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Three Years Examination in the Secondary Screening Test in a Project for Selecting *Cryptomeria japonica* Resistant to *Semanotus japonicus* (Coleoptera: Cerambycidae) Conducted in Kanto Breeding Region

Kazutaka Kato[1] and Toru Taniguchi[2]

Summary: The secondary screening test, in which trees were inoculated with newly-hatched *Semanotus japonicus* larvae, was conducted for 3 years on 15 candidate *Cryptomeria japonica* clones that were selected from the Kanto Breeding Region by the primary screening test for the resistance to *S. japonicus*. Two *C. japonica* varieties, Bokasugi and Kumotoshi, that are resistant and susceptible to *S. japonicus*, respectively, were also tested as controls. Every year, 30 or 60 larvae were inoculated to each clone (3 or 6 ramets) in the spring and the inoculation sites were debarked in the autumn to observe the condition of larvae. More than 10 larvae entered the bark in almost all the clones for 3 years. In the first year, the index of susceptibility (the proportion of larvae that survived to the adult stage to the number of larvae entering bark) was significantly higher in 5 clones than in Bokasugi. Two or more *S. japonicus* adults emerged from 2 other clones. Therefore, these 7 clones were considered to be non-resistant and eliminated from further test. In the second year, the index of susceptibility was not significantly higher in the 8 remaining clones than in Bokasugi. In the third year, however, the index of susceptibility was significantly higher in one clone than in Bokasugi. Three candidate clones consistently showed 0% in the proportion of emerging adults for 3 years and were confirmed resistant to *S. japonicus*

1. Introduction

Cryptomeria japonica (L.f.) D. Don. has been planted for timber production extensively in Japan and it covers more than 4 million ha (Forest agency 1993). Extensive plantations of a single species are often subject to insect damage, and many *C. japonica* stands suffer insect infestations (Kobayashi and Taketani 1994). *Semanotus japonicus* Lacordaire is one of the most important insect pests of *C. japonica* because their larvae enter and damage the wood (Kobayashi 1982).

S. japonicus larvae enter the bark and feed on the phloem and adjacent wood (Kobayashi 1985). During the feeding period, larvae are frequently killed by a host resistance response, oleoresin exudation from traumatic resin canals (Fujishita et al. 1968; Hagiwara and Ogawa 1970; Okuda 1982; Shibata 1987). The larval mortality from the host resistance differs among *C. japonica* varieties (Kishi et al. 1973; Kawarai and Kitazawa 1974; Okada 1980). This suggests the possibility of selecting trees highly resistant to *S. japonicus*.

(1) Forest Tree Breeding Center
3809-1, Ishi, Juo, Hitachi, Ibaraki 319-1301, Japan
林木育種センター
〒319-1301　茨城県日立市十王町伊師 3809-1
(2) Kyusyu Regional Breeding Office, Forest Tree Breeding Center
2320-5, Suya, Nishigoshi, Kikuchi, Kumamoto 861-1102, Japan
林木育種センター九州育種場
〒861-1102　熊本県菊池郡西合志町須屋 2320-5

Under the leadership of the Forestry Agency Japan, a project to select *C. japonica* resistant to *S. japonicus* began in 1985. The selection procedure consisted of the following four steps (Kato and Taniguchi 2003): surveys of heavily infested stands for candidate individuals, pin prick test, adult release test (primary screening test) and larval inoculation test (secondary screening test). As a result of the pin prick test, a total of 222 individual trees were selected as candidates in Kanto Breeding Region. The adult release test was applied to 200 of these trees from 1990 through 2000, showing that 21 clones were little damaged by *S. japonicus* larvae (Kato and Taniguchi 2003).

The final step, larval inoculation test, in which each tree is inoculated with newly- hatched *S. japonicus* larvae, is a more rigorous test for the detection of resistance than the first screening test because in the former test considerable number of larvae certainly enter and begin to feed on the bark. The larval inoculation test has been conducted for 3 years in the Forest Tree Breeding Center. Here, we summarize the results of the test.

2. Material and Methods

2.1 Study area

The secondary screening test was carried out in the experimental forests at the Forest Tree Breeding Center in Ibaraki Prefecture (50 m a.s.l.), east Japan (36° 20'N, 140° 27'E) from 2001 to 2003. The mean annual temperature and precipitation from 1994 to 2000 were 14.0℃ and 1404 mm, respectively.

