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Role of birds in dissemination of the thread blight disease caused by *Cylindrobasidium argenteum*

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The thread blight disease of broad leaved trees caused by *Cylindrobasidium argenteum* occurred in laurel forests over 20 yr old after clear cutting in Kyushu. Finding fruit bodies of the causal fungus was possible but very difficult in the forests. In inoculation tests using spores and infected branches harvested in the forests, we succeeded in transmission only with branches. In the forests, the disease is transmitted almost entirely by contact with infected branches. During 1989 to 1992 we found four brown-eared bulbul nests containing branches infected with the disease. In one nest, mycelia of the fungus had been transmitted to the host tree. The occurrence of the disease in forests over 20-yr-old was considered to be due to the nesting of birds in trees which provided the necessary height and closed environment.

Key Words——bird nests transmission; brown-eared bulbul; *Cylindrobasidium argenteum*; *Hypsipetes amaurotis*; thread blight disease.

In lowland areas of Kyushu, one of the four main islands of Japan, there are vast warm-temperate evergreen broad-leaved forests called laurel forests. In the forests, a succession of canopy tree species, represented by *Castanopsis cuspidata* Schottky, *Quercus gilva* Blume, *Distylium racemosum* Sieb. et Zucc., *Machilus thunbergii* Sieb. et Zucc., occur with the passage of time after clear cutting. These forest trees have been harvested repeatedly every 30 to 80 yr by means of clear cutting and used for various purposes such as lumber and pulp (Taoda, 1989). Therefore, there are secondary forests of various ages, from those which have just been cut to those with no record of cutting for more than 160 yr in Minamata City, Kumamoto Prefecture; Okuchi, Sendai City, Kagoshima Prefecture and Aya Cho, Miyazaki Prefecture. In those secondary forests, *C. cuspidata* constructs canopy about 15 to 80 yr after clear cutting, but later it declines yr by yr (Taoda, 1989).

The thread blight disease fungus infects branches and trunks of trees and shrubs including *Quercus glauca* Thunb., *C. cuspidata*, *D. racemosum* and *Eurya japonica* Thunb. (Ito, 1951; Kobayasi, 1971), and finally causes stem break and collapse of the infected trees. The causal fungus of the thread blight disease was first identified as *Cyphella pulchra* Berkeley et Broome (Ito, 1951), but Kobayasi (1971) described it as a new species *Corticium argenteum* Y. Kobayashi. Maekawa (1993) recently proposed *Cylindrobasidium argenteum* (Y. Kobayasi) Maekawa comb. nov. for the fungus. The northernmost extent of the thread blight disease is Chiba Prefecture on Honshu island (Ito, 1951) and its distribution stretches south all the way to Iriomote island in Okinawa Prefecture (Kobayasi, 1971). Usually the disease is transmitted to healthy trees through contact with infected branches (Ito, 1951). The ultimate purpose of our study is to clarify how the thread blight disease is related to the declining process of *C. cuspidata* after canopy formation. But in this paper, we report the unique mode of dissemination of the disease and discuss the ecological role of the vector.

Materials and Methods

Field observations To examine the relationship between the rate of infection and forest age, sites were selected in forests of various ages located in Minamata, Okuchi, Sendai and Aya. About 1,000 trees more than one meter in height in each research site were inspected to see whether or not they had been infected with the thread blight disease. Inspections were done on March 5, October 2–4, 14–16, 21 and 22, 1991; March 2, 9 and 10, April 2, July 9, September 27, 1992; and February 23 and 24, 1993. In the course of inspection, we noticed many new infections showing expansion of white mycelia from an old infected branch to a new host tree.

To confirm the major mode of transmission of the disease, every infected tree in a 25 m^2 plot set up in an 85-yr-old forest in Okuchi was marked with a plastic tag in March 1992. Additional transmissions, if any, in the plot were recorded in September 1992 and February 1993.

To explain why the disease occurred in forests over 20 yr old, we advanced two hypotheses: 1) strong winds such as typhoons convey infected branches from reserved forest areas that are situated along ridges; 2) birds convey branches infected with the pathogen to trees for nest-building. Searches for bird nests were carried out in the same sites and at the same times as described above. Additional searches for bird nests were done in Minamata, Okuchi, Sendai City, Kagoshima Prefecture and in Ebino City, Miyazaki Prefecture, in 1989 and 1990. At the same time, the geographical features of each site and its peripheral areas were observed in the forests and by using management maps published by Forestry Agency.

