Original Article

Infection and propagation of *Perenniporia subacida* in stands of *Chamaecyparis obtusa* (Japanese cypress)

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Summary

The incidence of butt rot and the invasion of *Perenniporia subacida*, the pathogen of butt rot of *Chamaecyparis obtusa* (Japanese cypress, hinoki in Japanese), were studied at 30-yr-old, 38-yr-old, and 43-yr-old hinoki stands in Kagawa Prefecture, Japan. Butt rot was found in 53%, 28%, and 49% of the trees in the 30-yr-old, 38-yr-old, and 43-yr-old stands. *Perenniporia subacida* was frequently isolated from the decayed wood of roots and the tissues of white mycelial mats on the bark of roots. A somatic incompatibility test of isolates showed there were at least five genets of *P. subacida* and the fungus spread to neighboring trees by mycelial growth through root contacts. Wounds which caused decay were classified into the following 6 types: 1) mutual root contacts, 2) stones, 3) root-feeding of larvae of *Mimela costata*, 4) small-size forwarder, 5) cracks of swelling, and 6) decayed absorbing root. Most wounds associated with butt rot were formed at the base of the stumps and in roots less than 36cm below ground level. These wounds were caused by the root-feeding of the chafer, root contacts, and stones.

Key Words: butt rot, *Chamaecyparis obtusa*, distribution, infection, *Perenniporia subacida*

Introduction

Chamaecyparis obtusa (Sieb. et Zucc.) Endlicher (Japanese cypress, hinoki in Japanese), has been extensively planted, from Kyushu to Tohoku District in Japan. Hinoki plantations represent about 23% of the artificial forest area in Japan and butt rots are sometimes found in these plantations. *Tinctoporellus epimiltinus* (Berk. et Br.) Ryv. is one of the fungi that causes butt rot in hinoki in the Kyushu District of Japan (Katsu 1971; Kubayashi et al. 2001). In 1997, butt rot was found at a hinoki plantation in Kagawa Prefecture where thinning had taken place. Tabata et al. (2002) conducted a field survey, identified the pathogenic fungus, and described the damage of hinoki by *Perenniporia subacida* (Pk.) Donk.

Perenniporia subacida is one of the most common and widespread polypores in Asia, Europe, and North America (Ryvarden and Gilbertson 1994). In Asia, it is found in Japan, Russia, and China (Aoshima 1950; Bondartsev 1971; Zhao 1998). The fungus causes white stringy rot in dead conifers and hardwoods, and butt rot in living trees (Buckland 1946; Gilbertson and Ryvarden 1987; Hansen and Lewis 1997; McCallum 1928; Tabata et al. 2002; Whitney 1995).

Kubayashi (1997) studied the infection route of butt rot fungi in the stands of hinoki and found wounds by the root-feeding of the larvae of a kind of chafer and decayed absorbing root as an invasion site of the fungi. We also studied the route of *P. subacida* and found root-feeding of larvae of *Mimela costata* Hope (larger striated chafer), wounds of mutual root contacts, stones, root-feeding of the chafer, small-size forwarder, and decayed absorbing roots as the invasion site of the fungus (Tabata et al. 2002, 2004, 2009). There is, however, a scarcity of information on infection and distribution of the fungus in the hinoki stands. We therefore conducted a field survey on the decay damage and the rot-infected stumps at the 30-yr-old, 38-yr-old, and 43-yr-old hinoki stands, and studied the genets at the stands.

Material and methods

Study site

The study was carried out in the 30-yr-old (site A), 38-yr-old (site B), and 43-yr-old (site C) hinoki stands in northern Kagawa Prefecture from where Tabata et al. (2002, 2004) had reported the damage. Site A formerly was a broad-leaved forest but was cleared and replaced by a hinoki plantation around 1923. Hinoki was harvested in 1964 through 1968 and replanted in 1969 at a density of 4,000 trees/ha. The site is located at an elevation of about 650 m at a slope of 10-21°.

