda et al., 1991; Mori et al., 2008; Oku et al., 1989) and chemical components of a pine tree (Hinode et al., 1987; Suga et al., 1993). Previous studies have shown that compared to the reduction in the population of *B. mucronatus*, the population of *B. xylophilus* (S-10) had drastically multiplied in 2-year-old *P. densiflora* seedlings on 9 days after inoculation (Odani et al., 1985). Futai (1980) had shown similar results, in which that the numbers of *B. xylophilus* in 3-year-old *P. densiflora* and *P. thunbergii* seedlings had increased, whereas those of *B. mucronatus* had decreased during the experimental period.

In this study, the pathogenicity of *B. xylophilus* and European and East Asian types of *B. mucronatus* was evaluated by inoculation test using 4-year-old *P. thunbergii* seedlings. The distribution and multiplication of those pine wood nematodes were then investigated at 25 weeks after inoculation. To differentiate the migration and multiplication abilities of the 3 kinds of pine wood nematodes, the distributions and population numbers of the 3 nematode types were investigated using 20-cm long one-year-old stem cuttings of 5-year-old *P. thunbergii*.

Verification of pathogenicity of *B. xylophilus* and European and East Asian types of *B. mucronatus*. On July 19, 2011, 3,000 *B. xylophilus* (collected in Yeosu City, Chyoen-nam Province, South Korea) and European and East Asian types of *B. mucronatus* (collected in Kwangrung, Namyangju City, Kyeongki Province and an unidentified area in South Korea, respectively) which suspended in 20 µl of distilled water respectively were inoculated at a notch, which was cut to expose the xylem at the bottom of current-year stems of 4-year-old *P. thunbergii* seedlings by using a razor blade. As a control, 20 µl of distilled water without pine wood nematodes were inoculated into pine seedlings. Forty pine seedlings were planted in pots and placed at a low hill at the Korea Forest Research Institute on May 19, 2011. Ten pine seedlings were used for each inoculum and the control group, respectively. The 3 kinds of pine wood nematode inoculi were prepared using nematodes that were incubated for 11 days at 25°C on *Botrytis cinerea* Pers. mycelial mats maintained on potato dextrose agar for 7 days.

Leaf discoloration in pine seedlings was observed every week for up to 8 weeks, and on 10 and 25 weeks after inoculation. On January 10, 2012, 5 pine seedlings from each inoculum group were cut into 5-cm segments from the inoculation point in both upward and downward directions, from which the pine wood nematodes were extracted using

the Baermann funnel method and counted using a stereomicroscope. Since the up and down ends of some seedlings were less than 5 cm, these were counted as separate segments.

Inoculation test for distributions of *B. xylophilus* and European and East Asian types of *B. mucronatus* in *P. thunbergii* stem cuttings. On April 18, 2012, 30 of the 20-cm long one-year-old stem cuttings (mean diameter: 10.29 mm) were collected from 5-year-old *P. thunbergii*. 20 µl of distilled waters contained 1,000 *B. xylophilus* or European or East Asian types of *B. mucronatus* were inoculated onto the cut surfaces of 10 pine stem cuttings, respectively. Previously, the nematodes were reared on *B. cinerea* at 25°C for 6–10 days. One and 5 days incubation after inoculation at 25°C, the pine stem cuttings were cut into 8 pieces of 2.5-cm length, and mounted on funnels for one day to extract pine wood nematodes. The collected nematodes were counted using a stereomicroscope.

Inoculation test for multiplications of *B. xylophilus* and European and East Asian types of *B. mucronatus* in *P. thunbergii* stem cuttings and on *B. cinerea* fungal mats. For inoculation, the number of each nematode type was adjusted to 200 in 20 µl of distilled water. As previously described, each kind of pine wood nematodes was inoculated onto 10 one-year-old stem cuttings (mean diameter of 30 pine stem cuttings: 10.16 mm) of 20-cm length that were cut from 5-year-old *P. thunbergii*. The nematodes were also inoculated onto *B. cinerea* mycelial mats maintained on PDA in 9-cm Petri dishes at 25°C for 7 days. The pine stem cuttings and *B. cinerea* mycelial mats, which were inoculated with each of the 3 types of pine wood nematodes, were incubated during 7 days at 25°C, and then nematodes were extracted using the Baermann funnel method and counted.

Statistical analysis. One-way ANOVA and Tukey's honestly significant difference method (with 5% significant level) were used for multiple comparisons of the distribution and proliferation of each nematode type in *P. thunbergii* stem cuttings and on *B. cinerea* fungal mats.

