Compatibility Among Host-Specialized Isolates of *Heterobasidion annosum* from Western North America

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ABSTRACT

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Host-specialization of isolates of *Heterobasidion annosum* from California was previously demonstrated by inoculating *Pinus ponderosa* and *Abies concolor* (white fir) seedlings in the greenhouse. In the present study, 18 of these isolates were tested for sexual compatibility in heterokaryon-homokaryon pairings with six S and four P tester strains of *H. annosum* from Europe representing two intersterility groups. Compatibility was determined by the presence of clamp connections in subcultures from the homokaryotic tester mycelia taken 5 wk after pairing. The nine isolates specialized to fir seedlings were compatible with S testers, and the nine isolates specialized to pine seedlings were compatible with P testers. Some P-compatible isolates formed clamps with a few S testers. Heterokaryotic isolates and homokaryotic (single-basidiospore) strains from another 18 trees and stumps in Pacific Coast states also fell into the S and P groups based on compatibility; those from diseased pine trees were P-compatible and those from other host genera were S-compatible. However, compatibility of isolates and strains from stumps with S and P testers was not consistent with the host genus, indicating that stump colonization can be non-host specific. When North American strains were paired among themselves or with heterokaryotic isolates, the two compatibility groups were less evident than when European strains were used. Reports thus far suggest that both the S-compatible and P-compatible groups are widespread in western North America, but only the P group has been reported in eastern North America.

Additional keywords: Annosum root rot, fungal genetics, host specialization.

Heterobasidion annosum (Fr.) Bref. (syn. *Fomes annosus* (Fr.) Cke.) causes an important root and butt rot of conifers throughout the northern temperate zone. Although the host range of the fungus is broad, including some hardwood species, mortality is most significant on pine (*Pinus*) species, in which the fungus is capable of spreading rapidly in the cambium and phloem tissues in advance of sapwood colonization and girdles the lower stem (4). In contrast, colonization of most other conifer hosts, such as species of fir (*Abies*) and spruce (*Picea*), is frequently restricted to heartwood tissues or the oldest growth rings of the sapwood in the largest lateral roots and at the base of the tree. Losses due to decay of the butt log in these non-pine species can be substantial, and mortality may occur, though not as rapidly as in pine species. Differences in host colonization patterns appear to be host-mediated (10). In addition to pathogenic colonization, saprophytic colonization may result from deposition of basidiospores onto freshly cut stump tops (8). Clones of the fungus can spread from root systems of colonized stumps or diseased trees to those of adjacent trees via root contacts or grafts (2,8).

Host preferences of isolates of *H. annosum* from pines and white fir (*Abies concolor* (Gord. & Glend.) Lindl.) were demonstrated in a seedling inoculation study. In two experiments, Worrall et al (10) found that isolates from *Pinus* spp. infected and killed more ponderosa pine (*P. ponderosa* Laws.) seedlings than fir seedlings; isolates from diseased fir trees, in contrast, infected and killed about the same proportion of pine and fir seedlings. A preliminary study of allozyme variation among California fir and pine isolates also suggested differences between the two groups (7). Indirect evidence for host specialization had been obtained in earlier mating experiments by Korhonen (6).

The pathogen is heterothallic over most of its range, and mated, secondary mycelia form clamp connections on at least some of the hyphae (6). Sexual compatibility among isolates can be tested by pairing primary (homokaryotic) mycelia against either primary or secondary (heterokaryotic) mycelia and examining hyphae of the homokaryon for clamp connections. In pairings among homokaryons derived from single basidiospores (herein referred to as strains), Korhonen found intersterility between two Finnish populations of *H. annosum*, and designated the two populations as S and P types. Presumably heterokaryotic isolates from decay (herein referred to as isolates) in a number of hosts around the world were tested for compatibility with homokaryotic testers of the S and P groups by looking for changes in cultural morphology of the testers after pairing. Most of the isolates from *Pinus* species were compatible with P tester strains, and most isolates from Norway spruce (*Picea abies* (L.) Karst.) were compatible with S tester strains. Of the 10 isolates tested from North America, all were from pines and compatible with P tester strains. Chase (1) examined a number of North American homokaryotic strains of *H. annosum*, and nearly all were compatible with either S or P strains from Finland. Most of the North American strains compatible with P testers were from species of pine, and most of the strains compatible with S testers were from non-pine species.

In our initial pairings among California isolates and strains, a high frequency of compatibility was found between strains from fir and pine trees. Although we tentatively concluded that the two intersterility groups found in Europe were not homologous with the two host-specialized groups in California, Chase's (1,3) results presented an alternative interpretation. For compatibility, two strains must be heterogenic at the mating type locus, as demonstrated by Korhonen (6), and must share a particular allele at one or more of five previously unknown compatibility loci. Alleles at two of these loci (the S and P loci) appeared to correlate with the capacity of the Finnish tester strains to differentiate the S and P intersterility groups. With Chase's genetic model and Finnish tester strains, we reexamined compatibility among the isolates from California that were demonstrated to be host-specialized (10).