Site B is 800 m away from site A and formerly was a broad-leaved forest but was cleared and replaced by a hinoki plantation around 1925. Hinoki was harvested in 1963 through 1966 and replanted in 1967 at a density of 3,500 trees/ha. The site was located at an elevation of about 670 m at a slope of 11-15°.

Site C is 40 m away from site A and formerly was a *P. densiflora* forest, cut and replanted with hinoki in 1958 at a density of 3,500 trees/ha. The site was the same elevation as site A on a slope of 4-10°.

In the area, the mean annual precipitation is about 1,200 mm/year and the average temperature is about 12°C. The sites are covered by a moderately moist brown forest soil (drier subtype: B_D(d)) according to the Japanese forest soil classification system (Forest Soil Division 1976). The soil has a shallow topsoil (A horizon) with a thin litter layer, and a compact subsurface soil (B horizon) of heavy clayey texture. The subsurface soil is not well drained and less aerated.

Field surveys

Two hundred and nineteen of the 30-yr-old trees, 114 of the 43-yr-old trees, and 100 of 38-yr-old trees were cut in the summers of 1999, 2001, and 2004, respectively. When decay was found in a tree, stem disks 2 to 3 cm thick were taken at 10 cm intervals from the bases to the heights where decay could no longer be detected.

Four stumps of the 30-yr-old trees that had been cut in 1999 were randomly selected between November and December 2000, 4 stumps of the 43-yr-old trees cut in 2001 were selected between November and December 2002, and 3 stumps of the 38-yr-old trees cut in 2004 were selected between November and December 2005. Their root systems were excavated by hand up to a 1.2 m radius around the base of the tree and dissected to ascertain whether any wounds were related to the root decay. We examined the shape and size of wounds with root decay and recorded their depth below the ground level.

Isolation and somatic incompatibility test (SIT)

Pieces were taken from decayed stumps and roots of cut trees to isolate fungal strains. White mycelial mats were also collected from stumps and roots of cut trees. Isolation was conducted as described previously, and cultures were identified by their cultural characteristics (Tabata et al. 2002). The cultures isolated from stumps, decayed wood, and mycelial mats were identified by comparing them morphologically with cultures from basidiocarps of the fungus. All cultures showed the same characteristics. As the characteristics were the same in all basidiomycetous cultures, they were considered to represent a single species. Eighty one isolates from site A in the area 30 x 60m, 35 from site B in the area 20 x 60m, and 36 from site C in the area 20 x 60m were used for SIT. The isolates were stored at the Forestry and Forest Products Research Institute.

Two isolates were inoculated 2 - 3cm apart on potato dextrose agar (PDA) plates in 9cm Petri dishes and incubated at 25°C in the dark for 2 - 3 weeks. We examined the presence or absence of a zone line between the isolates after 2 - 3 weeks of incubation. Self-pairings were also tested for each isolates. Two replicates were made for each combination.

Results

Field surveys

After thinning, butt rot was found in 53 %, 28 %, and 49 % of the trees in the 30-yr-old, 38-yr-old, and 43-yr-old hinoki stands. The maximum height of rot was 3.0m aboveground in the 30-yr-old trees. We collected 116 decayed stem disks from 116 trees at site A, 28 decayed stem disks from 28 trees at site B, and 56 decayed stem disks from 56 trees at site C. Two hundred samples of decayed stems were collected from the height of 0.1 - 3 m.