2.2 Test trees

15 of the 21 candidate clones that had 3 or more ramets available for the test and two varieties, Bokasugi and Kumotoshi, were tested. Bokasugi is highly resistant to *S. japonicus* and Kumotoshi is susceptible (Kato and Taniguchi 2003). Three or 6 ramets were used for the screening test. All the ramet trees were propagated by cutting and planted in 1993. Mean diameter at breast height in each clone in spring 2001 is shown in Table 1.

Table 1. Mean DBH (cm±SD) in each clone in spring 2001

Clones	No. of test ramets	DBH
Chiba 19	3	7.1±1.0
Chiba 15	3	8.0±3.0
Ibaraki 39	3	11.6±3.6
Ibaraki 29	3	9.1±1.4
Kasama mashiko 10	6	6.8±0.9
Aichi 20-5	3	10.4±1.4
Chiba 1	3	8.0±1.4
Ibaraki 2	3	11.0±2.2
Shirakawa sumito 2	3	4.7±1.7
Gifu 1	3	11.4±0.7
Ibaraki 27	3	10.2±1.1
Ibaraki 38	3	12.7±2.7
Ibaraki 22	3	8.9±2.0
Ibaraki 24	3	8.3±2.4
Ibaraki 35	3	10.1±0.5
Bokasugi	3	10.1±1.8
Kumotoshi	3	10.9±0.3

2.3 Inoculation of larvae and screening method

The survivorship of *S. japonicus* larvae depends not only on the resistance of trees but also on the larval vigor and environmental conditions such as temperature and precipitation (Kobayashi 1982; Kato et al. 2000). Therefore, the resistance of each clone to *S. japonicus* was tested for 3 years. Before beginning the test in each year, each candidate clone was inspected for the presence of damage arising from natural oviposition by *S. japonicus*. When 2 or more exit holes of *S. japonicus* were found, the clone was eliminated from further test.

Between mid-March and mid-April of each year, to obtain *S. japonicus* eggs newly emerged adults were collected from infested trees by the band-trapping method (Shibata 1983) in the experimental forest or in Omi seed orchard located 60 km southwest of Juo Town. Small female adults were not collected because larval survivorship of the progeny of small females is lower than that of large females (Kato et al. 2000). After a short storage at 5℃ in a refrigerator, adults were reared under laboratory condition. Each adult female was placed in a plastic container (diameter: 9 cm, height: 2 cm) in which a small piece of *C. japonica* bark was placed on a filter paper moistened with water. An adult male was added into each container for mating. After the females began to lay eggs, the males were removed and the eggs were collected. Immediately after hatching, the larvae were inoculated onto the bark of the trees by using inoculation papers that were 2×2 cm in size and had holes 0.5 cm in diameter in the center (Kawamura et al. 1982). Each tree was inoculated with 10 larvae on 5 inoculation sites (2 larvae per site). The inoculation sites in a tree were separated from each other vertically at least 30 cm because overcrowding of larvae would reduce their survivorship (Ito 2000).

In autumn of each year, the inoculation sites were debarked to observe the condition of larvae. The larvae were divided into three categories: died in the bark, died in the wood and developed to adults. When larvae were dead in the wood and the length of their feeding galleries in the wood was less than 5cm, new phloem tissue healed the galleries and no damage was left (Kato personal observation). Therefore, we considered these cases to be no damage to the trees.

From these data, proportions of larvae that entered the bark, larvae that entered the wood, larvae that damaged the wood and larvae that developed to the adult stage were calculated in each clone. The definition of each proportion was shown in Tables 2 and 3. The proportion of larvae that developed to the adult stage was used as an index of susceptibility because this proportion was closely related to deterioration of wood quality. If the index was significantly higher than that in Bokasugi (one-sided χ^2-test), those clones were eliminated from further test. All proportional data were analyzed statistically after the original data were arcsine-transformed.