MATERIALS AND METHODS

Ten single-basidiospore strains from Finland or Sweden were supplied by Korhonen, who had selected the strains for the capacity to differentiate S and P group isolates. The origin and collection numbers of these European tester strains are shown in Table 1. The majority of the field isolates from the earlier seedling inoculation study (10) were available for compatibility testing. Other isolates from decayed wood of stumps or diseased trees and single basidiospore strains were obtained from California, Oregon, Washington, and New Hampshire (see Table 2 for fir group isolates and strains, and Table 3 for pine group isolates and strains). All heterokaryotic isolates were from separate localities so that no genotype (clone) was represented more than once, but two single-basidiospore strains from a single basidiocarp were generally used.

TABLE 1. Collection numbers, host, and location of origin of homokaryotic S and P tester strains of *Heterobasidion annosum* from Europe

Isolate no.	Alternate no.[a]	Host of origin	Host condition[b]	Location
P1	750124.2.1/1	*Pinus sylvestris*	sapling	Finland
P2	821201.1.5/3	*Picea abies*	butt rot	Finland
P3	831102.1.6/2	*Pinus sylvestris*	root rot	Finland
P4	831102.1.6/3	same tree as P3		
S1	791205.1.1	*Pinus sylvestris*	stump	Finland
S2	831031.1.1/5	*Picea abies*	butt rot	Finland
S3	831031.1.1/6	same tree as S2		
S4	831102.2.1/5	*Picea abies*	butt rot	Finland
S5	841003.1.6	unknown	unknown	Sweden
S6	841119.2.8/1	*Picea abies*	butt rot	Sweden

[a] Strain numbers assigned by Korhonen (6).
[b] Host trees on which basidiocarps were produced were diseased saplings, mature trees with root or butt rot or were from stumps that may have been saprophytically colonized.

Most homokaryotic strains were obtained by collecting fresh basidiocarps from the field, placing portions of the hymenophore on the lid of petri dishes over water agar, and after the spore shower, transferring pieces of the agar with germinated single spores. In an attempt to derive more single-basidiospore strains, field isolates were incubated at room temperature on malt agar plates (MEA, 10 mg/ml of malt extract and 15 mg/ml of agar in 90-mm-diameter plastic petri dishes) with small blocks of pine wood, but only some of the pine isolates (JS1, PT1, PT2, PT3, and PT4) produced basidiocarps in vitro. Single-basidiospore strains were obtained from these basidiocarps as described above. Isolates and single-basidiospore strains were stored on malt extract agar or potato-dextrose agar slants at 4 C.

All pairings and subculturing from paired cultures were made on MEA plates and incubated at room temperature and lighting. In homokaryon-homokaryon pairings, mycelial plugs of the two strains were placed about 1 cm apart and allowed to grow together for 7 days, and subcultures were obtained by removing mycelial plugs from three locations along the confrontation zone. These mycelial plugs were transferred to MEA plates and incubated at room temperature for 3–5 days before examining for clamp connections (2). Examinations were conducted at 100 or 200× by inverting the plate on the microscope stage and viewing the hyphae through the bottom of the plate. All pairings were attempted at least twice, and a single clamp connection in either of two trials constituted a positive reaction.

Field isolates, most of which formed clamp connections, were tested at least twice for compatibility with single basidiospore strains in heterokaryon-homokaryon pairings. The paired cultures were incubated for 5 wk before subculturing. Mycelial plugs were

TABLE 2. Collection numbers, host, host condition, and location of origin of fir group strains and isolates of *Heterobasidion annosum* from western North America

Strain or isolate no.[a]	Alternate no.[b]	Host	Host condition[c]	Location[d]
CT1	BF1	*Calocedrus decurrens*	tree	San Bernardino Nat. For.
DS1	LW1	*Pseudotsuga menziesii*	stump	Lake of the Woods, OR
FS1A	FS1C	*Abies concolor*	stump	Northern Sierra Nevada
FS1B	FS2B	same stump as FS1A		
FT1A	FT12D	*Abies concolor*	tree	Northern Sierra Nevada
FT1B	FT12E	same tree as FT1A		
FT2A	FT13A	*Abies concolor*	tree	Northern Sierra Nevada
FT2B	FT13F	same tree as FT2A		
FT3	35-3-3*	*Abies concolor*	tree	San Bernardino Nat. For.
FT4	LAS7F*	*Abies concolor*	tree	Lassen Nat. For.
FT5	LAS17F*	*Abies concolor*	tree	Lassen Nat. For.
FT6	28-4-2*	*Abies concolor*	tree	San Bernardino Nat. For.
FT7	21-4-3*	*Abies concolor*	tree	San Bernardino Nat. For.
FT8	LAS1F*	*Abies concolor*	tree	Lassen Nat. For.
FT9	LAS4F	*Abies concolor*	tree	Lassen Nat. For.
FT10	LAS21F*	*Abies concolor*	tree	Lassen Nat. For.
FT11	LAS22F	*Abies concolor*	tree	Lassen Nat. For.
FT12	MOD14F*	*Abies concolor*	tree	Modoc Nat. For.
FT13	MOD15F*	*Abies concolor*	tree	Modoc Nat. For.
FT14	DG1	*Abies concolor*	tree	Bly, OR
GT1	DG8	*Abies grandis*	tree	Tieton, WA
HT1	DG7	*Tsuga heterophylla*	tree	Quilcene, WA
HT2	WH1	*Tsuga heterophylla*	tree	Mapleton, OR
PS1A	PS1B	*Pinus ponderosa*	stump	Northern Sierra Nevada
PS1B	PS1D	same stump as PS1A		
PS2A	PS2E	*Pinus ponderosa*	stump	Northern Sierra Nevada
PS2B	PS2F	same stump as PS2A		
SS1	SP3	*Pinus lambertiana*	stump	San Bernardino Nat. For.
SS2	SP4	*Pinus lambertiana*	stump	Stanislaus Nat. For.

