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A note on the discoloration and fungal infiltration processes on wood tissues surrounding the gallery system of scolytid beetles

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Abstract

The discoloration and fungal infiltration processes on wood tissues surrounding the galleries in a red pine log excavated by the ambrosia scolytid beetle, *Xyleborus varidus* EICHHOFF, were examined histologically under light microscopy. Many fungal hyphae and spores were found in a dark-colored area surrounding the gallery. The observations suggested that the lesions on wood tissues resulting from gallery construction by the scolytid beetle would cause discoloration associated with secondary resinosis and enhance subsequent rapid invasion of externally comtaminating fungi into the sapwood.

Key words : scolytid beetles, gallery, discoloration, fungal infiltration, wood tissue, resinosis.

I. Introduction

Most of the scolytid beetles attack stressed trees and wood during thinning and felling operations, and breed in their phloem (bark beetles) or in sapwood (ambrosia beetles) parts (BEAVER, 1989). Many species of these beetles are also known to be closely associated with fungi in their galleries. The ambrosia beetles, in particular, bore deep into the sapwood, making specific gallery systems (PREBBLE and GRAHAM, 1957) for cultivation of the ectosymbiotic "ambrosial fungi" which constitute the major part of food resources of both adults and larvae (*e.g.*, BATRA, 1966; NAKASHIMA, 1971; BERRYMAN, 1989; KINUURA *et al.*, 1991). The infestation of the fungi by the beetles, as well as their deep tunnelling, give rise to degradation in quality of logs and timber : the penetration of fungal hyphae into the wood tissues surrounding galleries and the dark discoloration most likely induced by fungi cause severe loss in the economic value (BORDEN, 1988). Galleries from which the beetles have emerged or those abandoned for some reason are also highly vulnerable to invasion by other contaminating fungi.

In the present brief report, we reveal histologically part of the discoloration and fungal infiltration processes on wood tissues surrounding the galleries excavated by the ambrosia beetle, *Xyleborus validus* EICHHOFF, in a red pine (*Pinus densiflora* SIEB. *et* ZUCC.) log, by means of light microscopy.

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II. Materials and Methods

A scolytid-infested red pine log (15yr-old; 45 cm long and 22 cm in diameter), was collected in July 1988 in the Nagoya University Forest at Inabu, Aichi Pref. The tree was felled in early spring 1988 and subsequently infested with scolytid beetles. Most of the beetles bored into the log were dominated by the ambrosia beetle, *X. validus*, followed by other bark beetles such as *Cryphalus fulvus* NIIJIMA, *Orthotomicus angulatus* (EICHHOFF), *Hylurgops interstitialis* (CHAPUIS).

For light microscopy, small wood blocks (20×20×20 mm) containing active galleries were taken from the log in late October 1988, when most of the beetles entered hibernation. The blocks were sampled only from the part near the entry holes of the galleries, where no beetles stayed around. The block samples were trimmed into thin sections (20 -50 μm) with a microtome at radial, transverse, or tangential face, and subsequently stained with picro-aniline blue, safranine, and fast-green solutions alternately. All the sections were subjected to repeated dehydration treatments with ethanol and xylene prior to enclosure in balsam. The multi-staining treatment facilitated differentiation of infiltrated fungal hyphae from wood tissues for the microscopy.

III. Results and Discussion

X. validus (Fig. 1) constructs the "forked" mother gallery system, which originates in an entry hole and infiltrates into the sapwood towards the heartwood, ramifying a few branch tunnels horizontally (Fig. 2). Attack density on the present log was considerably

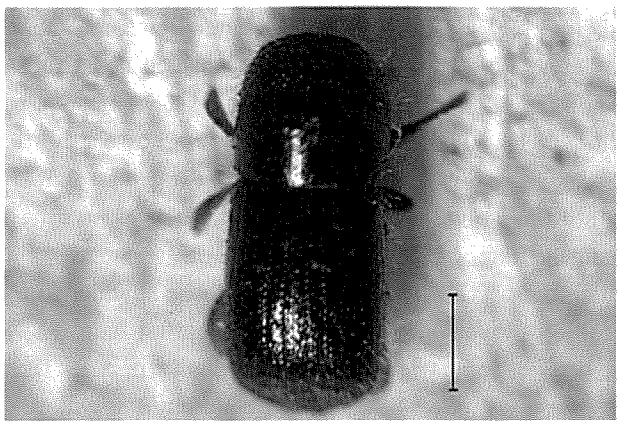

Fig. 1. Adult of the ambrosia beetle, *Xyleborus validus*. Bar=1 mm.