Table2. Proportion (%) of larvae entering bark and larvae entering wood in each clone in each year or in total

Clones	2001		2002		2003		Total	
	Bark	Wood	Bark	Wood	Bark	Wood	Bark	Wood
Chiba19	50.0 (15)	33.3 (5)	66.7 (20)	0 (0)	23.3 (7)	0 (0)	46.7 (42)	11.1 (5)
Chiba15	43.3 (13)	15.4 (2)	60.0 (18)	11.1 (2)	53.3 (16)	6.3 (1)	52.2 (47)	10.9 (5)
Ibaraki39	73.3 (22)	4.5 (1)	50.0 (15)	0 (0)	46.7 (14)	0 (0)	56.7 (51)	1.5 (1)
Ibaraki29	53.3 (16)	6.3 (1)	53.3 (16)	0 (0)	56.7 (17)	11.8 (2)	54.4 (49)	6.0 (3)
Kasama mashiko 10	53.3 (16)	37.5 (6)	53.3 (32)*	25.0 (8)	43.3 (13)	53.8 (7)	50.0 (61)	38.7 (21)
Aichi 20-5	36.7 (11)	36.3 (4)	60.0 (18)	16.7 (3)	13.3 (4)	0 (0)	36.7 (33)	17.7 (7)
Chiba 1	33.3 (10)	40.0 (4)	56.7 (17)	23.5 (4)	46.7 (14)	42.8 (6)	45.6 (41)	35.4 (14)
Ibaraki 2	43.3 (13)	7.7 (1)	50.0 (15)	26.7 (4)	40.0 (12)	25.0 (3)	44.4 (40)	19.8 (8)
Shirakawa sumito 2	53.3 (16)	18.8 (3)					53.3 (16)	18.8 (3)
Gifu 1	40.0 (12)	25.0 (3)					40.0 (12)	25.0 (3)
Ibaraki 27	40.0 (12)	41.7 (5)					40.0 (12)	41.7 (5)
Ibaraki 38	50.0 (15)	20.0 (3)					50.0 (15)	20.0 (3)
Ibaraki 22	50.0 (15)	53.3 (8)					50.0 (15)	53.3 (8)
Ibaraki 24	63.3 (19)	26.3 (5)					63.3 (19)	26.3 (5)
Ibaraki 35	73.3 (22)	0 (0)					73.3 (22)	0 (0)
Bokasugi	66.7 (20)	10.0 (2)	60.0 (18)	11.1 (2)	66.7 (20)	0 (0)	64.5 (58)	7.4 (4)
Kumotoshi	40.0 (12)	33.3 (4)	50.0 (15)	20.0 (3)	40.0 (12)	16.7 (2)	43.3 (39)	23.3 (9)

*, inoculated with 60 larvae: ( ), Number of larvae

Proportion of larvae entering bark: (number of larvae entering bark/ number of larvae inoculated) × 100

Proportion of larvae entering wood: (number of larvae entering wood/ number of larvae entering bark) × 100

Table 3. Proportion (%) of damage of wood and emwerging adults in each clone in each year or in total

Clones	2001				2002				2003				Total	
	Damage	χ^2	Adults	χ^2	Damage	χ^2	Adults	χ^2	Damage	χ^2	Adults	χ^2	Damage	Adults
Chiba19	4.7 (1)	1.4	0 (0)	–	0 (0)	–	0 (0)	–	0 (0)	–	0 (0)	–	1.6 (1)	0 (0)
Chiba15	7.7 (1)	1.6	0 (0)	–	0 (0)	–	0 (0)	–	0 (0)	–	0 (0)	–	2.6 (1)	0 (0)
Ibaraki39	4.5 (1)	0.9	0 (0)	–	0 (0)	–	0 (0)	–	0 (0)	–	0 (0)	–	1.5 (1)	0 (0)
Ibaraki29	6.3 (1)	1.3	0 (0)	–	0 (0)	–	0 (0)	–	5.9 (1)	1.2	5.9 (1)	1.2	12.1 (2)	2.0 (1)
Kasama mashiko 10	12.5 (2)	2.7	0 (0)	–	6.3 (2)	0.0	3.1 (1)	0.6	7.7 (1)	1.5	0 (0)	–	8.4 (5)	1.0 (1)
Aichi 20-5	0 (0)	–	0 (0)		16.7 (3)	1.1	5.6 (1)	1.0	0 (0)	–	0 (0)	–	5.8 (3)	2.9 (1)
Chiba 1	10.0 (1)	2.1	10.0 (1)	2.1	5.8 (1)	0.0	0 (0)	–	7.1 (1)	1.4	0 (0)	–	7.6 (3)	3.3 (1)
Ibaraki 2	0 (0)	–	0 (0)	–	13.3 (2)	0.6	6.7 (1)	1.2	16.7 (2)	3.4 *	16.7 (2)	3.4 *	10.0 (4)	7.8 (1)
Shirakawa sumito 2	18.8 (3)	4.1 **	18.8 (3)	4.1 **									18.8 (3)	18.8 (3)
Gifu 1	16.7 (2)	3.7 *	16.7 (2)	3.7 *									16.7 (2)	16.7 (2)
Ibaraki 27	41.7 (5)	9.9 ***	41.7 (5)	9.9 ***									41.7 (5)	41.7 (5)
Ibaraki 38	20.0 (3)	4.4 **	20.0 (0)	4.4 **									20.0 (3)	20.0 (0)
Ibaraki 22	46.7 (7)	11.7 ***	40.0 (6)	9.7 ***									46.7 (7)	40.0 (6)
Ibaraki 24	5.3 (1)	1.1	5.3 (1)	1.1									5.3 (1)	5.3 (1)
Ibaraki 35	0 (0)	0	0 (0)	–									0 (0)	0 (0)
Bokasugi	0 (0)		0 (0)		5.6 (1)		0 (0)		0 (0)		0 (0)		1.9 (1)	0 (0)
Kumotoshi	16.7 (2)		16.7 (2)		6.7 (1)		0 (0)		7.1 (1)		7.1 (1)		10.2 (4)	7.9 (3)