Old hinoki stumps cut in 1994 at site A and C, and in 2000 at site B were found to be colonized by *P. subacida.* White

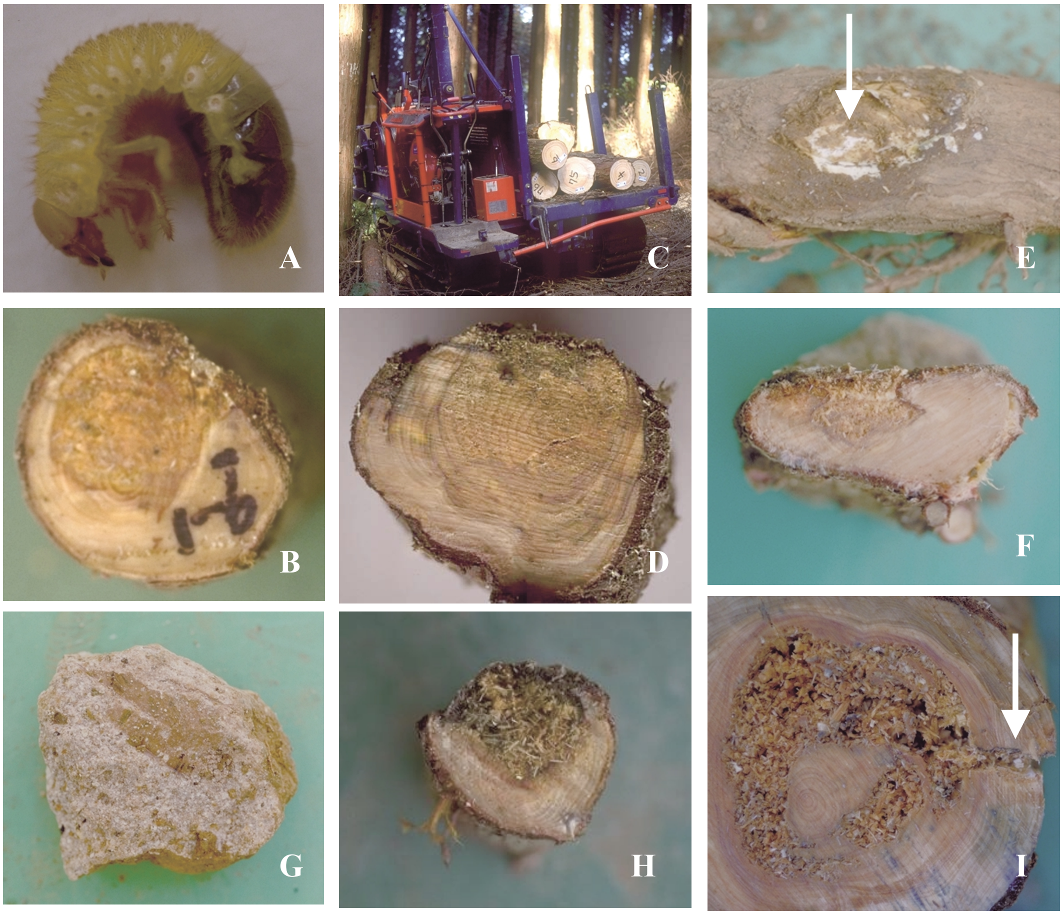

Fig. 1 (A) Larva of root-feeding insect, *Mimela costata.* (B) Cross section of decay from the wound by larva of *M. costata.* (C) Small-size forwarder used for carrying logs. (D) Cross section of decay from the wound by small-size forwarder. (E) Mutual root contact and white mycelial mat spread on the bark of root (arrow). (F) Cross section of decay from the root contact. (G) Stones collected in field survey. (H) Cross section of decay from the wounds by stones. (I) Cross section of decay from the wound by crack (arrow).

mycelial mats were sometimes observed on the bark near the base of stems and on root bark of felled trees as well as living trees. They also spread from the root of decayed stumps to those of neighboring trees through root contact.

Larvae of the root-feeding insect, *Mimela costata* (larger striated chafer), sometimes were found on decaying roots on which they fed, leaving feeding wounds at site A and C (Fig.1A, B). Some roots apparently had been wounded by small-size forwarder used for carrying logs at site A (Fig. 1C, D). Wounds were caused by mutual root contacts or stones at all sites (Fig. 1E, F, G, H). Wounds were also caused by cracks of swelling at site A and C (Fig. 1I). Few absorbing roots were found in the clayey subsurface soil of the high dry bulk density and less soil aeration at site C. Some absorbing roots caused root rot and decay spread from the base of absorbing root to the central part of woody root.

