Distribution of Genets of *Perenniporia subacida* in Stands of *Chamaecyparis obtusa* (Japanese Cypress) Determined by AFLP Fingerprints and Somatic Incompatibility

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ABSTRACT

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The clonal structure of *Perenniporia subacida*, a wood-rotting basidiomycete, was studied in a 30-year-old stand (site A; 1 ha) and a 43-year-old stand (site B; 0.5 ha) of *Chamaecyparis obtusa* in northern Kagawa Prefecture, Japan. A total of 110 isolates from decayed trees, stumps, wooden fences, logs, and mycelial mats were analyzed by amplified fragment length polymorphism (AFLP) and somatic incompatibility test (SIT). The results of AFLP, which agreed with those of SIT, revealed that the *P. subacida* population consisted of at least 17 genets at site A and 6 genets at site B. Individual genets were found in 1 to 9 trees at site A and in 1 to 7 trees at site B. One particular genet had spread over a distance of 70 m. Root contacts were observed among roots of decaying stumps and living trees at both sites. White mycelial mats were often found where roots made contact between stumps and neighboring trees. These results suggest that *P. subacida* spreads vegetatively over significant distances through root contacts.

Chamaecyparis obtusa (Siebolt & Zucc.) Endl. (Japanese cypress, "hinoki" in Japanese) is extensively planted in Japan, from the warm-temperate to the cool-temperate zones. Hinoki is endemic to Japan and one of the most important tree species for construction, especially for timber frames of Japanese houses. Hinoki plantations represent about 23% of the artificial forest area, and butt rots are sometimes found in these plantations. *Tinctoporellus epimiltinus* (Berk. & Broome) Ryvarden is one of the fungi that cause butt rot of hinoki in the Kyushu District of Japan (16,21). In 1997, butt rot was found at a hinoki plantation in Kagawa Prefecture where thinning operations had taken place. Afterward, Tabata et al. (32) conducted a field survey on butt rot, studied the causal fungus, and demonstrated that it was caused by *Perenniporia subacida* (Peck) Donk (Basidiomycota, Polyporales, Polyporaceae).

P. subacida is one of the most common and widespread polypores in Asia, Europe, and North America (27). In Asia, it has been found in Japan, Russia, and China (1,3,41). The fungus occurs predominantly in cool-temperate zones (1,9,27), although *T. epimiltinus* is widespread in warm-temperate and tropical zones (9,14,28). It

sometimes produces basidiocarps on the surface of decayed stumps, logs, or dead trees from summer to autumn. Basidiocarps are white to cream, resupinate, becoming widely effused, up to 10 mm thick; the surface is composed of small pores, 3 to 5 per mm. Basidiospores are hyaline, ovoid to broadly ellipsoid, smooth, thin-walled, 4 to 6 × 3 to 5 µm. Anamorph of the fungus has not been known.

The fungus causes white stringy rot, mostly in dead conifers and occasionally in hardwoods, and butt rot in living trees (4,9,11,22,32,38). The early stage of decay appears as a light brown stain. Later, small white pits develop and coalesce to form a mass of white spongy fibers. Black flecks sometimes appear in the white fibers (32). However, how the fungus attacks living trees has not been known. Closely planted trees are sometimes found to have been decayed by the fungus, but it is uncertain whether they were infected individually by spores or invaded by mycelia from adjacent decayed trees through root contacts. To clarify the situation, it is necessary to identify and distinguish genets of *P. subacida* in the wild. Individual genets have been identified in other root and butt rot fungi including *Armillaria mellea* (Vahl) P. Kumm. s. l. (18), *Heterobasidion annosum* (Fr.) Bref. s. l. (8,19,35,36), and *Phellinus noxius* (Corner) G. Cunn. (12). These fungi can spread from one tree to an adjacent tree by vegetative growth of the mycelia through host root systems.

A variety of methods have been used to elucidate fungal population structures of basidiomycetous fungi that infect forest trees, including somatic incompatibility

(2,12,17,20,31), isozyme profiles (10,12,13,31), random amplified polymorphic DNA (RAPD) analysis (10,20), microsatellite (15,40), and amplified fragment length polymorphism (AFLP) analysis (34). Somatic incompatibility tests (SIT) do not require marker development but may not distinguish closely related genotypes (17). Isozyme profiles are relatively easy to establish but tend to underestimate levels of polymorphisms (31). RAPD analysis is a fast and simple technique to examine polymorphisms but suffers from a lack of reproducibility (10). Microsatellite markers are well known as powerful tools for population genetic studies; however, it is said that fungal microsatellites appeared harder to isolate and to exhibit fewer polymorphism than in other organisms (5). On the other hand, AFLP is a particularly useful marker when DNA is pure and from cultures, but it is a much more limited and challenging method when the fungi cannot be cultured.