[a] The first letter of the collection number designates the common name of the host, and the second letter designates tree (T) or stump (S) isolations. Collection numbers ending in letters are single-basidiospore strains, and those ending in a numeral are isolates from decayed wood.
[b] Collection numbers of various cooperators. Asterisks (*) denote those isolates used in the seeding inoculation study by Worrall et al (10).
[c] All isolates and strains are from stumps or diseased trees. The former may have been saprophytically colonized.
[d] All locations are in California except for those from Oregon (OR) and Washington (WA).

removed from three places on the homokaryotic side of the plate, at approximately 1 cm behind the line of confrontation. The subcultures were incubated and examined as in the homokaryon-homokaryon pairings.

RESULTS

Homokaryon-homokaryon pairings. Pairings among homokaryotic tester strains from Europe confirmed the intersterility of the S and P groups of Korhonen (6). All 24 pairings between the six S and four P tester strains were negative; the six within-group pairings of P strains resulted in clamp connections; 13 of 15 within-group pairings of the S strains yielded clamps (S6 failed to form clamps with S3 and S5).

Two intersterility groups, a pine and a fir group, were also suggested by the pairings among homokaryotic strains from western North America (Table 4). Only 12 of 120 pairings between strains of the fir and pine groups resulted in clamp connections. In contrast, 34 of 45 pairings within the fir group were positive, and three of the 11 negative pairings were between single-basidiospore strains derived from the same basidiocarp. In these three cases, incompatibility could be attributed to homoallelism at the mating

TABLE 3. Collection numbers, host, host condition, and location of origin of pine group strains and isolates of *Heterobasidion annosum* from North America

Strain or isolate no.[a]	Alternate no.[b]	Host	Host condition[c]	Location[d]
JS1	JL1*	*Pinus jeffreyi*	stump	San Bernardino Nat. For.
JS1A	JL1-1	same tree as JS1		
JS1B	JL1-3	same tree as JS1		
JS2	JP6*	*Pinus jeffreyi*	stump	San Bernardino Nat. For.
JT1	INYOJP	*Pinus jeffreyi*	tree	Inyo Nat. For.
PT1	LAS3P*	*Pinus ponderosa*	tree	Lassen Nat. For.
PT1A	LAS3P-5	same tree as PT1		
PT1B	LAS3P-9	same tree as PT1		
PT2	LAS11P*	*Pinus ponderosa*	tree	Lassen Nat. For.
PT2A	LAS11P-A	same tree as PT2		
PT2B	LAS11P-B	same tree as PT2		
PT3	LAS8P*	*Pinus ponderosa*	tree	Lassen Nat. For.
PT3A	LAS8P-E	same tree as PT3		
PT4	MOD12P*	*Pinus ponderosa*	tree	Modoc Nat. For.
PT4A	MOD12P-8	same tree as PT4		
PT5A	23-8	*Pinus* sp.	tree	Placerville
PT5B	23-59	same tree as PT5A		
PT6	PP1*	*Pinus ponderosa*	tree	San Bernardino Nat. For.
PT7	BOG3P*	*Pinus ponderosa*	tree	Boggs Mountain
PT8	BOG7P*	*Pinus ponderosa*	tree	Boggs Mountain
RT1A	12P-A	*Pinus resinosa*	tree	Durham, NH
RT2A	6AP-A	*Pinus resinosa*	tree	Durham, NH
SS3A	2001-13	*Pinus lambertiana*	stump	Boggs Mountain
SS3B	2001-16	same tree as SS3A		

[a] The first letter of the collection number designates the common name of the host, and the second letter designates tree (T) or stump (S) isolations. Collection numbers ending in letters are single-basidiospore strains, and those ending in a numeral are isolates from decayed wood.
[b] Collection numbers of various cooperators. Asterisks (*) denote those isolates used in the seedling inoculation study by Worrall et al (10).
[c] All isolates and strains are from stumps or diseased trees. The former may have been saprophytically colonized.
[d] All locations are in California except those from New Hampshire (NH).