Wounds by stones was higher in number than the other wounds and the rate of the wounds by stones was 72%, 75%, and 33% at site A, B, and C, respectively. Most wounds associated with butt rot were found at the base of the stumps and in roots less than 36cm below ground level. These wounds were caused by the root-feeding of the chafer, root contacts, and stones.

The size of the invasion site of *P. subacida* and its depth below the ground level is shown in Table 1. The size from the wound by cracks was larger than the other wounds (13 x 0.5cm), while that from the wound by the feeding of the chafer was smaller (3.5 x 0.5cm). The wounded-roots by the small-size forwarder was near the ground surface, while the roots by stones had the invasion sites that were deep, that is, 48 - 67cm below the ground level.

Table 1 The size of invasion site of *Perenniporia subacida* and its depth below the ground level

	Size (cm)*			Depth (cm)		
Type of invasion	Site A	Site B	Site C	Site A	Site B	Site C
Wound by small-size forwarder	4.5x1	-	-	7-8	-	-
Wound by root-feeding of chafer	3.5x0.5	-	5 x0.5	8-20	-	14-33
Wound by root contact	5.3x1.5	3.7x1.5	5.9x0.8	8-41	22-53	16-40
Wound by stone	7.1x1.2	6.9x1.6	6.3x1.3	6-48	11-65	9-67
Wound by crack of swelling	8 x0.7	-	13 x0.5	14-30	-	15-50
Decayed absorbing root	-	-	-	-	-	33-48

*: length x width (average)

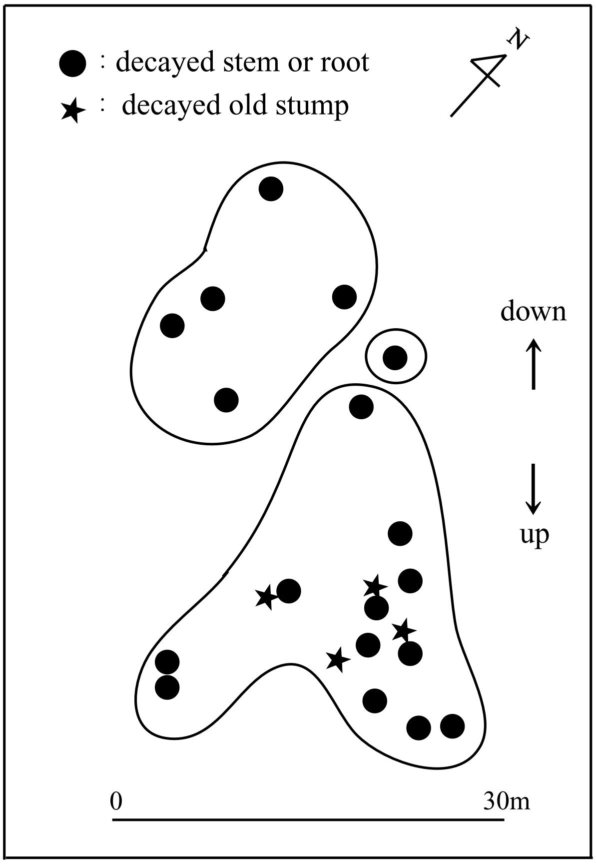

Fig. 2 Spatial distribution of three genets of *P. subacida* inferred from somatic incompatibility test (SIT) at site A.