Pathogenicity of *B. xylophilus* and European and East Asian types of *B. mucronatus* to *P. thunbergii* seedlings. The number of *P. thunbergii* seedlings showing leaf discoloration is presented in Table 1. Leaf discoloration appeared in six 2-year-old stems inoculated with *B. xylophilus* on 2 weeks after inoculation. Five weeks after inoculation, red leaf discoloration was observed at the basal part of leaves of current and one-year-old stems and the entire leaves of the 2-year-old stems. Twenty-five weeks after

Table 1. The number of 4-year-old *Pinus thunbergii* seedlings showing leaf discoloration after inoculated with *Bursaphelenchus xylophilus* and European type and East Asian type of *B. mucronatus* on 19 July 2011

Weeks after inoculation	<i>Bursaphelenchus xylophilus</i>			European type of <i>B. mucronatus</i>			East Asian type of <i>B. mucronatus</i>			Control		
	No change	Partial discoloration	Whole discoloration	No change	Partial discoloration	Whole discoloration	No change	Partial discoloration	Whole discoloration	No change	Partial discoloration	Whole discoloration
1	10	-	-	10	-	-	10	-	-	10	-	-
2	4	6	-	10	-	-	10	-	-	10	-	-
3	4	6	-	10	-	-	10	-	-	10	-	-
4	4	6	-	10	-	-	10	-	-	9	1	-
5	4	4	2	9	1	-	10	-	-	9	1	-
6	2	4	4	7	3	-	9	1	-	9	2	-
7	1	2	7	6	4	-	7	3	-	8	2	-
8	1	1	8	4	6	-	5	5	-	7	3	-
10	1	1	8	4	6	-	3	7	-	4	6	-
25	1	0	9	4	6	-	3	7	-	4	6	-

inoculation, 9 seedlings had wilted, and only one seedling was alive without discoloration. In the seedlings inoculated with European and East Asian types of *B. mucronatus* and the control group, leaf discoloration was observed only in 2-year-old stems of one seedling on 5, 6, and 4 weeks after inoculation, respectively. Thereafter partial leaf discoloration appeared in 6, 7, and 6 seedlings inoculated with the 2 types of *B. mucronatus* and of the control group, respectively, on 25 weeks after inoculation. No seedling showed entire discoloration or dead until the 25th week after inoculation.

Reisolations and distributions of *B. xylophilus* and European and East Asian types of *B. mucronatus* in *P. thunbergii* seedlings. *B. xylophilus* was reisolated from 4 dead seedlings (No. 1–4), but not from the 1 living seedling (No. 5) on 25 weeks after inoculation (Fig. 1), whereas only a few European and East Asian types of *B. mucronatus* were reisolated from 3 (No. 3–5) and 2 (No. 1–2) pine seedlings. No nematodes were found in the two examined pine seedlings from the control group.

B. xylophilus was distributed throughout the pine seedlings, with most of them thriving in the lower part of seedlings (No. 1, 2, and 4) (Fig. 1). A few European and East Asian types of *B. mucronatus* existed in only 1 or 2 parts of a seedling and were distributed at 50–60 cm and 35–40 cm away from the inoculation site, respectively.

Distributions of *B. xylophilus* and European and East Asian types of *B. mucronatus* in *P. thunbergii* stem cuttings. Nematode distribution in 20-cm long pine stem cuttings on 1 and 5 days after inoculation is shown in Table 2. In 0–2.5 cm of stem cuttings on 1 day after inoculation, the

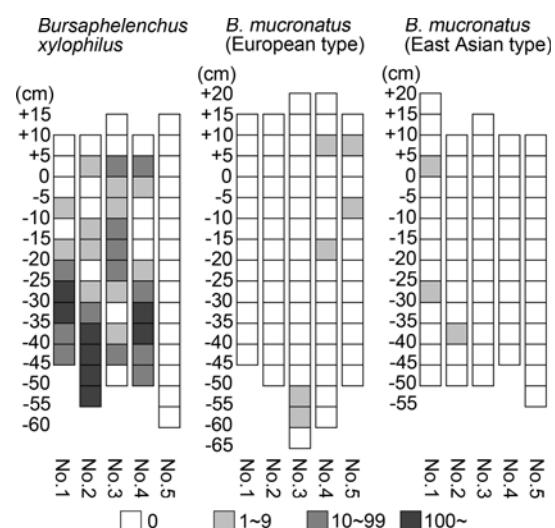


Fig. 1. The distribution of pine wood nematode of *Bursaphelenchus xylophilus* and European type and East Asian type of *B. mucronatus* in each seedling of 4-year-old *Pinus thunbergii* on 25 weeks after inoculation. Numbers below columns show seedling number. Each column represents the number of extracted pine wood nematodes from each 5-cm stem segments. Numbers at left side of columns indicate the distance from inoculated point of the seedlings.