TABLE 4. Compatibility among homokaryotic strains of *Heterobasidion annosum* from western North America

Strain	Pine group strains												Fir group strains									
no.	SS3B	SS3A	PT5B	PT5A	PT4A	PT3A	PT2B	PT2A	PT1B	PT1A	JS1B	JS1A	PS2B	PS2A	PS1B	PS1A	FT2B	FT2A	FT1B	FT1A	FS1B	FS1A
FS1A	−[a]	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	+	+	+	+	+	−
FS1B	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	
FT1A	−	+	−	−	+	−	−	−	−	−	−	−	+	−	+	+	−	+	−	−		
FT1B	−	+	+	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−			
FT2A	−	−	−	+	+	−	−	−	−	−	−	−	+	+	+	−	−	−				
FT2B	−	+	−	−	+	−	−	−	−	−	+	+	+	+	−	+	−					
PS1A	−	−	−	−	+	−	−	−	−	+	−	−	+	+	−	−						
PS1B	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−							
PS2A	−	−	−	−	−	−	−	−	−	−	−	−	−									
PS2B	−	−	−	−	−	−	−	−	−	−	−	−										
JS1A	+	+	+	+	+	+	+	+	+	+	+	−										
JS1B	+	+	+	+	+	+	+	+	+	+	−											
PT1A	+	+	+	+	+	+	+	+	−	−												
PT1B	+	+	+	+	+	+	+	+	−													
PT2A	−	+	+	+	+	+	−	−														
PT2B	+	+	+	+	+	+	−															
PT3A	+	+	+	+	+	−																
PT4A	+	+	+	+	−																	
PT5A	+	+	−	−																		
PT5B	+	+	−																			
SS3A	−	−																				
SS3B	−																					

[a] Compatibility determined by the presence (+) or absence (−) of clamp connections on subcultures taken from the zone of confrontation between homokaryotic mycelia after 7 days.

type locus (the A locus). Similarly, 61 of 66 pairings within the pine group resulted in clamps, and four of the five negative pairings were between strains derived from the same basidiocarp.

Four homokaryotic strains from basidiocarps on two diseased fir trees (FT1 and FT2) gave positive interactions more frequently with fir group strains (22 of 28 positive) than with pine group strains (10 of 48 positive) (Table 4). Likewise, eight strains from basidiocarps on five diseased pine trees (PT1–PT5) were more frequently compatible with strains of the pine group than with strains of the fir group (44 of 45 vs. 7 of 80 positive). Two strains from a fir stump (FS1) were compatible with only strains of the fir group (13 of 16 positive). However, of eight strains from pine stumps, four (from stumps JS1 and SS3) were more often compatible with strains of the pine group (20 of 20 vs. 2 of 20 positive), one strain (PS1A) was more often compatible with strains of the fir group (2 of 12 vs. 7 of 9 positive), and three strains (PS1B, PS2A, and PS2B) were compatible with only strains of the fir group (18 of 24 positive).

Of the 17 North American homokaryotic strains that formed clamps with European testers, all but one were compatible with either S or P tester strains but not with both (Table 5). Fifty-eight of 60 pairings between strains of the fir group and S testers from Europe resulted in clamp connections, but none of the 40 pairings of fir group strains with P testers was positive. Four of the North American pine group strains failed to form clamps with European testers, but seven other North American pine strains formed clamps with only P testers (23 of 28 positive). One (SS3A) of two strains from a basidiocarp produced on a sugar pine stump formed clamp connections after pairing with the four P testers and three of six S testers.

Heterokaryon-homokaryon pairings. Compatibility of field isolates from stumps and diseased trees in California, Oregon, and Washington was tested with S and P testers from Europe (Table 6). Clamp connections in subcultures from the homokaryotic testers was presumed to indicate dikaryotization of the tester by the heterokaryon. However, invasive growth of the original heterokaryotic hyphae into the homokaryon could give the same result.

Like the homokaryotic strains, all North American isolates from pine trees were compatible with more P testers than S testers in heterokaryon-homokaryon pairings (36 of 40 vs. 7 of 60 positive); two isolates from sugar pine stumps (SS1 and SS2), however, were compatible with only S testers (11 of 12 positive).

TABLE 5. Compatibility of western North American strains (homokaryons) of *Heterobasidion annosum* with S and P tester strains from Europe

Strain no.	P tester strains				S tester strains					
	P1	P2	P3	P4	S1	S2	S3	S4	S5	S6
FS1A	—[a]	–	–	–	+	+	+	+	+	+
FS1B	–	–	–	–	–	+	+	+	+	+
FT1A	–	–	–	–	+	+	+	+	+	+
FT1B	–	–	–	–	+	+	+	+	+	+
FT2A	–	–	–	–	+	+	+	+	+	–
FT2B	–	–	–	–	+	+	+	+	+	+
PS1A	–	–	–	–	+	+	+	+	+	+
PS1B	–	–	–	–	+	+	+	+	+	+
PS2A	–	–	–	–	+	+	+	+	+	+
PS2B	–	–	–	–	+	+	+	+	+	+
PT1A	–	–	–	–	–	–	–	–	–	–
PT1B	–	–	–	–	–	–	–	–	–	–
PT2A	–	–	–	–	–	–	–	–	–	–
PT2B	–	–	–	–	–	–	–	–	–	–
JS1A	+	+	+	+	–	–	–	–	–	–
JS1B	+	+	+	+	–	–	–	–	–	–
PT3A	–	–	+	–	–	–	–	–	–	–
PT4A	+	+	+	+	–	–	–	–	–	–
PT5A	+	+	+	+	–	–	–	–	–	–
SS3A	+	+	+	+	–	+	+	+	–	–
SS3B	+	–	+	–	–	–	–	–	–	–

[a] Compatibility determined by the presence (+) or absence (–) of clamp connections on subcultures taken from the zone of confrontation between homokaryotic mycelia after 7 days.