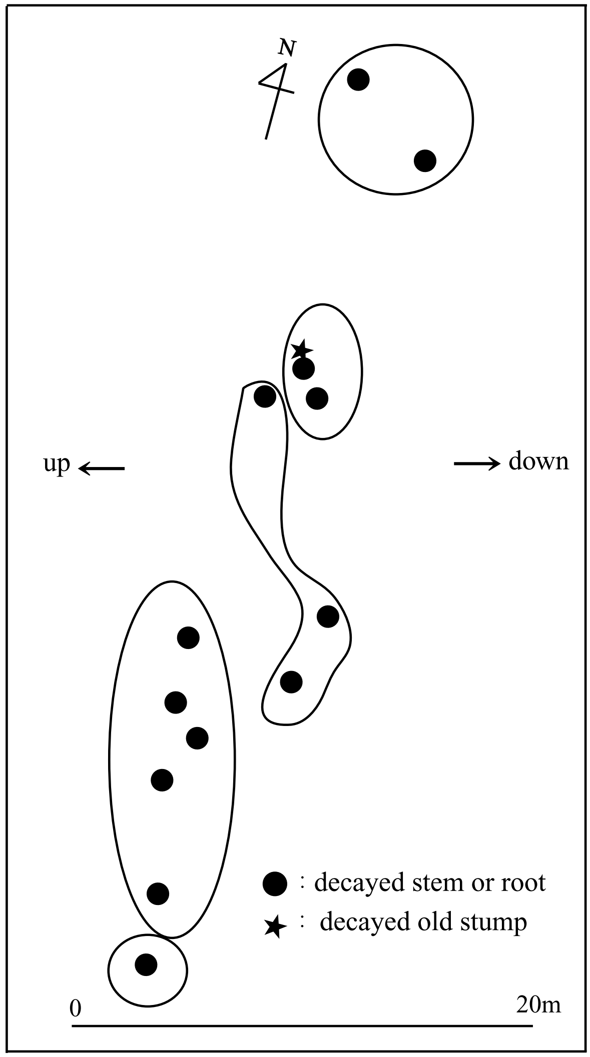

Fig. 3 Spatial distribution of five genets of *P. subacida* inferred from SIT at site B.

Isolation and SIT

White mycelial mats of *P. subacida* were often observed to spread on the bark of roots in almost thinned trees with butt rot. They also spread from the roots of decayed stumps to those of neighboring living trees through root contact. Roots contacts were also observed between roots of thinned trees and decayed old stumps at all sites. The fungus was isolated at a rate of 30 - 100% from the decayed wood of roots and the tissues of white mycelial mats on the roots.

Clear zone lines indicating somatic incompatibility were formed in some pairings, while they were absent in others, suggesting somatic compatibility. The 81 isolates from site A, 35 isolates from site B, and 36 isolates from site C were divided by three, five, and two groups by SIT. The spatial distribution of the three genets at site A is shown in Fig.2. One genet was found