In this study, we studied the distribution of *P. subacida* genets in two stands of hinoki using AFLP analysis and SIT. We evaluated the usefulness of AFLPs as genetic markers in *P. subacida* populations and clarified the population structure of *P. subacida* in these stands.

MATERIALS AND METHODS

Study site. The study was conducted in 30-year-old (site A) and 43-year-old (site B) stands of hinoki in northern Kagawa Prefecture, where Tabata et al. (32,33) had reported the root rots. 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 to 21 degrees and has about 1 ha total area.

Site B is 40 m away from site A and formerly was a *Pinus densiflora* forest, cut and replanted with hinoki at a density of 3,500 trees/ha in 1958. It is at the same elevation as site A, located on a slope of 4 to 10 degrees, and covers about 0.5 ha.

In the area, the mean annual precipitation is about 1,200 mm 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 (7). The soil has a shallow topsoil (A

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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 not well aerated.

Field surveys. One hundred thirty-two of the 30-year-old trees and 131 of the 43-year-old trees were cut in the summers (May to September) of 1999 and 2001, respectively. Cut trees were classified into trees with and without decay in the butt, and their locations in the 30-year-old and 43-year-old stands were plotted on a map. 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. The heights of decay were examined.

Eighteen stumps of 30-year-old trees that had been cut in 1999 were randomly selected between October and December 2000, and 17 stumps of 43-year-old trees cut in 2001 were selected between October to December 2001. 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.

Roots were dug up for 11 living trees and 8 stumps in a small plot A1 (8 × 8 m) at site A, and for 9 living trees and 2 stumps in a small plot B1 (6 × 6 m) at site B, to check for root contact with fungi and fungal colonization.

Isolation. To isolate fungal strains, pieces were taken from decayed stems and

roots of cut trees, old stumps, wooden fences, and felled logs. White mycelial mats were also collected from stems and roots of cut and living trees. Isolation was conducted as described previously, and cultures were identified by their cultural characteristics (32). The cultures isolated from stumps, decayed wood, and mycelial mats were identified by morphological characteristics following Nobles (24) and Stalpers (30), and also by comparing them morphologically with cultures derived from basidiocarps of the fungus. All cultures showed the same characteristics: colonies are white to whitish cream, cottony to wooly, with growth 60 to 70 mm in radius at 25°C in 14 days; generative hyphae are 1 to 7 µm wide, with clamps (dikaryon); skeletal hyphae are thick-walled, 1 to 2 µm wide. As the characteristics were the same in all basidiomycetous cultures, they were considered to represent a single species. Ninety-six isolates from site A (K1 to 96, Table 1) were used both for AFLP analysis and SIT. SIT was also conducted using 109 isolates from site B (K116 to 224, Table 2) and 30 isolates from plots A1 and B1 (K97 to 115, K225 to 235, Tables 1 and 2). Fourteen isolates were used for AFLP analysis from site B (K116, 152, 160, 168, 180, 182, 183, 185, 187, 190, 191, 207, 208, 212; Table 2). The isolates were stored at the Forestry and Forest Products Research Institute.

DNA extraction and AFLP procedure.

AFLP analysis was conducted using 110 of the isolates described above. Mycelia were grown at 25°C for 2 to 3 weeks on potato dextrose agar (PDA) plates. Fresh mycelial mats were collected with a pestle and ground using a Mixer Mill MM300 (Qiagen). DNA was extracted using the DNeasy Plant Mini Kit (Qiagen).

AFLP analysis was conducted according to the protocol of Perkin-Elmer's AFLP microbial fingerprinting. In the preselective amplification step, we used the preselective primer without selective nucleotide (*Mse*I+0 and *Eco*RI+0). In the selective amplification step, we used a pair of selective primers (*Mse*I+AC and *Eco*RI+C) which were identified as the most informative and reliable during a preliminary investigation (data not shown). Because the selected primer pair produced more than 80 markers and the difference among genets was clear (see results), we decided to use only one pair of selective primers in this study. We conducted the restriction digestion, ligation of adaptors, preselective amplification, and selective amplification using an iCycler thermal cycler (Bio-Rad Laboratories). AFLP profiles were obtained with an ABI PRISM 310 Genetic Analyzer and the GeneScan Analysis software (Applied Biosystems), and were compared regarding the presence and intensity of fragments.