high, reaching 12-13 per 100 cm^2-bark surface area. Cross section of the mother gallery at tangential face is shown in Figure 3, which demonstrates that damaged tissues surrounding the gallery are intensively stained dark-brown with resin-like substances. A light-brown discoloration spreads wood tissues surrounding the heavily stained (dark-

brown) parts along the direction of the axis, where no fungal hyphae and spores could be found.

A magnified detail of broken and discolored wood tissues around the tunnel is given

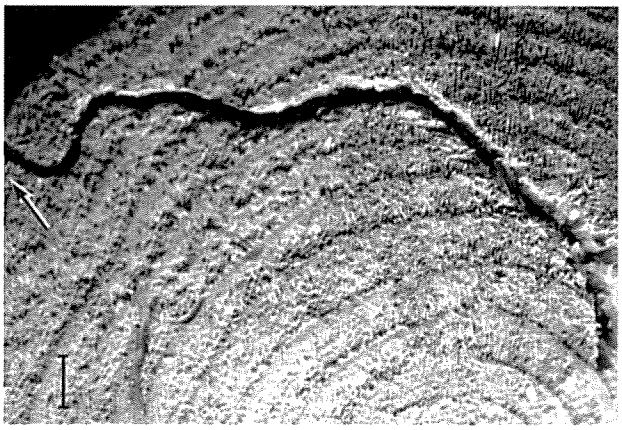

Fig. 2. Mother gallery bored into the sapwood of a red pine log. Arrow shows the entry hole of the gallery. Bar=10 mm.

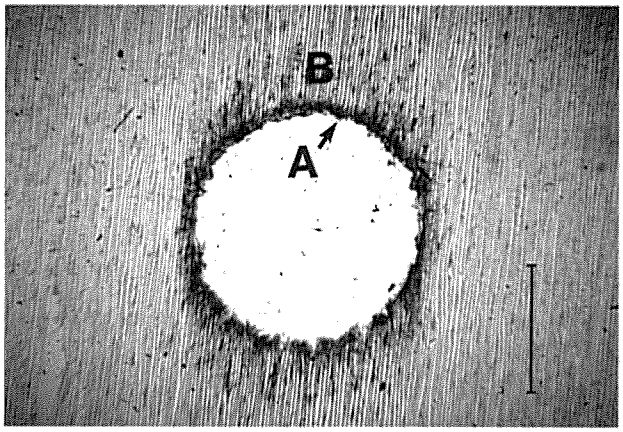

Fig. 3. Cross section of the mother gallery (tangential face : 20×), showing longitudinal discoloration of the wood originating the tunnel. In a dark-brown discoloration area (A), both masses of resin and fungal hyphae and spores are found in the wood tissues. No fungi occur over a light-brown discoloration area (B). Bar=1 mm.

in Figure 4. This figure shows that dark stains, which seems to be induced by secondary resinosis, concentrate exclusively in ray parenchyma cells, which is also shown by Figure 5 taken at transverse face. In Figure 5, the dark stains are observed significantly in ray parenchyma cells and damaged wood tissues surrounding the gallery. Figure 5 also shows that some tracheids adjacent to the rays were filled with fungal spores.

Invading processes of the fungi into normal wood tissues could be explained in part

by Figures 6-8. Fungal hyphae originating in the broken-down tissues around the gallery infiltrate actively into tracheids through pitting, ramifying and making several masses of spores (Fig. 6). Figure 7 is an enlarged view of a fungal hypha that penetrated into an adjacent tracheid through a bordered pit. This process is also demonstrated by Figure