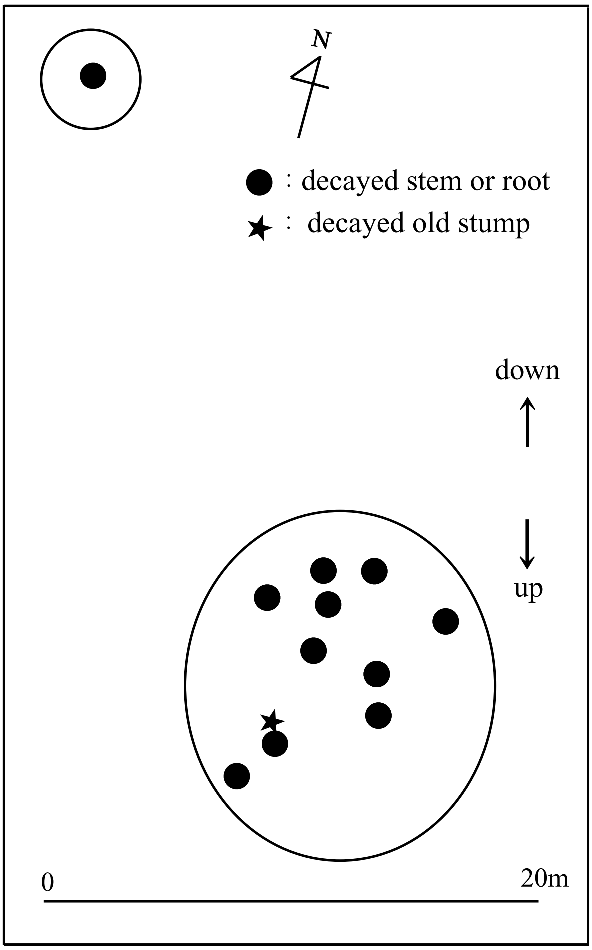

Fig. 4 Spatial distribution of two genets of *P. subacida* inferred from SIT at site C.

in 12 trees at site A and spread over a distance of 23.9m. The spatial distribution of the five genets at site B is shown in Fig.3. One genet was found in five trees at site B and spread over a distance of 18.2m. The spatial distribution of the two genets at site C is shown in Fig.4. One genet was found in 10 trees at site C and spread over a distance of 13.8m. The same genets were isolated from decayed stumps and roots or stems of adjacent trees at all sites. All isolates from roots and stems of the same trees were identical.

Discussion

Butt rot caused by *P. subacida* was found in 46% of the hinoki trees felled by thinning at three sites. No other species of wood-rotting fungi like *T. epimiltinus* were isolated from decaying wood in this area.

Several genets were found in the population of *P. subacida*, three genets at site A, five genets at site B, and two genets at site C. There were at least five infection centers at site B from which the fungus appears to have spread to neighboring trees by mycelial growth through root contacts such as root-rot fungi, *Heterobasidion annosum* (Fr.) Bref. s. l. (Stenlid 1985) and *Phellinus noxius* (Corner) G. Cunn. (Hattori et al. 1996).

Decayed old stumps were present at all sites where genets of *P. subacida* were collected. Old stumps appear to be the infection center from which the fungus grows along the decaying root system, spreading to adjacent trees through root contacts (Tabata et al. 2002, 2004, 2009).

Our observations in rot-infected hinoki stumps showed that root decay mainly occurred in the wounds by root-feeding of larva of the chafer, root contacts, stones, and cracks. The results of isolation and SIT test of isolates suggested that the wounds acted as entrance sites for invasion by *P. subacida.* The wounds by stones become sites for invasion by *Phaeolus schweinitzii* (Fr. : Fr.) Pat., *Oligoporus balsameus* (Pk.) Gilbn. & Ryv., and *Sparassis crispa* (Wulfen : Fr.) Fr. of Japanese larch (Koiwa 2002). Therefore, it is thought that the wounds, which occur in the root systems by various causes, play an important role providing the invasion site for the root-decay fungi in the hinoki stands.

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ヒノキ根株腐朽病を起こすキンイロアナタケの感染と伝染

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要　旨

30・38・43年生ヒノキ林内で発生しているヒノキ根株腐朽病について病原菌のジェネット構造や侵入口を調べ，病原菌の感染と伝染について検討した。キンイロアナタケによるヒノキ根株腐朽病の被害率は，30・38・43年生ヒノキ林においてそれぞれ53%，28%，49%であった。キンイロアナタケは根の腐朽部や樹皮上の白色菌糸体から頻繁に分離された。分離菌株の対峙培養の結果，38年生ヒノキ林で少なくとも５個のジェネットが見つかり，病原菌は根系接触によって隣接木に栄養繁殖で広がっていた。伐根の掘り取り調査の結果，腐朽が原因となる傷は６つのタイプ（根系接触，石レキ，根切虫オオスジコガネの幼虫の食害，林内作業車，根の亀裂，根腐れ）に分類された。伐根につながる傷はほとんど地下36cm未満で発生し，根系接触，石レキ，オオスジコガネ幼虫の食害が関係していた。

キーワード：根株腐朽，ヒノキ，分布，感染，キンイロアナタケ