mean percentage numbers and standard deviations of *B. xylophilus* and European and East Asian types of *B. mucronatus* were 83.6 ± 12.7 , 91.7 ± 2.7 , and 87.5 ± 6.7 , respectively, and no significant differences among the 3 pine wood nematode types were observed at the 5% level by Tukey's honestly significant difference method. The other segment groups showing different distances were also found to have no statistical significance. Five days after inoculation, the mean percentage numbers of *B. xylophilus* and

Table 2. Mean percentage number of pine wood nematodes of *Bursaphelenchus xylophilus* and European type and East Asian type of *B. mucronatus* distributed in pine stem segments of 20-cm long one-year-old stem cuttings excised from 5-year-old *Pinus thunbergii* seedlings on 1 and 5 days after inoculation (n = 5)

Distance from inoculation surface of segments (cm)	1 day after inoculation			5 days after inoculation		
	<i>Bursaphelenchus xylophilus</i>	European type of <i>B. mucronatus</i>	East Asian type of <i>B. mucronatus</i>	<i>Bursaphelenchus xylophilus</i>	European type of <i>B. mucronatus</i>	East Asian type of <i>B. mucronatus</i>
0–2.5	83.6 ± 12.7 a ^a	91.7 ± 2.7 a	87.5 ± 6.7 a	67.0 ± 17.0 a	59.6 ± 17.3 a	79.4 ± 14.7 a
2.5–5	1.5 ± 1.9 a	1.3 ± 1.0 a	3.7 ± 2.8 a	11.9 ± 16.2 a	7.3 ± 5.2 a	4.5 ± 2.4 a
5–7.5	1.4 ± 1.5 a	1.0 ± 0.3 a	1.7 ± 0.8 a	5.3 ± 3.0 a	6.0 ± 2.9 a	3.1 ± 2.5 a
7.5–10	3.0 ± 2.5 a	1.0 ± 0.8 a	1.9 ± 1.2 a	3.6 ± 1.3 a	6.0 ± 4.4 a	3.2 ± 2.6 a
10–12.5	2.8 ± 2.2 a	1.0 ± 0.7 a	1.9 ± 1.5 a	2.2 ± 0.4 a	5.4 ± 4.1 a	2.6 ± 1.8 a
12.5–15	2.1 ± 1.5 a	0.7 ± 0.4 a	1.1 ± 1.1 a	3.8 ± 3.3 a	5.6 ± 3.3 a	3.0 ± 2.2 a
15–17.5	2.8 ± 2.0 a	1.0 ± 0.4 a	1.3 ± 0.7 a	3.9 ± 4.3 a	5.4 ± 3.1 a	2.6 ± 2.4 a
17.5–20	2.7 ± 2.6 a	2.3 ± 2.2 a	1.0 ± 1.4 a	2.3 ± 1.8 a	4.6 ± 1.7 a	1.6 ± 2.5 a

^aMean numbers followed by same letters in each row on each day are not significantly different at the 5% level by Tukey's honestly significant difference method. SD = Standard deviation.

Table 3. Mean number of pine wood nematodes of *Bursaphelenchus xylophilus* and European type and East Asian type of *B. mucronatus* distributed in 0–5 cm and 5–20 cm of one-year-old stem cuttings of 5-year-old *Pinus thunbergii* seedlings on 1 and 5 days after inoculation (n = 5)

Portion of pine stem cuttings	1 day after inoculation			5 days after inoculation		
	<i>Bursaphelenchus xylophilus</i>	European type of <i>B. mucronatus</i>	East Asian type of <i>B. mucronatus</i>	<i>Bursaphelenchus xylophilus</i>	European type of <i>B. mucronatus</i>	East Asian type of <i>B. mucronatus</i>
0–5 cm	668.4 ± 362.4	398.4 ± 106.9	478.8 ± 147.9	260 ± 97.4	175.8 ± 73.4	363 ± 230.9
(%)	(85.6 ± 11.2)	(93.0 ± 1.8)	(91.8 ± 5.8)	(77.2 ± 10.5)	(65.6 ± 12.9)	(91.8 ± 5.8)
5–20 cm	112.4 ± 97.1	30 ± 10.9	42.6 ± 20.7	76.8 ± 47.3	92 ± 65.1	59.4 ± 44.6
(%)	(14.4 ± 11.2) a ^a	(7.0 ± 1.8) a	(8.2 ± 5.8) a	(22.8 ± 10.5) a	(34.3 ± 12.9) a	(14.1 ± 13.1) a
Ratio ^b	0.112 ± 0.097 a	0.030 ± 0.011 a	0.043 ± 0.021 a	0.077 ± 0.047 a	0.092 ± 0.065 a	0.059 ± 0.045 a

^aMean numbers followed by same letters in each row on each day are not significantly different at the 5% level by Tukey's honestly significant difference method.