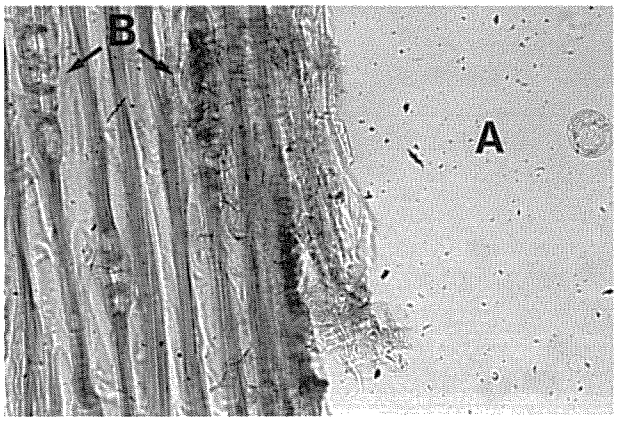

Fig. 4. Crushed and discolored wood tissues around the mother gallery (A) resulting from boring by the beetle (tangential face : 100×). Dark staining induced by secondary resinosis can be observed more significantly in ray parenchyma cells (B) than in tracheids.

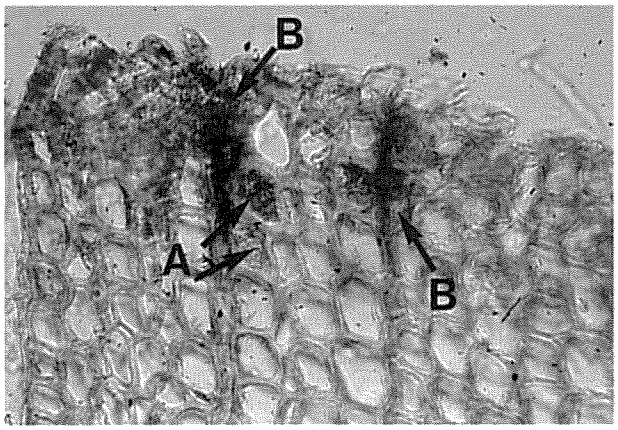

Fig. 5. Transverse section of the wood tissues near the gallery (cross face : 200×). Fungal spores are tightly packed in tracheids (A). Secondary resinosis causing dark staining are found exclusively in ray parenchyma cells (B).

8 taken at tangential face. Figure 9 shows one of spore masses in a tracheid, which are frequently observed also in other tracheids (Fig. 6).

The fungal species could not be identified in our present study, but it may probably belong to species of wood-decay fungi. Most wood-decay fungi are capable of penetration through the cell walls of wood tissues and the rate of penetration is closely

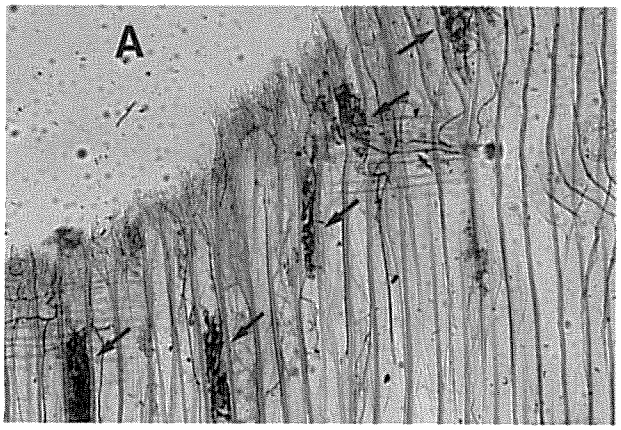

Fig. 6. Radial face of the wood near the gallery (A). Arrows show fungal hyphae and spores in tracheids.

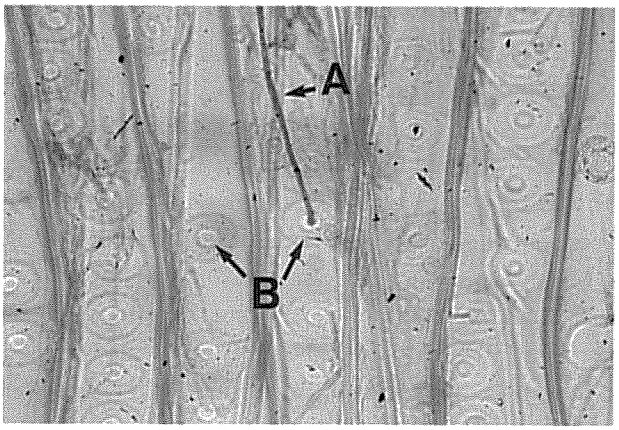

Fig. 7. Fungal hyphae (A) extending through bordered pits (B) (radial face : 400×).