Most heterokaryotic isolates of the fir group (including SS1 and SS2) were compatible with only S testers (94 of 114 pairings positive), but isolate FT9 was also compatible with tester P4 (Table 6). This subcultured dikaryon (from the P4 mycelium) was later paired with the 10 S and P tester strains, and it apparently dikaryotized only the four P testers, including tester P4.

Another unpredicted dikaryon was isolated from the pairing between a European tester (S3) and a Californian isolate (PT3), and it produced basidiocarps in vitro. Eleven single-basidiospore progeny were obtained, and homokaryon-homokaryon pairings among these progeny and with homokaryotic strains derived from basidiocarps of the PT3 parent isolate demonstrated that the synthesized dikaryon (S3 × PT3) carried both the mating type factors (A alleles) of the parent PT3 heterokaryon. When the 11 progeny were paired with five S and P testers, they were compatible with testers P1 and P3 but not S2, S3, or S4.

The distinction between the North American fir and pine groups was not readily apparent when heterokaryotic isolates were paired with homokaryotic strains from California and New Hampshire (Table 7). As expected, two of the fir group homokaryons (strains FS1A and PS2A) appeared to be dikaryotized only by heterokaryons that were compatible with S testers from Europe; one pine group homokaryon (strain PT1B) was apparently dikaryotized only by heterokaryons that were compatible with European P testers. The other eight homokaryotic strains from North America formed clamps after pairing with either fir or pine group heterokaryons. Isolate FT7 was unusual in that it was S-compatible with European testers (Table 6), but it apparently dikaryotized a higher proportion of North American pine group strains than fir group strains (3 of 7 vs. 1 of 4 positive) (Table 7).

Seven subcultures from fir group strains that had been putatively dikaryotized by isolates of the pine group and six subcultures of pine group strains that had been putatively dikaryotized by fir group isolates (Table 7) were paired with S and P tester strains. Twelve of these dikaryons appeared to be

TABLE 6. Compatibility of North American isolates (heterokaryons) of *Heterobasidion annosum* in heterokaryon-homokaryon pairings with S and P tester strains from Europe

Isolate no.	P tester strains				S tester strains					
	P1	P2	P3	P4	S1	S2	S3	S4	S5	S6
CT1	—[a]	–	–	–	+	+	+	–	+	–
DS1	–	–	–	–	+	+	+	+	+	+
FT3	–	–	–	–	–	+	–	+	+	+
FT4	–	–	–	–	–	+	+	+	+	+
FT5	–	–	–	–	+	+	+	+	–	–
FT6	–	–	–	–	–	+	–	+	+	+
FT7	–	–	–	–	–	+	+	–	–	+
FT8	–	–	–	–	+	+	+	+	+	+
FT9	–	–	–	+	+	+	+	+	+	+
FT10	–	–	–	–	–	+	+	+	–	–
FT11	–	–	–	–	–	+	+	+	+	–
FT12	–	–	–	–	+	+	+	+	+	+
FT13	–	–	–	–	–	+	–	+	+	+
FT14	–	–	–	–	+	+	+	+	+	+
GT1	–	–	–	–	+	+	+	+	+	+
HT1	–	–	–	–	+	+	+	+	+	+
HT2	–	–	–	–	+	+	+	+	+	+
SS1	–	–	–	–	–	+	+	+	+	+
SS2	–	–	–	–	+	+	+	+	+	+
JS1	+	+	+	+	–	–	+	–	–	–
JS2	+	–	+	+	–	–	–	–	–	–
JT1	+	+	+	+	–	–	–	–	–	–
PT1	+	+	+	–	–	–	–	–	–	+
PT2	+	+	+	+	–	–	–	–	–	–
PT3	+	+	+	+	–	–	+	–	–	–
PT4	+	+	+	+	–	–	+	–	–	–
PT6	+	+	+	+	–	–	–	–	–	–
PT7	+	–	+	+	–	–	+	+	–	–
PT8	+	–	+	+	+	–	–	–	–	–

[a] Compatibility determined by the presence (+) or absence (–) of clamp connections on subcultures taken from the homokaryotic mycelia 5 wk after pairing with the heterokaryotic isolate.