^bRatio of the number of nematodes in 5–20 cm pine stem segments to inoculated nematodes number.

European and East Asian types of *B. mucronatus* in all segment groups were also shown to have no statistical significance.

The mean percentages of *B. xylophilus* and European and East Asian type of *B. mucronatus* in 5–20 cm pine stems were 14.4, 7.0 and 8.2, respectively, with no significant differences observed at the 5% level by Tukey's honestly significant difference method on 1 day after inoculation (Table 3). The ratio of pine wood nematodes that migrated over 5 cm from the site of inoculation, i.e., the number of nematodes within 5–20 cm of pine stem segments, to an inoculation number of 1000 nematodes of *B. xylophilus* and European and East Asian types of *B. mucronatus* were 0.112, 0.030 and 0.043, respectively, with no significant differences among the 3 nematodes at the 5% level by Tukey's honestly significant difference method. Five days after inoculation, the mean percentage numbers and the ratio of three kinds of pine wood nematodes in the 5–20 cm stem cuttings were not significantly different from each

other.

Multiplications of *B. xylophilus* and European and East Asian types of *B. mucronatus* in *P. thunbergii* stem cuttings and on *B. cinerea* fungal mats. The mean numbers of reproducing nematodes in 20-cm *P. thunbergii* stem cuttings and on *B. cinerea* fungal mats are shown in Table 4. The average numbers of *B. xylophilus* and European and East Asian types of *B. mucronatus* thriving on fungal mats were 13227, 3954, and 3420, respectively, whereas those in pine stem cuttings were 172, 61, and 78, respectively. The mean number of *B. xylophilus* was significantly larger than those of the European and East Asian types of *B. mucronatus* both in pine stem cuttings and on fungal mats at the 5% Trendslevel by Tukey's honestly significant difference method.

From the mortality rate of 4-year-old *P. thunbergii* seedlings inoculated with *B. xylophilus* (collected from Yeosu City, Chyoen-nam Province, South Korea) and European and East Asian types of *B. mucronatus* (collected from Kwang-

Table 4. Mean number of pine wood nematodes of *Bursaphelenchus xylophilus* and European type and East Asian type of *B. mucronatus* multiplied in 20-cm long one-year-old stem cuttings excised from 5-year-old *Pinus thunbergii* seedlings and on *Botrytis cinerea* fungal mats during 7 days

Media for nematode multiplication	<i>Bursaphelenchus xylophilus</i>	European type of <i>B. mucronatus</i>	East Asian type of <i>B. mucronatus</i>
<i>Pinus thunbergii</i> stem cuttings	172 ± 119 a ^a (n = 10) ^b	61 ± 36 b (n = 9)	78 ± 59 b (n = 10)
<i>Botrytis cinerea</i> fungal mats	13227 ± 5496 a (n = 10)	3954 ± 817 b (n = 8)	3420 ± 1414 b (n = 10)

^aMean numbers followed by same letters in each row are not significantly different at the 5% level by Tukey's honestly significant difference method.

^bn is a replication number.

rung, Namyangju City, Kyeongki Province and an unidentified area in South Korea, respectively), only *B. xylophilus* showed pathogenicity and strong virulence to *P. thunbergii* seedlings with 90% mortality. This result was similar to that reported by Choi and Moon (1989) that *B. xylophilus* (isolated from Busan Province, South Korea) weakened 20% of 4-year-old *P. densiflora* seedlings and killed 33.3% on 14 days after inoculation, and thereafter, killed 100% of pine seedlings on 50 days after inoculation. In addition, Woo et al. (2010) reported that the mortality rates of 4-year-old *P. densiflora* seedlings inoculated with *B. xylophilus* and *B. mucronatus* on 75 days after inoculation were 90% and 0%, respectively, linking the virulence of pine wood nematodes to high gas exchange rates. Similar results were observed by Mamiya and Enda (1979) and Kanzaki et al. (2011) in Japan. In this study, the European type of *B. mucronatus* showed nonpathogenicity to *P. thunbergii* seedlings the same as East Asian type of *B. mucronatus*.