Table 1. Isolates of *Perenniporia subacida* used for amplified fragment length polymorphism (AFLP) and/or somatic incompatibility test (SIT) at site A^a

Isolate	Origin	Tree*	Isolate	Origin	Tree*	Isolate	Origin	Tree*	Isolate	Origin	Tree*
K1	RB	KA1	K31	RB	KA17	K61	FA		K91	SA	KA56
K2	BA		K32	SA	KA18	K62	BA		K92	SA	KA57
K3	SA	KA2	K33	FA		K63	SA	KA35	K93	RA	KA58
K4	SA	KA3	K34	RB	KA19	K64	SA	KA36	K94	LA	
K5	SA	KA4	K35	FA		K65	FA		K95	BA	
K6	FA		K36	SA	KA20	K66	SA	KA37	K96	RB	KA59
K7	FA		K37	SA	KA21	K67	RB	KA38	K97	MB	KA60
K8	FA		K38	SA	KA22	K68	SA	KA39	K98	BA	
K9	FA		K39	SA	KA23	K69	RB	KA40	K99	MB	KA61
K10	SA	KA5	K40	SA	KA24	K70	SA	KA41	K100	BA	
K11	SA	KA6	K41	MA	KA25	K71	BA		K101	BA	
K12	RB	KA7	K42	BA		K72	SA	KA42	K102	BA	
K13	SA	KA8	K43	RB	KA26	K73	FA		K103	MB	KA62
K14	RB	KA9	K44	BA		K74	RB	KA43	K104	MB	KA63
K15	SA	KA9	K45	RB	KA27	K75	SA	KA44	K105	MB	KA64
K16	SA	KA9	K46	RB	KA27	K76	RA	KA44	K106	BA	
K17	SA	KA9	K47	SA	KA28	K77	SA	KA45	K107	BA	
K18	BA		K48	SA	KA29	K78	SA	KA46	K108	MB	KA65
K19	RB	KA10	K49	BA		K79	RA	KA47	K109	MB	KA66
K20	SA	KA11	K50	SA	KA30	K80	SA	KA48	K110	BA	
K21	SA	KA12	K51	BA		K81	SA	KA49	K111	MB	KA67
K22	BA		K52	RA	KA31	K82	LA		K112	MB	KA68
K23	MA	KA13	K53	SA	KA32	K83	SA	KA50	K113	MB	KA69
K24	SA	KA13	K54	SA	KA32	K84	SA	KA51	K114	BA	
K25	SA	KA13	K55	SA	KA32	K85	RA	KA52	K115	MB	KA70
K26	SA	KA14	K56	SA	KA32	K86	SA	KA53			
K27	MA	KA15	K57	LA		K87	BA				
K28	BA		K58	SA	KA33	K88	SA	KA54			
K29	BA		K59	RA	KA34	K89	SA	KA55			
K30	SA	KA16	K60	FA		K90	BA				

^a Boxed isolates are from the same tree. BA: decayed old stump's root; FA: decayed wooden fence pole; LA: decayed log; MA: white mycelial mats formed on the bark of stem; MB: white mycelial mats formed on the bark of root; RA: *P. subacida*-infected root wounded by a small-size forwarder; RB: decayed root with unknown infection; SA: decayed stem from heights between 0.1 and 3.0 m; Tree*: decayed tree number.

Data analysis. Scores were determined as the presence of fragments (bands) in a reproducible range of fragment sizes. Dissimilarity of AFLP fingerprints between pairs of genets was calculated as $[b + c/(a + b + c)]$, where a is the number of shared bands, and b and c are the numbers of bands present in one sample but absent in the other. These measures were subjected to UPGMA analysis using PHYLIP version 3.57c (6).

Mantel tests were performed to determine the correlation between the degree of polymorphism for a pair of genets and the shortest geographic distance at which two genets had been found. The test was performed using the web-based software developed by Rodney Dyer (<http://dyerlab.bio.vcu.edu/cgi-bin/Mantel.cgi>).