*, $P < 0.1$: **, $P < 0.05$: ***, $P < 0.01$

Proportion of damage of wood: (number of larvae entering wood but dead before feeding wood less than 5 cm/ number of larvae entering bark) × 100

Proportion of emerging adults: (number of emerging adults/ number of larvae entering bark) × 100

3. Results

In 2001, the proportion of larvae entering bark in each candidate clone is over 30%; more than 10 larvae entered the bark (Table 2). However, the proportion of larvae entering wood considerably decreased and showed 0% in one clone (Ibaraki 29). The proportion of damage to wood and emerging adults further decreased and showed 0% in 3 and 7 candidate clones, respectively (Table 3). The proportion of emerging adults was 0% in Bokasugi while that was more than 10% in Kumotoshi, suggesting that these two varieties showed significantly different resistance to *S. japonicus* (one-sided χ^2-test, χ^2 = 3.7, respectively, $P < 0.10$).

In 5 candidate clones (Shirakawa sumito 2, Gifu 1, Ibaraki 27, Ibaraki 38 and Ibaraki 22), the proportion of emerging adults was significantly higher than that in Bokasugi (one-sided χ^2-test, $P < 0.10$). In other 2 clones (Ibaraki 24 and Ibaraki 35), although the proportion was not significantly higher than in Bokasugi (one-sided χ^2-test, $P > 0.10$), naturally emerging holes by *S. japonicus* adults, each 4 and 9 numbers respectively, were found in mid-April 2002. Therefore, we regarded these 7 clones as low resistant trees and eliminated them from the further test.

In 2002, in each of the 8 remaining candidate clones, more than 10 larvae entered the bark. Three clones (Chiba 19, Ibaraki 39 and Ibaraki 29) showed 0% in the proportion of larvae entering wood. No candidate clones showed significantly higher proportion of emerging adults than Bokasugi (one-sided χ^2-test, $P < 0.10$). Kumotoshi also showed 0% in the proportion of emerging adults.

In 2003, less than 10 larvae entered the bark in 2 candidate clones (Chiba 19 and Aichi 20-5). Three clones (Chiba 19, Ibaraki 39 and Aichi 20-5) showed 0% in the proportion of larvae entering wood; 2 clones of them showed 0% in the proportion of emerging adults in 2002 as well. One clone (Ibaraki 2) showed significantly higher proportion of emerging adults than Bokasugi (one-sided χ^2-test, $P < 0.10$).

All the candidate clones and Bokasugi did not consistently show 0% in the proportion of damage to wood for 3 years. On the other hand, 3 candidate clones (Chiba 19, Chiba 15 and Ibaraki 39) and Bokasugi consistently showed 0% in the proportion of emerging adults for 3 years.

There were significant correlations in the relationships between the proportion of larvae entering wood and damage to wood and between the proportion of damage to wood and emerging adults for all years ($P < 0.05$) (Table 4). However, significant correlation was not observed between the proportion of larvae entering wood and emerging adults for 2003.