TABLE 7. Compatibility of North American isolates (heterokaryons) of *Heterobasidion annosum* in heterokaryon-homokaryon pairings with North American strains

Isolate no.	Pine group strains							Fir group strains			
	PT1B	JS1A	PT4A	PT5A	RT1A	RT2A	SS3A	FS1A	PS2A	FT1B	PS1A
CT1	–[a]	–	–	–	+	+	–	+	+	+	+
FT3	–	–	–	–	–	+	–	+	+	+	+
FT4	–	–	–	–	+	+	+	+	+	+	–
FT5	–	–	–	–	–	+	–	+	+	+	+
SS1	–	–	–	–	–	+	–	+	+	+	+
DS1	–	–	+	–	+	+	+	+	+	+	+
FT6	–	–	–	–	–	–	+	+	+	+	–
FT7	–	+	–	–	+	–	+	–	–	–	+
FT8	–	–	–	–	–	–	–	+	+	+	+
FT9	–	–	–	–	+	+	+	+	+	+	+
FT10	–	–	–	–	+	+	+	+	–	+	+
FT11	–	–	+	–	+	+	+	+	+	+	+
FT12	–	+	–	–	+	+	+	+	+	+	+
FT13	–	–	–	–	+	+	–	+	+	+	+
FT14	–	+	+	–	+	+	+	+	+	+	+
GT1	–	+	+	–	+	+	+	+	+	+	+
HT1	–	+	+	–	+	+	+	+	+	+	+
HT2	–	+	+	+	+	+	–	+	+	+	+
SS2	–	–	–	–	–	–	+	–	+	+	+
JS2	+	+	+	+	+	+	+	–	–	–	+
PT1	+	+	+	+	+	+	+	–	–	–	–
PT2	+	+	+	+	+	+	+	–	–	–	+
PT7	+	+	+	+	+	+	+	–	–	–	–
JS1	+	+	+	+	+	+	+	–	–	+	+
JT1	+	+	+	+	+	+	+	–	–	–	+
PT3	+	+	+	+	+	+	+	–	–	+	+
PT4	–	+	+	+	–	–	+	–	–	+	+
PT6	+	+	+	+	+	+	+	–	–	+	+
PT8	+	+	+	+	+	+	–	–	–	–	+

[a] Compatibility determined by the presence (+) or absence (–) of clamp connections on subcultures taken from the homokaryotic mycelia 5 wk after pairing with the heterokaryotic isolate.

Table 8. Compatibility of anomalous dikaryons of *Heterobasidion annosum* synthesized from heterokaryon-homokaryon pairings with S and P tester strains from Europe

Heterokaryon parent	Homokaryon parent	P testers			S testers		
		P1	P3	P4	S2	S4	S5
Fir group × pine group							
FT12	PT4A	+[a]	+	+	–	–	–
FT12	RT1A	+	+	–	+	+	–
FT12	RT2A	+	+	+	+	+	+
HT1	JS1A	–	+	+	+	+	+
HT1	PT4A	+	+	–	+	+	+
HT1	RT1A	+	+	+	+	+	+
HT1	RT2A	+	+	+	+	+	+
Pine group × fir group							
PT3	FT1B	+	+	+	+	+	+
PT3	PS1A	+	+	–	+	+	+
PT4	FT1B	+	+	+	+	+	+
PT4	PS1A	+	+	–	+	+	+
PT6	FT1B	+	+	–	+	+	+
PT6	FT2B	+	+	–	+	+	+
PT6	PS1A	+	+	–	+	+	+

[a] Compatibility determined by the presence (+) or absence (–) of clamp connection on subcultures taken from the homokaryotic mycelia 5 wk after pairing with the synthesized dikaryon.

synthesized hybrids of the pine and fir type because they apparently dikaryotized both S and P testers (Table 8). The other dikaryon (a subculture of strain PT4A after pairing with FT12) dikaryotized only P testers. Isolate FT12, however, was compatible with S, but not P testers, in earlier pairings (Table 6).

Tester strains with V^+ loci. The 11 homokaryotic North American strains used in Table 7 and the S and P testers of Korhonen were also paired with a collection of North American strains provided by Chase. The latter reportedly carry a variety of positive alleles that allow compatibility between S and P type strains, i.e., they are positive at one or more of three loci (loci V1, V2, or V3) (1). Consistent with the results from homokaryon-homokaryon (Table 4) and heterokaryon-homokaryon pairings (Table 7) among the North American strains and isolates, strain PT1B was compatible with only Chase's P^+ strains, regardless of the positive alleles at the V loci (data not shown). Similarly, strain FS1A was compatible with only Chase's S^+ strains. However, strain PS2A (which was compatible with only fir group strains and isolates from North America [Table 4] and S testers from Europe [Tables 5 and 7]) was compatible with one of Chase's P^+ V^+ strains as well as most of his S^+ V^+ strains. The other eight North American strains from Table 7 (which were each compatible with both fir and pine group strains and isolates) were compatible with most of the S^+ and P^+ strains that were identified by Chase to have positive V loci.

The four P testers of Korhonen were compatible with only Chase's P^+ strains, and S5 and S6 were compatible with only Chase's S^+ strains. However, testers S1, S2, S3, and S4 were each compatible with at least one of Chase's P^+ V^+ strains.