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

RESULTS

Field surveys. After thinning, there were 2,000 trees/ha at site A and 1,200 trees/ha at site B. Butt rot was found in 58.3% of the felled trees at site A and in

49.6% at site B. Decay was not found in the felled trees that were located between the spur road and the waterway (Fig. 1). The maximum height of rot was 3.0 m aboveground in the 30-year-old trees. We collected 47 decayed stem disks from 41 trees at site A and 5 decayed stem disks from 5 trees at site B. Fifty-two samples of decayed stems were collected from heights of 0.1 to 3.0 m.

Larvae of the root-feeding insect, *Mimela costata* Hope (larger striated chafer), sometimes were found on decaying roots on which they fed, leaving feeding wounds. Some roots apparently had been wounded by small-size forwarder used for carrying logs. Wounds were also caused by mutual root contacts or stones. Of the 112 decayed woody roots, 30 roots (26.8%) showed wounds by mutual root contact, 28 (25%) were by chafer larvae, 9 (8%) by stones, 8 (7.1%) by forwarder, and 37 (33.1%) showed infections of unknown origin.

Old hinoki stumps cut in 1994 at sites A and B were found to be colonized by *P. subacida* (Tables 1 and 2). White 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 roots of decayed stumps to those of adjacent living trees through root contact. However, no rhizomorph was found in the soil around the

decayed stumps in the study sites. Basidiocarps of *P. subacida* were found on two of the stumps.

Wooden fences of hinoki, erected along the waterway to control erosion in 1995, were infected with *P. subacida* and decayed at site A. We found roots of infected trees to be in contact with the decayed fences. Some logs felled in 1996 had been left at site A. *P. subacida* grew on several of them and basidiocarps were found on a few of the decayed fallen logs.

Root contacts were observed between roots of living trees and decayed stumps in the small plots, A1 and B1. White mycelial mats were found on the surface of the roots of all living trees and decayed stumps in these plots, and were identified as *P. subacida* in culture studies.

Isolation. Three hundred and fifty isolates were taken from site A and 360 from site B; 235 isolates were selected for further study (Tables 1 and 2). The isolates were derived from 52 decayed stems and 112 decayed roots of felled trees, 29 decayed stumps, 10 decayed wooden fence poles, 3 decayed logs, and 29 white mycelial mats. They were identified as *P. subacida* by their morphological characteristics. Isolation rates of the fungus were 4.4 to 100% in decayed stems, 10 to 100% in roots, 30 to 100% in stumps, 11.1 to 100% in fences, 85.7 to 100% in fallen logs, and 28.6 to 90% in mycelial mats.

Table 2. Isolates of *Perenniporia subacida* used for amplified fragment length polymorphism (AFLP) and/or somatic incompatibility test (SIT) at the site B^a