Table 4. Correlations among the proportion of larvae entering wood (PL), damage of wood (F emerging adults (PE) in each clone in each year

Proportior	Year 2001		2002		2003	
	PL	PD	PL	PD	PL	PD
PD	0.65**		0.70**		0.65**	
PE	0.60**	0.96**	0.57*	0.85**	0.15	0.84**

*, $P < 0.10$: **, $P < 0.05$

4. Discussion

To determine the precise resistance levels of 15 *C. japonica* clones against *S. japonicus*, we conducted the secondary screening test for three years. When the number of larvae entering the bark is low, their survivorship is more influenced by their vigor or environmental factors than by the resistance level of host trees (Kobayashi 1982; Kato et al. 2000). On those clones, since the number of larvae entering bark was low (less than 10) in the primary screening test (Kato and Taniguchi 2003), resistance level of the clones may not be tested precisely. On the other hand, in the secondary screening test, more than 10 larvae entered bark so that the true resistant level of the clones can be detected.

As a result of the 2001 test, 5 clones were eliminated from the further test because the proportions of emerging adults in these clones were significantly higher than those in the resistant control variety, Bokasugi (Table 3). In the 8 remaining candidate clones except for Ibaraki24 and Ibaraki 35, although the proportion of entering wood varied among years (Table 2), the proportion of emerging adults was consistently less than 17% (Table 3). Although the larval condition was poorer in 2002 than in other years, as suggested by 0% proportion of emerging adults even on Kumotoshi in 2002, the resistance of 8 remaining clones is generally regarded to be high. However, if feeding conditions become favorable or larval vigor is more superior, some clones may permit the entering of larvae in the wood and sometimes permit adult emergence like Ibaraki 29, Kasama mashiko 10, Aichi 20-5, Chiba 1 and Ibaraki 2 clones. Thus, the resistance of these 5 clones is lower than other 3 clones (Chiba 19, Chiba 15 and Ibaraki 39).

Even in the highly resistant 3 clones and Bokasugi, the proportion of larvae entering wood could be high (Table 2). No correlation was observed between the proportion of larvae entering wood and emerging adults in 2003 (Table 4). These results suggest that the resistance of *C. japonica* trees to *S. japonicus* is not related to whether *S. japonicus* can enter wood or not. Arihara and Kawakami (1998) reported that the thickness of the inner bark in *C. japonica* is an important resistant factor against *S. japonicus*. In the present test, the thickness of the inner bark of candidate clones was probably thin because they were young and small (Table 1) (Arihara 2001), suggesting that *S. japonicus* larvae could easily enter wood on the trees.

Highly resistant *C. japonica* trees have the high ability to form traumatic resin canals (Kanazashi et al. 1987; Kato 2003). Larvae are hard to feed in highly resistant trees because of the oleoresin exuded from the traumatic resin canals. Thus, the proportion of damage to wood decreased greatly and the proportion of emerging adults was consistently 0 % on the 3 resistant clones and Bokasugi (Table 3). When the resistant clones are planted in the field, the trees would not suffer from infestations by *S. japonicus* because new adults do not emerge on the trees.

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スギカミキリ抵抗性育種事業において関東育種基本区で実施した二次検定の３年間の結果

加藤　一隆[1]・谷口　亨[2]

Kazutaka Kato[1] and Toru Taniguchi[2]

要旨：スギカミキリ抵抗性育種事業の一環として関東育種基本区において一次検定で選抜されたスギ 21 個体のうち 15 個体のクローンに対して，孵化直後のスギカミキリ幼虫を樹幹に接種する二次検定を 2001 年から 2003 年までの３年間にわたり実施した。スギカミキリ抵抗性品種であるボカスギと感受性品種であるクモトオシを対照として用いた。各年の春に，孵化直後のスギカミキリ幼虫を各クローンに 30 または 60 頭接種し，その秋に接種部を剥皮し幼虫の穿孔状況を観察した。３年とも，ほとんどのクローンにおいて 10 頭以上の幼虫が樹皮内に穿孔した。１年目の検定では，５クローンがボカスギに比べて高い羽化率を示した。また別の２クローンでは，自然産卵された幼虫に由来する複数の成虫が羽化した。これら７クローンのスギカミキリ抵抗性は低いと判断して，その後の検定から除外した。２年目では，残り８クローン全てがボカスギと同程度の羽化率を示した。さらに３年目では，１クローンがボカスギよりも高い羽化率を示した。３年とも羽化率が０％を示した３クローンを二次検定合格木と判定した。

(1) 林木育種センター
　〒319-1301　茨城県日立市十王町伊師 3809-1
(2) 林木育種センター九州育種場
　〒861-1102　熊本県菊池郡西合志町須屋 2320-5