Seedling pathogenicity. In the earlier inoculation study (10), ponderosa pine and white fir seedlings were wound-inoculated with isolates from stumps and diseased trees of pine and fir. The percentage of infected pine seedlings was compared with the percentage of infected fir seedlings, and this difference is illustrated in Figure 1. A bimodal distribution of the isolates is suggested, with one population infecting many more pine seedlings than fir seedlings (a difference greater than 30%), and another population infecting about the same percentage of pine and fir seedlings (a difference less than 30%). Of those isolates still available for compatibility testing (Tables 2 and 3), those compatible with European P testers each infected more pine than fir seedlings, whereas S-compatible isolates infected about the same percentage of pine and fir seedlings (Fig. 1). Of the isolates unavailable for compatibility testing (shown as triangles and circles in the figure), five from diseased pines trees and one from a pine stump infected more pine than fir seedlings. Conversely, four additional isolates from fir trees showed little host preference, but the three isolates from fir stumps infected more pine seedlings than fir seedlings.

DISCUSSION

The fir and pine groups of *H. annosum* in California that were demonstrated to be host-specialized in the seedling inoculation study (10) appear to correspond with the S and P intersterility groups identified by Korhonen (6). The S and P tester strains that we used had been selected by Korhonen for their capacity to differentiate the two intersterility groups in compatibility testing, and two intersterile groups were clearly seen when these 10 tester strains were paired among themselves. A similar trend was seen when North American strains were paired among themselves, but there were a number of anomalous positive reactions between strains of the North American fir and pine groups. Strains of the fir group were compatible with S but not P testers. Some strains of the pine group did not form clamps with any European tester strain, but most of those that did form clamps did so only with P testers. Heterokaryon-homokaryon pairings, likewise, indicated that there were two groups among the North American isolates and that these groups correspond to the S and P intersterility groups identified by Korhonen.

The fir and pine groups in California were not found to be strictly intersterile, but neither were the S and P groups in Europe (6). Positive pairings between S and P or fir and pine groups are consistent with Chase's (1,3) model of the genetic basis for intersterility in *H. annosum*. This model consists of a multi-locus, biallelic (+ or −) compatibility system that is superimposed on the bipolar (single-locus, A factor, and multiallelic) mating type system. Two strains heteroallelic at the A locus are capable of forming a dikaryon only if they have a common positive allele for at least one of five loci (S, P, V1, V2, and V3). All naturally derived strains tested by Chase were either positive at the S locus or the P locus, but not both, and this determined whether the strains belonged to Korhonen's S or P group. Many North American strains were positive at one or more of the other three loci (V1, V2, or V3), but Korhonen's S and P tester strains were mostly negative at the three identified V loci. Thus, pairings between North American strains that were $S^+ P^-$ and $S^- P^+$ (representing the S and P groups, respectively) frequently resulted in clamps because the two strains could have a common positive allele at one or more of the V loci.

Most of our fir and pine group strains from North America were apparently positive at one or more V loci. The P tester strains from Europe appeared to be negative at the V loci, but the S tester strains from Europe showed evidence of positive V loci. For instance, testers S2, S3, and S4 were compatible with SS3A, a pine group strain from California. Furthermore, in pairings with Chase's strains, evidence of V^+ alleles were found in S1, S2, S3, and S4.

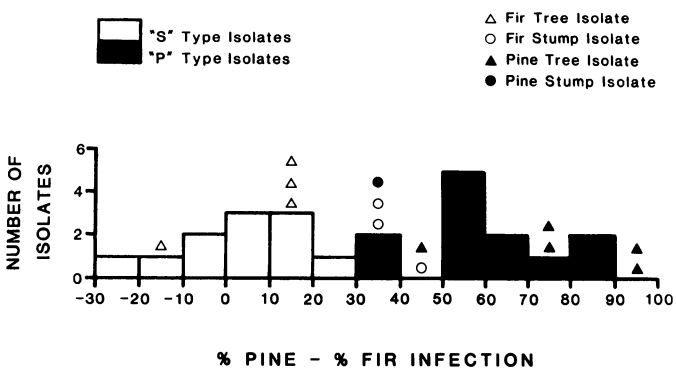

Fig. 1. Frequency of distribution of host preference for fir (S-type) and pine (P-type) isolates of *Heterobasidion annosum* from California. Percentage of seedlings of ponderosa pine that were infected minus the percentage of seedlings of white fir that were infected is used for host preference, data derived from Worrall et al (10). Data from two experiments are combined, and those isolates used in both experiments are plotted twice in the figure. S and P types (bars) were determined by compatibility with European tester strains (Table 6). Triangles and circles represent isolates unavailable for compatibility testing.

In heterokaryon-homokaryon pairings, the S and P testers supplied by Korhonen clearly differentiated fir and pine group isolates. However, three of the S testers (S1, S3, and S6) infrequently formed clamps with pine group isolates from North America. Four of the seven compatible reactions between S tester strains and pine group isolates were with tester S3. The basidiospore progeny of one such dikaryon (S3× PT3) were compatible in homokaryon-homokaryon pairings with P but not S testers. Perhaps both nuclear types from PT3 migrated into the homokaryotic mycelium of S3, maintained the dikaryon, and supplanted the S3 nucleus in the mycelium. More likely, heterokaryotic PT3 hyphae had grown across the confrontation zone into the tester without fusing, an occurrence noted rarely by Korhonen (6). In either case, the S3 strain may be particularly prone to such reactions, and this phenomenon may not be related to V^+ alleles.