Isolate	Origin	Tree*	Isolate	Origin	Tree*	Isolate	Origin	Tree*	Isolate	Origin	Tree*
K116	RB	KB1	K146	RC	KB3	K176	RD	KB5	K206	RB	KB15
K117	SA	KB2	K147	RE	KB3	K177	RD	KB5	K207	RB	KB16
K118	MB	KB2	K148	RB	KB3	K178	RD	KB5	K208	BA	
K119	RE	KB2	K149	RE	KB3	K179	MB	KB6	K209	SA	KB17
K120	RE	KB2	K150	RE	KB3	K180	RE	KB6	K210	RB	KB17
K121	RE	KB2	K151	RC	KB3	K181	RE	KB6	K211	RC	KB17
K122	RC	KB2	K152	RE	KB3	K182	SA	KB7	K212	RC	KB17
K123	RB	KB2	K153	RE	KB3	K183	BA		K213	RC	KB17
K124	RE	KB2	K154	MB	KB3	K184	RC	KB8	K214	RB	KB17
K125	RB	KB2	K155	RC	KB3	K185	RC	KB8	K215	RD	KB17
K126	RE	KB2	K156	RC	KB3	K186	BA		K216	RB	KB17
K127	RE	KB2	K157	RC	KB3	K187	BA		K217	RB	KB17
K128	RE	KB2	K158	RC	KB3	K188	RC	KB9	K218	RE	KB17
K129	RB	KB2	K159	RE	KB3	K189	RB	KB10	K219	RE	KB17
K130	RE	KB2	K160	MB	KB4	K190	RB	KB10	K220	RC	KB18
K131	RB	KB2	K161	RE	KB4	K191	RB	KB11	K221	RE	KB18
K132	RC	KB2	K162	RE	KB4	K192	RA	KB12	K222	RE	KB18
K133	RE	KB2	K163	MB	KB5	K193	RA	KB12	K223	RC	KB18
K134	RE	KB2	K164	RE	KB5	K194	BA		K224	RC	KB18
K135	RE	KB2	K165	RB	KB5	K195	RB	KB13	K225	MB	KB19
K136	RE	KB2	K166	RE	KB5	K196	SA	KB14	K226	MB	KB20
K137	SA	KB3	K167	RE	KB5	K197	RB	KB14	K227	MB	KB21
K138	RC	KB3	K168	RD	KB5	K198	RC	KB14	K228	MB	KB22
K139	RC	KB3	K169	RD	KB5	K199	RB	KB14	K229	MB	KB23
K140	RC	KB3	K170	RC	KB5	K200	RB	KB14	K230	MB	KB24
K141	MB	KB3	K171	RB	KB5	K201	RC	KB14	K231	BA	
K142	RC	KB3	K172	RD	KB5	K202	RB	KB14	K232	MB	KB25
K143	RC	KB3	K173	RD	KB5	K203	RC	KB14	K233	MB	KB26
K144	RC	KB3	K174	RD	KB5	K204	RB	KB14	K234	MB	KB27
K145	RB	KB3	K175	RE	KB5	K205	RC	KB14	K235	BA	

^a Boxed isolates are from the same tree. BA: decayed old stump's root; MB: white mycelial mats formed on the bark of root; RA: *P. subacida*-infected root wounded by a small-size forwarder; RB: decayed root with unknown infection; RC: *P. subacida*-infected root wounded by larger striated chafer; RD: *P. subacida*-infected root wounded by the stone contact; RE: *P. subacida*-infected root wounded by mutual contact; SA: decayed stem from heights between 0.1 and 3.0 m; Tree*: decayed tree number.

AFLP analysis. Distinct AFLP fingerprints were obtained from the cultures. For the 96 samples collected at site A, a primer pair produced 33 to 45 (average 41) fragments per sample with sizes in the range of 50 to 300 bp. In total, 87 different fragments with lengths in this range were detected, 83% of which were polymorphic. We used these fragments to distinguish genetically distinct isolates. Seventeen groups were detected, and each group consisted of 1 to 19 isolates. Eight to 32 (average 21.9) fragments differed (were present/absent) when pairs of genets were compared, and 9.2 to 39.8% (average 25.2%) of the fragments showed polymorphisms in these pairwise comparisons.

For the 14 samples collected from site B, the primer pair produced 38 to 46 (average 41) 50 to 300 bp fragments per sample in the range of 50 to 300 bp. In total, 87 such fragments were detected, and 77% were polymorphic. The fragments allowed us to define 6 groups of genetically distinct isolates; each group consisted of 1 to 3 isolates. In pairwise comparisons of genets, 13 to 24 (average 19.1) fragments differed (were present/absent) and 25.5 to 48% (average 37.9%) of them showed polymorphisms.

Results of UPGMA analyses based on the dissimilarity of AFLP profiles in pairs

of genets indicated few clearly defined genetic clusters of genets (Figs. 2 and 3). No significant correlation was found between the degree of polymorphism of each genet and the geographic distance to other genets by the Mantel test.

SIT. Clear zone lines indicating somatic incompatibility were formed in some pairings, while they were absent in others (suggesting somatic compatibility). The 96 isolates from site A were divided into 17 groups by SIT, in complete

Fig. 2. UPGMA clustering based on dissimilarities of amplified fragment length polymorphism (AFLP) profiles of the 17 genets (I to XVII) of *Perenniporia subacida* at site A.

Fig. 1. Spatial distribution of 17 genets (I to XVII) of *Perenniporia subacida* inferred from amplified fragment length polymorphism (AFLP) fingerprints and somatic incompatibility test (SIT) of the isolates K1 to 96 at site A.

Fig. 3. UPGMA clustering based on dissimilarities of amplified fragment length polymorphism (AFLP) profiles of the 6 genets (XVIII to XXIII) of *Perenniporia subacida* at site B.