The mechanism of infestation of *B. xylophilus* after entry into a susceptible pine tree involves migration and multiplication within the host tree (Fukuda et al., 1992; Ichihara et al., 2000; Kuroda and Ito, 1992; Mamiya, 1980). The migration and multiplication of *B. xylophilus*, however, are restricted to resistant pine species and families (Kawakuchi, 2006; Mori et al., 2008; Oku et al., 1989). In the case of *B. mucronatus*, the migration and multiplication were restricted within the susceptible pine tree or not in comparison with *B. xylophilus* (Fukuda et al., 1992; Togashi and Matsunaga, 2003). The distribution of the 3 nematode types in 4-year-old *P. thunbergii* seedlings on 25 weeks after inoculation showed that *B. xylophilus* could migrate, multiply, and survive within the entire tree, whereas both *B. mucronatus* types almost diminished under the same conditions. The proliferation of *B. xylophilus* was observed only in dead pine seedlings, indicating the importance of multiplying ability of pine wood nematode and resistance level of individual pine tree to the incidence of pine wilt disease. Woo et al. (2010) also reported that several *B. xylophilus* were recovered from 10 seedlings of *P. densiflora*, whereas

few *B. mucronatus* were recovered from only 3 seedlings. The results of this study showed that pathogenicity and nonpathogenicity of the 3 nematode types used in this study are influenced by their multiplication ability rather than migration ability.

Early migration was considered important to the progression of pine wilt disease because during migration, the surrounding pine cells are destroyed (Hogetsu et al., 1994; Ichihara et al., 2000; Mamiya, 1980). The distributions of *B. xylophilus* and European and East Asian types of *B. mucronatus* in 20-cm long stem cuttings of *P. thunbergii* were not significantly different on 1 and 5 days after inoculation, which shows that both types of *B. mucronatus* have similar migration abilities and were not significantly different from that of *B. xylophilus* at an early stage after inoculation, such as days 1 and 5. Further, in this study, the number of *B. xylophilus* in 5–20 cm pine stem segments which was equal to the number passing through 5 cm pine stem cuttings was not significantly different from that of the 2 types of *B. mucronatus* at 24 hours after inoculation. Togashi and Matsunaga (2003) showed similar results with no significant difference between the number of *B. xylophilus* (S-10) and that of *B. mucronatus* (Un-1) passing through 5-cm living branch sections at 24 hours after inoculation. Conversely, Ishida et al. (1993) reported that the number of *B. xylophilus* passing through 5-cm *P. thunbergii* stem cuttings was larger than that of *B. mucronatus* at 24 hours after inoculation. They further suggested that *B. xylophilus* invades and kills the cortical tissues before they form a wound periderm while *B. mucronatus* hardly invade cortical tissue; thus, *B. mucronatus* in 20-cm *P. thunbergii* stem cuttings never migrated 15–20 cm away from the inoculation site on 7 days after inoculation (Ishida et al., 1993). These conflicting observations suggest that migration ability may be a necessary condition but may not be the only requirement for the development of pine wilt disease.

In our study, the mean number of multiplied nematodes of *B. xylophilus* (Yeosu City) was significantly larger than that of the European and East Asian types of *B. mucronatus*

by thriving on *B. cinerea* fungal mats during 7 days, which is similar to that by Wang et al. (2005), who reported that the population rate increase of *B. xylophilus* (S-10) was higher than that of *B. mucronatus* because of high fecundity, long duration of egg laying, and short life cycle on fungal mats. Although the mean numbers of the 3 nematode types that were extracted from 20-cm *P. thunbergii* stem cuttings were smaller than the initial inoculation number of 200, the mean number of *B. xylophilus* was significantly larger than those of the European and East Asian types of *B. mucronatus*. This obvious difference in nematode populations may be attributed to the inhibitory effect of pine trees, which was removed by boiling and short culture period for pine trees (Aikawa and Kikuchi, 2007; Togashi and Matusnaga, 2003). *B. xylophilus* generally multiplies in pine trees within 10 days after infestation, eventually resulting in the death of the pine trees (Futai, 1980; Ichihara et al., 2000; Odani et al., 1985). It is thus possible that the higher proliferative ability of *B. xylophilus* (Yeosu City) than that of both types of *B. mucronatus* contributed greatly to *B. xylophilus* pathogenicity.

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