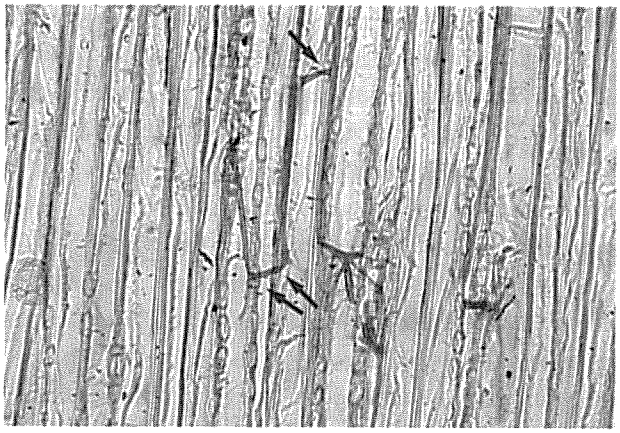

Fig. 8. Fungal hyphae extending across tracheids through bordered pits (arrows) (tangential face : 100×).

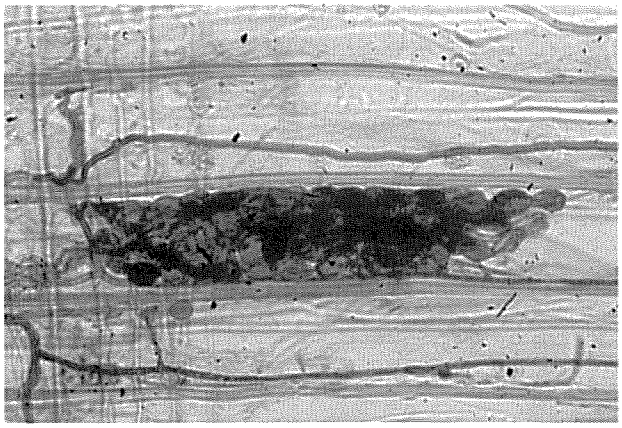

Fig. 9. Fungal-spore mass and hyphae in tracheids (radial face : 400×).

correlated with the ability to degrade cellulose (COOKE and RAYNER, 1984). The present fungus extended its hyphae exclusively through bordered pits, which suggests that it was one of the species with relatively lower cellulolytic abilities.

Discoloration spreading wood tissues has been understood as a physiological resistance of trees to attack by scolytid beetles and their fungal associates adhering to external body parts of the beetles (MOLNER, 1965; REID *et al.*, 1967). In vigorously growing trees, in particular, the invasion by both scolytids and fungi into the stem generally elicits a severely resinous resistance of the tissue that hinders further beetle colonization and fungal penetration (*e.g.*, REID *et al.*, 1967; BERRYMAN, 1969; RAFFA and BERRYMAN, 1983; RAFFA and SMALLEY, 1988). However, such primary resinosis (REID *et al.*, 1967), a resistance to the boring stimulus by scolytid beetles or to the progressive penetration of pathogenic fungi into sapwood, would be little expected in fallen or felled trees. In such trees, secondary resin originated within living parenchyma cells of the sapwood is likely to be more important in discoloration of wood tissues (REID *et al.*, 1967).

Besides their mutualistically associated fungi, ambrosia beetles, as well as bark beetles, often carry other fungal pathogens of trees and/or bacteria into their gallery systems (*e.g.*, WHITNEY, 1982; BEAVER, 1989). The successful growth and dominance of their symbiotically associated ambrosial fungi would be ensured only at the egg niches or larval cradles whose conditions are well controlled by the adult beetles, where other fungi, in most cases pathogenic or wood-decay fungi, are likely to be elimitated (*e.g.*, BEAVER, 1989). However, wood tissues surrounding galleries on which no adult beetles stay, or old galleries without tending by adult beetles, would be highly vulnerable to attack by pathogenic or wood-decay fungi as seen in the present report.

The lesions on wood tissues resulting from the beetles' tunnelling cause discoloration of the wood tissues associated with secondary resinosis and enhance subsequent rapid invasion by external pathogenic or wood-decay fungi into the sapwood through tracheids, both of which would cause combined damages on the quality of timber and logs.