Fig. 4. Distribution of genets of *Perenniporia subacida* inferred from somatic incompatibility test (SIT) of isolates K97 to 115 in plot A1 and isolates K225 to 235 in plot B1, and root contacts between living trees and decayed stumps. Bars between trees and stumps indicate that the same genet of *P. subacida* had been found on both and that root contact between them had been observed.

Fig. 5. Spatial distribution of 6 genets (XVIII to XXIII) of *Perenniporia subacida* inferred from amplified fragment length polymorphism (AFLP) fingerprints and/or somatic incompatibility test (SIT) of isolates K116 to 224 at site B.

agreement with the results from AFLP analysis.

The 109 isolates from site B were divided into 6 groups by SIT; with respect to the 14 samples that had been analyzed by AFLP, there was a complete agreement between the results from AFLP and SIT. SIT also showed that the 19 isolates (K97 to 115) from plot A1 and the 11 isolates (K225 to 235) from plot B1 consisted of a single group each (X in Fig. 4A1 and XXIII in Fig. 4B1).

Spatial distribution of genets of *P. subacida*. The spatial distribution of the 17 genets at site A is shown in Figure 1. Four genets were found only in one decayed tree or one decayed stump, while 13 genets were found in at least two places. One genet (XIV) was found in 9 trees and spread over a distance of 34.5 m (K81 and K89), while another genet (X) was found at locations more than 70 m apart (K41 and K58). The spatial distribution of the 6 genets at site B is shown in Figure 5. One genet (XXII) was found in 7 trees and another one (XXI) spread over a distance of 13.5 m (K184, K185, and K188). Same genets were sometimes isolated from decayed stumps and roots or stems of neighboring trees, for example K1 and K2 in the genet I, and K182 and K183 in the genet XXI (Figs. 1 and 5). Same genets were found in decayed wood fences and felled neighboring trees (genets V, VIII, XI, and XII). All isolates from roots and stems of the same trees were identical, for example K14 to 17 in the genet V (Fig. 1) and K117 to 136 in the genet XVIII (Fig. 5).

DISCUSSION

Butt rot caused by *P. subacida* was found in 54% of the hinoki trees felled by thinning activities at two sites, and was randomly distributed. No other species of wood-rot fungi like *Tinctoporella epimiltinus* or *Phaeolus schweinitzii* were isolated from decaying wood in this area.

On the other hand, many genets were found in the population of *P. subacida*, 17 genets at site A and 6 genets at site B. A single genet was sometimes found in a single decayed tree or stump, but in other cases was found in more than two trees, nine different trees at maximum. There were at least 17 infection centers at site A from which the fungus appears to have spread by vegetative growth of mycelia. Root rot fungi are known to spread to neighbor trees by mycelial growth. *Armillaria* spp. often spread to neighboring trees by forming rhizomorphs (25,26,29,39). On the other hand, *Heterobasidion annosum*, *Phellinus noxius*, and *P. weirii* do not produce rhizomorphs, but they spread via root contact of trees (23,31,37). *P. subacida* does not produce any rhizomorphs in soil and appears to spread to neighboring trees by mycelial growth through root contacts.

The soil in the study sites was clay-rich, which means high dry bulk density and an

air phase of low volume, leading to frequent occurrences of excess soil moisture (32). These soil properties seemed to promote the growth of the pathogen.

An analysis (Figs. 2 and 3) and the Mantel test based on the dissimilarity of AFLP profiles in pairs of genets indicated no significant correlation between the degrees of polymorphism and the locations where samples had been collected.

Decayed old stumps were present at most sites where genets of *P. subacida* were collected. Old stumps appear to be infection centers from which the fungus grows along the decaying root system, spreading to neighboring trees through root contacts (32,33).

As wood fences were colonized by *P. subacida* and roots of neighboring trees came into contact with them, the fences appeared to be infection centers for neighboring trees. The fences had not been treated with preservatives, and their decay was more advanced than that in neighboring newly cut stumps. We suggest that wooden fences should not be used where root and butt rot damage exists.

Many types of wounds could occur in the root system of a single tree due to various causes such as root and stone contacts (33). It was expected that different genets of *P. subacida* would invade the same tree, however, only one genet was found per tree in every case. We assume that once a genet has invaded a tree through wounded roots, it spreads on the bark of the roots and prevents invasion by different genets. After colonization of a stump or tree, the fungus appears to spread to neighboring trees by vegetative growth of mycelia through root contacts.

