林育研報 Bull. Natl. For. Tree Breed. Center No.14, 1996

Comparison of resistance in elite hinoki trees (*Chamaecyparis obtusa* (SIEB. et ZUCC.) ENDL) to *Seiridium unicorne* (COOKE et ELLIS) SLLIS

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Summary: Resistance in elite hinoki clones (*Chamaecyparis obtusa* (SIEB.et ZUCC.) ENDL) to *Seiridium unicorne* (COOKE et ELLIS) SUTTON was compared by inoculating the disease to 203 1-, 2-, and 3-year-old hinoki tree clones at the Kansai Regional Breeding Office, National Forest Tree Breeding Center. Overall proportion of infection by *S. unicorne* per hinoki clone rapidly increased until 6 months after the inoculation (nearly 70%), then gradually increased until 18 months after the inoculation (nearly 80%). Infection in 3-year-old trees spread faster than in younger trees. Values for resinosis (index of susceptibility) also increased over the investigated period and were highest for 3-yearold trees. The infection level in each clone was highly correlated with the value of resinosis.

There were 3 hinoki clones which were not infected at 18 months after the inoculation. These result suggest that most elite hinoki trees are susceptible to *S.unicorne* and further observation will be needed for forestation of elite hinoki trees with resistance to this disease.

### **1** Introduction

Seiridium unicorne is one of the most serious diseases in young hinoki trees (*Chamaecyparis obtusa* (S<sub>IEB</sub>. et Z<sub>UCC</sub>.) ENDL) because terminal parts of the trees are broken by strong winds or killed by infestation, making it aserious problem for forestry management. Therefore, seedlings with resistance to this disease should be planted for future forestation of hinoki trees.

Although it has been well known that resistance of hinoki trees to *S. unicorne* differs greatly among tree clones<sup>1)</sup>, only 30 elite hinoki tree clones were examined for resistance to the disease by artificial inoculation<sup>2)</sup>, and all of those clones were susceptible to the disease. Therefore, if elite hinoki trees with resistance to the disease are not found, the program will not be able to proceed with its forestation objectives.

In this study, 203 kinds of elite hinoki tree clones were inoculated with *S. unicorne* and the resistance of the clones to the disease was compared.

## 2 Materials and methods

Observations were carried out at the Kansai Regional Breeding Office, National Forest Tree breeding Center in Okayama Pref. (150 m a.s.l.) located 150 km northwest of Osaka, Japan. Annual mean temperature and mean precipitation from 1983 through 1992 were 13.  $1^{\circ}$ C and 1540 mm, respectively.

Inoculation tests were conducted for 1-, 2-, and 3-year-old grafted hinoki elite tree clones. All the trees were grown in unglazed pots, but the pots for 3-year-old trees were buried in the soil. The number of elite tree clones for each age group was 67, 72, and 67, respectively (4 or 5 ramets per clone). S. unicorne for the noculation was provided by the Kansai Research Center Forestry and Forest Products Research Institute in April 1993. They were cultured for the inoculation with PDA (potato dextrose agar) medium under 23 °C for about one month. After that, conidia were suspended insterilized water and regulated at 20000 conidiaper 1 ml. Twenty-five ml of the inoculum suspension was sprayed on each hinoki tree in May 1993. The spraying was conducted

over theentire surface of the 1-and 2-year-old trees and on the upper 50 cm of each of the 3-year-old trees. To avoid killing the conidia by desiccation and outflow by rainfall, all the trees were covered with polyethylene bags, and the 1-and 2-year-old trees were further covered with lawn over the bags for a week after the inoculation.

The value of resinosis (index of susceptibility) in each tree was calculated 1, 3, 6, 9, 12, 18 months after the inoculation. The relative proportion of infection by *S. unicorne* in each clone was calculated by dividing the number of trees infected by the total number of trees. Although it was reported that infected branches were sometimes killed<sup>2)</sup>, the number of branches killed by the infection could not becounted because some branches had been killed by ther factors such as desiccation or burning.

### **3 Results**

In the summer of 1994, about 10% of the 1-and 2-year-old trees were killed by extreme desiccation. Forconvenience, values of resinosi sof the dead trees were also calculated 18 mon ths after the inoculation and added to the data. Some trees saw a decrease in resinosis values over time because the constructed resin osis parts sometimes exfoliated from the trees of fused together.

#### 3.1 Rate of infection

In all tested trees, the mean proportion of infection by S. unicorne per clone was nearly 10% at 1 month after the inoculation, but the proportion inoceased rapidly by 6 months after the inoculation (Fig. 1). The mean proportion of the infection in 3-year-old trees was significantly higher that those at 3, 6, and 9 months after the inoculation in 1-yearold trees and at 3, 6, and 12 months after the inoculation in 2-year-old trees (t-test, P<0.05). However, there was no difference in the mean proportion of the infection between the age groups at 18 months after the inoculation (t-test, P > 0.05). These results suggest that although susceptibility of hinoki trees to S. unicorne does not differ between age groups, the rate of infection in 3-year-old trees by S.



Fig. 1 Changes in mean proportion of infection by S. unicorne per clone in eachage group. Vertical bars indicate  $\pm$  SE.

*unicorne* is faster than in the other year-old trees.