Inoculation tests To determine if the disease can be transmitted by spores, inoculation tests were conducted at the Kyushu Research Center, Forestry and Forest Products Research Institute, 4-11-16, Kurokami, Kumamoto Prefecture. Ten seedlings of *C. cuspidata* and 10 of *D. racemosum*, planted in pots about 50 cm high and 7–8 mm in diam at the base, were used in both contact and spore inoculation tests. Cuttings (50 × 8 mm) of infected branches of *Camellia japonica* L. harvested in Okuchi were used as inocula for contact inoculation tests. As a control, cuttings of the same size taken from healthy branches were used. Cuttings were bound to stems of seedlings with cotton thread (Fig. 6).

For spore inoculation tests, basidiospores produced from infected branches (Fig. 7) were collected and macerated in a mortar with 1.2% glucose solution. Spore suspension adjusted to 1,000 spores/ml was sprayed onto seedlings. As a control, 1.2% glucose solution was sprayed. Both the spore-inoculated seedlings and the control seedlings were kept in high humidly for 1 wk by covering them with plastic bags.

All of the inoculated seedlings in pots were transferred to the shade of tall trees and watered once a week for further observation.

Results

Field observations The relationship between the rate of infection by the thread blight disease and forest age is shown in Fig. 1. Infected trees appeared in forests at least 20 yr after clear cutting. The rates of infection were higher in stands near streams than in other stands.

A total of five bird nests including four of the brown-eared bulbul (*Hypsipetes amaurotis* Temminck) were found between 1989 and 1992. One nest was at an inaccessible height on the tree, so we could neither determine the bird species of the nest nor gather any data. Branches infected by the thread blight disease were contained in all brown-eared bulbul nests (Figs. 2, 4, 5). Two of the four bulbul nests had fallen to the ground after typhoon number 19 in 1991, and both contained branches infected with the thread blight disease and also mycelia of the genus *Marasmius* (Fig. 2). In one nest remaining on a living tree (*Q. glauca*) at approximately 7-8 m in height, mycelia of the thread blight disease had been transmitted from nest twigs to the branches of the nest tree (Fig. 4). In another nest built in a deciduous broad-leaved tree, infected branches were observed but we could not see whether mycelium had been transmitted to the tree (Fig. 5).

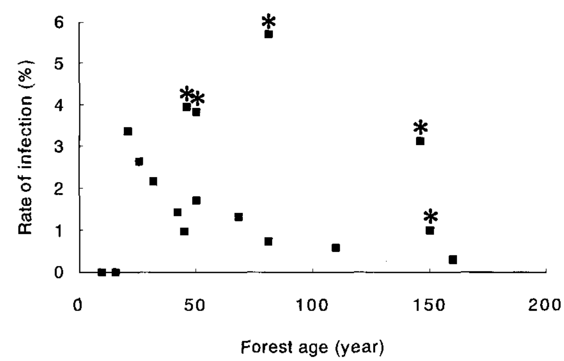
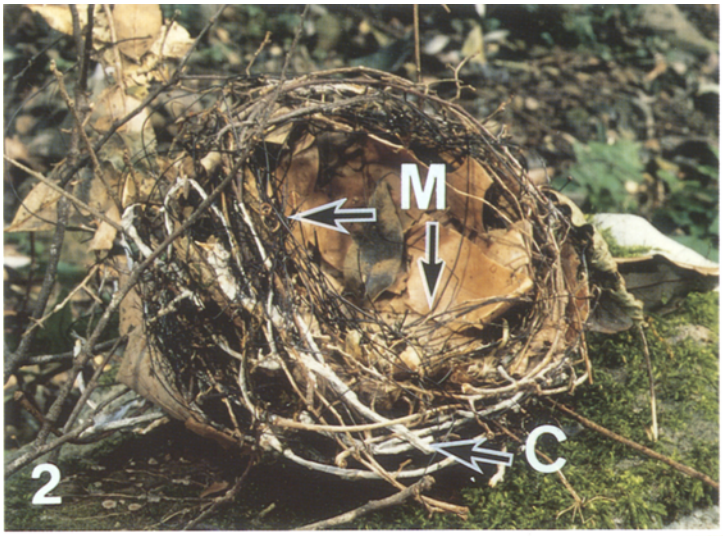


Fig. 1. Relationship between the rate of infection and the age of forest.
Only trees of more than one meter in height were inspected. Stars show the rate in stands near streams.

In the plot set up in Okuchi, 42 infected trees and shrubs including small seedlings were recorded in March 1992, and 30 of these showed traces of contact transmission. Cause of transmission was unclear in the other 12. In September 1992, 14 new transmissions were observed, all of them due to contact. An additional 7 new transmissions were observed in February 1993, all as a result of contact.