The largest *P. subacida* genet spread up to 70 m in length (K41 and K58). This distance is similar to those found for *Armillaria* spp. by Rishbeth (25) (70 m), Rizzo and Harrington (26) (35 m), Smith et al. (29) (70 m), Worrall (39) (40 m), for *H. annosum* by Stenlid (31) (30 m), and for *Phellinus noxius* by Hattori et al. (12) (20 m). The studies show that genets of these fungi may expand through root-to-root contacts or by rhizomorphs for more than several years, causing root and butt rot of conifers or hardwoods.

One genet (X) was detected in isolated places more than 40 m apart (K42 and K48). Since many living trees and decayed stumps were present between the places where K42 and K48 were isolated, it is plausible that the infection spread through root contacts in living trees.

LITERATURE CITED

1. Aoshima, K. 1950. Wood-rotting *Por*ia from Japan (I). (In Japanese, with English summary.) Bull. Gov. For. Exp. Stn. 46:155-168.

2. Barrett, D. K., and Uscuplic, M. 1971. The field distribution of interacting strains of *Polyporus schweinitzii* and their origin. New Phytol. 70:581-598.
3. Bondartsev, A. S. 1971. The Polyporaceae of the European USSR and Caucasia. (Translated from Russian.) Israel Program for Scientific Translations Ltd., Jerusalem.
4. Buckland, D. C. 1946. Investigation of decay in western red cedar in British Columbia. Can. J. Res. Sec. C. 24:158-181.
5. Dutech, C., Enjalbert, J., Fournier, E., Delmotte, F., Barres, B., Carlier, J., Tharreau, D., and Giraud, T. 2007. Challenges of microsatellite isolation in fungi. Fungal Genet. Biol. 44:933-949.
6. Felsenstein, J. 1995. PHYLIP (Phylogeny Inference Package) Ver. 3.57c. Department of Genetics, University of Washington, Seattle.
7. Forest Soil Division. 1976. Classification of forest soil in Japan (1975). Bull. Gov. For. Exp. Stn. Tokyo 280:1-28.
8. Garbelotto, M., Cobb, F. W., Bruns, T. D., Otrerosina, W. J., Popenuck, T., and Slaughter, G. 1999. Genetic structure of *Heterobasidion annosum* in white fir mortality centers in California. Phytopathology 89:546-554.
9. Gilbertson, R. L., and Ryvarden, L. 1987. North American Polypores. Vol. 2. Fungiflora, Oslo.
10. Guillaumin, J. J., Anderson, J. B., Legrand, P., Ghahari, S., and Berthelay, S. 1996. A comparison of difference methods for the identification of genets of *Armillaria* spp. New Phytol. 133:333-343.
11. Hansen, E. M., and Lewis K. L. 1997. Compendium of Conifer Diseases. American Phytopathological Society, St. Paul, MN.
12. Hattori, T., Abe, Y., and Usugi, T. 1996. Distribution of clones of *Phellinus noxius* in a windbreak on Ishigaki Island. Eur. J. For. Pathol. 26:69-80.
13. Huttermann, A., Volger, C., Schorn, R., Ahnert, G., and Ganser, H. G. 1979. Studies on isoenzyme polymorphism in *Fomes annosus*. Eur. J. For. Pathol. 9:265-274.
14. Ito, K. 1974. Pathology of forest trees III. (In Japanese.) Norin Shuppan, Tokyo.
15. Johannesson, H., and Stenlid, J. 2004. Nuclear reassortment between vegetative mycelia in natural populations of the basidiomycete *Heterobasidion annosum*. Fungal Genet. Biol. 41:563-570.
16. Katsu, Z. 1971. Butt rot of *Chamaecyparis obtusa*. (In Japanese.) For. Pests 20:141-146.
17. Kile, G. A. 1983. Identification of genotypes and the clonal development of *Armillaria luteobubalina* Walting and Kile in eucalypt forests. Aust. J. Bot. 31: 657-671.
18. Korhonen, K. 1978. Interfertility and clonal size in the *Armillaria mellea* complex. Karstenia 18:31-42.
19. Korhonen, K. 1978. Intersterility groups of *Heterobasidion annosum*. Commun. Inst. For. Fenn. 94(6):1-25.