## 3.2 Value of resinosis

Mean value of resinosis per hinoki tree in each clone increased with the investigated period after the inoculation (Fig. 2). However, the mean value of resinosis differed greatly be-



Fig. 2 Changes in mean value of resinosis per hinoki clone in each age group. Vertical bars indicate  $\pm$  SE.

tween ages; for example, the mean value of resinosis in 3-year-old trees was significantly larger than in younger trees starting at 6 months after the inoculation (t-test, P < 0.05). Although the mean value of resinosis in 1-yearold trees was significantly larger than in 2year-old trees at 6 months after the inoculation (t-test, t = 2.54, P < 0.05), the mean value in 1-year-old trees was lower than that in 2-year-old trees at 9 and 12 months, and significantly lower at 18 months after the inoculation (t-test, t = 2.96, P < 0.01). These results suggest that the mean value of resinosis per hinoki clone is positively correlated with the age of the trees.

### 3.3 Relative proportion of infection levels

At 1 month after the inoculation, the relative proportion of clones whose infection levels ranged from 0 to 20% were dominant in all tested trees (Fig. 3). However, the relative proportion of higher infection level increased gradually with time and, at 18 months after the inoculation, the relative proportion of clones ranging from 60 to 80% or 80 to 100% were dominant in each age group (Fig. 3). These results suggest that even if some clones are extremely susceptible to *S. unicorne*, it takes much time for resinosis to appear on the trees.

There were one 1-year-old clone and two 2year-old clones which were 0% infected at 18 months after the inoculation.

# 3.4 Relationship between infection level and value of resinosis

Figure 4 indicates that the mean value of resinosis per hinoki clone increases with infection levels in each period after the inoculation. Especially, clones with an infection level of 80 to 100% consistently had a higher value of resinosis than those of any other infection level in each period. This result suggests that the infection level is highly correlated with the intensity of infection (value of resinosis) by *S. unicorne*.

### **4** Discussion

The fact that both the proportion of the infection by *S. unicorne* and value of resinosis



Fig. 3 Relative proportion of infection levels in each age group. The 6 levels of the proportion of infection are as follows:  $0.0 < \le 20$ ,  $20 < \sim \le 40$ ,  $40 < < \le 60$ ,  $60 < \sim \le 80$ ,  $80 < \sim \le 100$  (=100) (%).

at 18 months were higher than at 12 months after the inoculation (Figs. 1 and 2) indicates that resinosis should be observed until 18 months after the inoculation to determine precisely the resistance of hinoki trees to the disease. Although further observations could reveal an increase in the proportion of *S. unicorne* infection (due to the slower infection rate of the disease) (Fig. 3). they would be pointless because testing should be done as early as possible.

Another problem concerns the most suitable age of hinoki trees for the inoculation test. Although the infection rate in 3-year-old trees by S. unicorne is faster than in the other age groups (Fig. 1). there is no difference in the mean proportion of the infection between the age groups at 18 months after the inoculation (Fig. 1). Therefore, it is likely that using 1year-old trees is the most suitable for the inoculation test because it takes less time to cultivate those seedlings. However, if not only





Fig. 4 Changes in the relationship between mean value of resinosis per hinoki tree and infection levels in each clone. Vertical bars indicate  $\pm$  SE. The 5 levels of the proportion of infection are as follows:  $0 < \sim \le 20$ ,  $20 < \sim \le 40$ ,  $40 < \sim \le 60$ ,  $60 < \sim \le 80$ ,  $80 < \sim \le 100$  (= 100) (%).

proportion of the infection by *S. unicorne* but also value of resinosis on a tree are used as anindex of resistance to the disease, it is likely that 3-year-old trees would be a more suitable indicator because there is a great difference in the value of resinosis between susceptible and less susceptible clones (Fig. 4). To resolve this problem, it is necessary to investigate the relationship between the intensity of susceptibility (value of resinosis) and the proportion of trees whose terminal parts are broken or dead.

The present study indicates that most elite hinoki tree clones are susceptible to *S. unicorne* (Figs. 1 and 3). However, it is noteworthy that there were one 1-year-old clone and two 2-year-old clones which were 0% infected at 18 months after the inoculation. (Fig.3). Yet, it may be impossible for forestation because very few number of elite hinoki tree clones can not reveal forest stand with high genetic variation. Therefore, it is necessary to do further examination of other elite hinoki tree clones or to use less susceptible clones for forestation. Moreover, it is not clear whether the seemingly less susceptible clones actually do have resistance to the disease. Hence, further inoculation tests were conducted on these clones in May 1995. Identification of hinoki clones resistant to S. unicorne and the use of such clones for forestation will need more time.

### Acknowledgements

I wish thank Mr. T. HANDA for providing hinoki seedlings. I am also grateful to the staff of the Forestry and Forest Products Research Institute Kansai Research Center for providing *S. unicorne*.

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# ヒノキ樹脂胴枯病に対するヒノキ精英樹の抵抗性の比較

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要旨:ヒノキ樹脂胴枯病に対するヒノキ精英樹の抵抗性を比較するために、林木育種センター関西育種場に おいて1,2,および3年生の接ぎ木203クローンにこの菌の接種を行った。全体の感染率は、接種後6 カ月までは急激に増加したが(およそ70%)、その後は18カ月目までは徐々に増加した(およそ80%)。

3年生の苗木への感染スピードは、他の年生への感染スピードよりも早かった。感受性の指標である病班 数は、調査時期とともに増加していき、3年生の苗木で最も多かった。各クローンでの感染レベルは病班数 と相関が高かった。

接種後18カ月で全く病班の見られなかったクローンが3つあった。

これらの結果から,ほとんどのヒノキ精英樹はヒノキ樹脂胴枯病に対して感受性であることがわかり,こ の菌に抵抗性のある精英樹を造林に用いるためにはさらなる研究が必要である。