Inoculation tests We regarded as infection the observation of new growth of mycelia from the inocula to the inoculated seedlings. Six months after the inoculation, all inoculated stems were inspected for infection. Results of inoculations are summarized in Table 1. Infection occurred in most of the contact inoculations using infected branches (Fig. 6), but not in the spore inocula and controls.

Fig. 2. A nest fallen to the ground after a typhoon. The nest material contained branches infected with the thread blight disease (C) and mycelia of *Marasmius* (M).
Fig. 3. Stem break of *C. cuspidata* at the point of infection with the thread blight disease.
Fig. 4. Expansion of mycelia (arrow) from a removed nest to healthy branches of *Q. glauca*.
Fig. 5. A nest built on a deciduous broad-leaved tree having branches infected with the thread blight disease (arrow).
Fig. 6. Dead stem of *D. racemosum* seedling after contact inoculation using a naturally infected branch.
Fig. 7. Fruit bodies of *C. argenteum* produced on infected dead branch.
Fig. 8. Contact transmission of the thread blight disease commonly observed in the forests.


M
C
2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5


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7


8

Table 1. Inoculation tests using spores and infected branches of *C. argenteum*.

Inoculum	Inoculated plant	
	C. cuspidata	*D. racemosum*
Spores	0[a)]/10[b)]	0/10
Control (1.2% glucose solution)	0/10	0/10
Infected branches	9/10	10/10
Control (Healthy branches)	0/10	0/10

a) Number of infected seedlings.
b) Number of inoculated seedlings.

Discussion

The results of inoculation tests showed that the disease is not readily transmissible by spores but only by mycelia on infected branches (Table 1). The results do not rule out the possibility of disease dissemination by spores (Sanui and Kuroki, 1995), but we conclude that in most cases the disease is disseminated by contact with infected branches. This conclusion is partly supported by the following inspection. Despite close inspection of several hundred infected trees during 1986–1992, fruit bodies of *C. argenteum* were located on only three occasions (unpublished data). We observed the thread blight disease in many secondary forests that did not have reserved forest areas in close proximity, so it was thought unlikely that infection could have been initiated by wind-blown infected twigs. We found evidence of transmission of the disease from bird nests (Figs. 2, 4, 5) through observations in the forests. The trees in forests over 20-yr-old after clear cutting had gained sufficient height and density of canopy to provide favorable nesting sites.

Many insect vectors are known to be involved in the occurrence of tree disease (Manion, 1981), but only a few examples of vertebrate vectors are known, e.g., squirrels transmitting the oak wilt fungus, *Ceratocystis fagacearum* (Bretz) Hunt (Engelhard and Bragonier, 1960; Himelick et al., 1953) and birds carrying the chestnut-blight fungus, *Cryphonectria parasitica* (Murrill) Barr (Heald and Studhalter, 1914). Birds were presumed to be conveyors of the fungus *Marasmius* causing the hair blight disease of tea plants by building their nests in the plants, but no evidence was provided (Petch, 1923). Transmission via infected branches contained in bird nests is a unique mode of fungal dissemination.

The role of fungi inhabiting forests has been emphasized by mycologists (Imazeki, 1978), and fungal involvement in gap formation (Imazeki and Aoshima, 1955; Taoda, 1987) or enhanced growth of host trees due to the symbiont fungi (Marx and Bryan, 1975) has been reported. Inoculated *C. cuspidata* branches less than 10 mm in diam were killed within 130 d in summer in highly humid conditions (Kusunoki et al., 1992). The higher rate of infection in stands near streams or lakes is considered to be due to the highly humid conditions. In larger *C. cuspidata* of ca. 200 mm d.b.h., mycelia of *C. argenteum* had expanded on the surface of the bark about 80 cm longitudinally after 51 mo, and had grown all around the girth of the tree trunks. Near the point of inoculation, cambia of the host trees had been killed and sapwood tissue had been decayed in the early days of inoculation (Kusunoki et al., 1992). Later, the fungus causes stem rot and subsequent death followed by stem break and falling of the infected trees (Fig. 3). *Castanopsis cuspidata* is generally reported to be susceptible to invasion by wood rot fungi (Taoda, 1984). Besides the thread blight disease, a trunk rot disease is known which also induces decline of *C. cuspidata* (Kawabe et al., 1988). But the trunk rot disease is not distributed as widely as the thread blight disease. The causal fungus of the thread blight disease is also considered to contribute to gap formation and influence the succession of tree species.

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