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References

BATRA, L. R. : Ambrosia fungi : extent of specificity to ambrosia beetles. Science **153** : 193-195, 1966.

BEAVER, R. A. : Insect-fungus relationship in the bark and ambrosia beetles. *In* Insect-fungus interactions (WILDING, N., COLLINS, N. M., HAMMOND, P. M., and WEBBER, J. F., eds.). 121-143, Academic Press, New York & London, 1989.

BERRYMAN, A. A. : Responses of *Abies grandis* to attack by *Scolytus ventralis* (Coleoptera : Scolytidae). Can. Entomol. **101**: 1033-1041, 1969.

——— : Adaptive pathways in scolytid-fungus associations. *In* Insect-fungus interactions (WILDING, N., COLLINS, N. M., HAMMOND, P. M., and WEBBER, J. F., eds.). 145-159, Academic Press, New York & London, 1989.

BORDEN, J. H. : The striped ambrosia beetle. *In* Dynamics of forest insect populations - patterns, causes, implications (BERRYMAN, A. A. ed.). 579-596, Plenum Press, New York & London, 1988.

COOKE, R. C. and RAYNER, A. D. M. : Ecology of saprotrophic fungi. 416pp, Longman, London & New York, 1984.

KINUURA, H., HIJII, N., and KANAMITSU, K. : Symbiotic fungi associated with the ambrosia beetle, *Scolytoplatypus mikado* BLANDFORD (Coleoptera : Scolytidae) -succession of the flora and fungal phases in the gallery system and the mycangium in relation to the developmental stages of the beetle-. J. Jpn. For. Soc. **73**: 197-205, 1991.

MOLNER, A. C. : Pathogenic fungi associated with a bark beetle on alpine fir. Can. J. Bot. **43** : 563-570, 1965.

NAKASHIMA, T. : Notes on the associated fungi and the mycetangia of the ambrosia beetle, *Crossotarsus niponicus* BLANDFORD (Coleoptera: Platypodidae). Appl. Entomol. Zool. **6**: 131-137, 1971.

PREBBLE, M. L. and GRAHAM, K. : Studies of attack of ambrosia beetles in softwood logs on Vancouver Island, British Columbia. For. Sci. **3**: 90-112, 1957.

RAFFA, K. F. and BERRYMAN, A. A. : Physiological aspects of lodgepole pine wound responses to a fungal symbiont of the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera : Scolytidae). Can. Entomol. **115**: 723-734, 1983.

——— and SMALLEY, E. B. : Seasonal and long-term responses of host trees to microbial associates of the pine engraver, *Ips pini*. Can. J. For. Res. **18**: 1624-1634, 1988.

REID, R. W., WHITNEY, H. S., and WATSON, J. A. : Reactions of lodgepole pine to attack by *Dendroctonus ponderosae* HOPKINS and blue stain fungi. Can. J. Bot. **45**: 1115-1126,

1967.

WHITNEY, H. S.: Relationships between bark beetles and symbiotic organisms. *In* Bark beetles in North American conifers (MITTON, J. B. and STURGEON, K. B., eds.). 183-211, Univ. of Texas Press, Austin, 1982.

キクイムシ坑道周辺の木材組織にみられる変色および菌の侵入様式について

肘井直樹・梶村　恒・西部雄司

伐倒直後のアカマツ材に穿孔したキクイムシの一種，トドマツオオキクイムシの坑道について，その形状，および穿孔によってひきおこされたと考えられる坑道周辺組織の変色と材内への菌の侵入様式を，光学顕微鏡によって観察した。最も強い変色は，坑道周辺の放射柔細胞から浸出した二次性樹脂によるものと思われ，この変色域では菌の菌糸，胞子も多数観察された。菌糸は障害を受けた組織周辺から有縁壁孔を通って活発に伸長しており，坑道に最も近いところでは，多くの仮導管内に胞子塊が認められた。キクイムシによる坑道形成は，樹脂浸出がもたらす材の変色のみならず，木材腐朽菌を含む外部からの菌の侵入と増殖を容易にするという点からも，木材生産上重要な意味を持つものと考えられる。