Only one pairing between a P tester from Europe and a fir group isolate (P4 × FT9) resulted in a dikaryon, and when this newly synthesized dikaryon was paired with the S and P testers, it dikaryotized only P testers. The dikaryotization of P4 by FT9 appeared to result in a dikaryon carrying P^+ but not S^+ alleles. A mutation at the A locus of P4 or, more likely, recombination between the genomes of P4 and FT9 that included incorporation of one of the mating type alleles of FT9 into a P4 nucleus could explain this anomalous dikaryotization.

In most cases of dikaryotization of homokaryons by heterokaryons, it would appear that one of the nuclei from the heterokaryon paired with the nucleus of the homokaryon to establish a new dikaryon. Because we subcultured from only 1 cm behind the confrontation line, it is possible that a few of our dikaryotic subcultures resulted from invasive ingrowth of heterokaryotic hyphae before subculture. As noted above, European tester S3 may have been particularly prone to such invasive growth. However, when we subcultured at 3–4 cm from the confrontation zone, we obtained results similar to those with subculturing from 1 cm away, except that there were fewer positive reactions. Of the tested 13 dikaryons putatively synthesized by heterokaryon-homokaryon pairings of North American isolates and strains, hybridization was demonstrated in 12 of the dikaryons by their compatibility with both S and P tester strains in further heterokaryon-homokaryon pairings. Close agreement in results between heterokaryon-homokaryon pairings and homokaryon-homokaryon pairings indicates that there is a common mechanism of dikaryotization in these two tests, i.e., nuclear migration into homokaryotic hyphae and establishment of a new pair of sexually compatible nuclei. Korhonen's (6) observations on changes in mycelial morphology of homokaryotic testers after prolonged pairing with heterokaryotic isolates also supports the conclusion that heterokaryons of *H. annosum* are capable of dikaryotizing homokaryons through the Buller Phenomenon.

When Korhonen used heterokaryon-homokaryon pairings to differentiate S and P group isolates, he usually chose changes in morphology of tester mycelia as his criterion rather than microscopic examination of hyphae for clamp connections. We attempted to use his morphological criterion, but with the homokaryons we employed, it was subjective and inconsistent with results based on microscopic examination. This may have been due to the age of the strains and isolates that we used, many of which may have deteriorated, as evidenced by extremely slow growth and very rare clamp formation after pairing with compatible isolates.

Success in using heterokaryon-homokaryon pairings to differentiate fir and pine group isolates from North America allowed us to test the heterokaryotic isolates used in the earlier seedling-inoculation study. Those isolates demonstrated to be host-specialized to pine seedlings were shown to be compatible with Korhonen's P intersterility group, and the others were compatible with Korhonen's S group. Stenlid and Swedjemark (9) recently presented evidence of host-specialization of European isolates of the S and P intersterility groups to Norway spruce and Scotch pine.

Based on compatibility testing of 780 isolates from Finland, Korhonen (6) reported that P isolates were found killing pines of all ages, causing butt rot of spruce, and mortality of some

hardwoods and *Juniperus communis* L. S group isolates were found causing butt rot of spruce and killing only saplings of pine. Too few North American isolates of *H. annosum* from diseased trees have been tested to draw strong conclusions on host ranges of the two groups on this continent, but the reports to date indicate relatively distinct host ranges.

Thus far, only pine group isolates have been identified in eastern North America. Using reports from diseased trees only and disregarding isolates and strains from possibly saprophytically colonized stumps, we find that pines are the primary host. Korhonen (6) identified as P-compatible 10 isolates from *Pinus* spp. in eastern North America, and Chase (1) identified strains from two diseased pine trees. Likewise, we have found only the pine group in northeastern North America: six isolates from diseased pines, one from dying *J. virginiana* L., and one from a *Picea abies* tree with butt rot (*unpublished*).

Chase (1) identified strains of S-compatible *H. annosum* from nine trees in western North America: five were *Abies* spp., two were *Thuja plicata* Donn., and two were *Tsuga heterophylla* (Raf.) Sarg. P-compatible strains were identified from six trees of *Pinus ponderosa* in Montana and one in Oregon. In the present study, all of the isolates and strains from diseased trees of *Pinus* spp. were of the pine group, and all those from diseased hosts of other genera were of the fir group. It is clear, however, that isolates from stumps can be either S- or P- compatible, regardless of the species of the stump. The same can be seen in the results of the seedling-inoculation study (10), in which three isolates from fir stumps showed a host preference for pine seedlings.

The intersterility groups of *H. annosum* may represent the early stages of speciation (1,3). Slight differences in morphology (5,6), physiology (6,9,10), geographic distributions (6), and ecology between these two groups are coincidental with a specific genetic background determining within-group compatibility (1,3). Further collections and testing of field isolates and strains will be needed to clearly determine the geographic distributions and host ranges of the fir and pine groups in North America and elsewhere. Field and greenhouse inoculations of a range of hosts with S-compatible and P-compatible isolates and their hybrids will also be needed to understand the nature of host-specialization in this important forest pathogen.

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