20. Kubayashi, T., and Hattori, T. 2002. Clone distribution of two causal fungi of root and butt rot in a Japanese cypress (*Chamaecyparis obtusa*) plantation. (In Japanese, with English summary.) J. Jpn. For. Soc. 84:104-110.
21. Kubayashi, T., Hattori, T., and Kawabe, Y. 2001. *Tinctoporellus epimiltinus*, a causal fungus of butt rot of Japanese cypress. For. Pathol. 31:43-52.
22. McCallum, A. W. 1928. Studies in forest pathology. I. Decay in balsam fir (*Abies balsamea* Mill.). Can. Dep. Agric. Bull. (n. s.) 104:1-25.
23. Nandris, D., Nicole, M., and Geiger, J. P. 1987. Root rot diseases of rubber trees. Plant Dis. 71:298-306.
24. Nobles, M. K. 1948. Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi. Can. J. Res. C. 26:281-431.
25. Rishbeth, J. 1991. *Armillaria* in an ancient broadleaved woodland. Eur. J. For. Pathol. 21:239-249.
26. Rizzo, D. M., and Harrington, T. C. 1993. Delineation and biology of clones of *Armillaria ostoyae*, *A. gemina* and *A. calvesens*. Mycologia 85:164-174.
27. Ryvarden, L., and Gilbertson, R. L. 1994. European Polypores Part 2: Meripilus-Tyromyces. Synopsis Fungorum 7:395-743.
28. Ryvarden, L., and Johansen, I. 1980. A preliminary polypore flora of East Africa. Fungiflora, Oslo.
29. Smith, M. L., Bruhn, J. N., and Anderson, J. B. 1994. Relatedness and spatial distribution of *Armillaria* genets infecting red pine seedlings. Phytopathology 84:822-829.
30. Stalpers, J. A. 1978. Identification of wood-inhabiting Aphyllophorales in pure culture. Stud. Mycol. 16:1-248.
31. Stenlid, J. 1985. Population structure of *Heterobasidion annosum* as determined by somatic incompatibility, sexual incompatibility, and isoenzyme patterns. Can. J. Bot. 63:2268-2273.
32. Tabata, M., Kato, T., Ohkubo, M., Abe, Y., and Yoshinaga, S. 2002. Butt rot of *Chamaecyparis obtusa* (Sieb. et Zucc.) Endlicher trees caused by *Perenniporia subacida* in Shikoku District, Japan: Pathogen, distribution of damaged trees in the stand, and soil investigation. J. For. Res. 7:105-112.
33. Tabata, M., Takemoto, M., Toda, N., and Abe, Y. 2004. Infection of *Perenniporia subacida* in *Chamaecyparis obtusa*. Proc. 11th Int. Conf. Root Butt Rots. Poznan-Bialowieza, Poland.
34. Terashima, K., Cha, J. Y., and Miura, K. 2001. Detection of genetic variation among single-spore isolates and identification of genets of *Armillaria ostoyae* by AFLP analysis with Texas Red TM-labeled selective primer. Mycoscience 42:123-127.
35. Vasiliauskas, R., and Stenlid, J. 1998. Spread of S and P group isolates of *Heterobasidion annosum* within and among *Picea abies* trees in central Lithuania. Can. J. For. Res. 28:961-966.
36. Vollbrecht, G., and Stenlid, J. 1999. Transfer of the P-type of *Heterobasidion annosum* from old-growth stumps of *Picea abies* to *Picea abies* and *Larix x eurolepis*. Eur. J. For. Pathol. 29:153-159.
37. Wallis, G. W., and Reynolds, G. 1965. The initiation and spread of *Por*ia *weirii* root rot of Douglas fir. Can. J. Bot. 43:1-9.
38. Whitney, R. D. 1995. Root-rotting fungi in white spruce, black spruce, balsam fir in northern Ontario. Can. J. For. Res. 25:1209-1230.
39. Worrall, J. J. 1994. Population structure of *Armillaria* species in several forest types. Mycologia 86:401-407.
40. Worrall, J. J., Sullivan, K. F., Harrington, T. C., and Steimel, J. P. 2004. Incidence, host relations and population structure of *Armillaria ostoyae* in Colorado campgrounds. For. Ecol. Manag. 192:191-206.
41. Zhao, J. D. 1998. Flora Fungorum Sinicorum Vol. 3. Polyporaceae. (In Chinese.) Science Press, Beijing.