Host Specialization of *Heterobasidion annosum*

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ABSTRACT

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A total of 30 isolates of *Heterobasidion annosum* (*Fomes annosus*) from *Pinus ponderosa* and *Abies concolor* were inoculated into seedlings of both hosts in two greenhouse experiments. Two-way analyses of variance showed significant differential interaction between isolate groups and host species. Isolates from one host were more virulent on that host than isolates from the other host. Mortality data and tissue colonization patterns parallel observations of *H. annosum* on the two hosts in nature.

The host ranges of many plant pathogens, particularly obligate parasites, are restricted by physiological specialization. Important control methods such as crop rotation and use of resistant cultivars rely on this phenomenon (22). Fungi causing root and butt rots of forest trees are classified as ecologically obligate symbionts (4) because, although they are necrotrophic and can survive for considerable periods in dead host tissue, they generally compete poorly as saprophytes and usually colonize only fresh host material. Thus, despite the very wide host ranges of fungi such as *Armillaria mellea* (Vahl) Kummer and *Heterobasidion annosum* (Fr.) Brefeld (*Fomes annosus* (Fr.) Cooke), the assumption that no host-specialized forms exist may not be warranted.

H. annosum is considered one of the most important forest pathogens worldwide and attacks many species of trees (7,8,18). Isolates vary in several physiological characters (1,5,7,18,20), including virulence (6,13,16), but these characters have not been correlated with host of origin. In Finland, Korhonen (14) discovered two intersterility groups that correlated somewhat with host of origin. His S group was found primarily on spruce, although it was also isolated from pine saplings, particularly those adjacent to spruce stumps. It also was isolated from many exotic conifers. The P group was isolated commonly from pine as well as from pure stands of spruce.

Over much of California, white fir (*Abies concolor* (Gord. & Glend.) Lindl.) and ponderosa pine (*Pinus ponderosa* Laws.) are common hosts of *H. annosum*. Our observations suggest that at higher elevations, where pine and fir occur together and provide ample opportunity for the fungus to attack both species within a single infection center, *H. annosum* is commonly found only on fir and mortality in such centers rarely includes both species. *H. annosum* occurs on pine more often at lower elevations where fir is less frequent. Host specificity was considered as a possible explanation for such observations. Thus, the study reported here was designed to investigate the existence of pathogenic variants on these hosts.

MATERIALS AND METHODS

The experiment was performed twice in the greenhouse by inoculating pine and fir seedlings with isolates collected from both pine and fir growing in six widely separated forests in California (Table 1); only one isolate was collected from each disease locus. Isolates used in experiment 1 were from both stumps and diseased trees; those in experiment 2 were from diseased trees only and were collected by the authors within 6 mo prior to the experiment, except for three each from pine and fir, which were used in both experiments. Isolates were grown on PDA (first experiment) or lima bean agar (2) (second experiment) in jars for 10 days before adding autoclaved wedges (1.2 × 2.5 cm) of sapwood from ponderosa pine (first experiment) or beech (second experiment). These were grown for about 80 days with occasional shaking. Two-year-old bare-root seedlings of white fir and ponderosa pine were potted and grown for 6 mo prior to inoculation in a mixture of peat, sand, and Redwood Soil Conditioner (Berkeley Horticultural) (1:1:2, v/v) before inoculation. Using aseptic technique, we made an oblique incision 3 cm above the soil line to about one-third of the stem diameter, placed a colonized wedge into the wound, and tightly wrapped the area with thermoplastic film (Parafilm, American Can Co., Greenwich, CT). Seedlings were dormant when inoculated in December (first experiment) and October (second experiment). In the first experiment, 27–30 seedlings of each host were inoculated; in the second experiment, 28 seedlings were inoculated with each isolate. Control seedlings (30 in experiment 1 and 28 in experiment 2) were inoculated with sterile blocks.

Seedlings were watered as needed and examined every 4–5 days for symptoms. When symptoms were sufficiently advanced (see Results), seedlings were removed from the soil, stripped of branches, lateral roots and needles, swabbed with an ethanol-bleach-water (1:1:8, v/v) solution, split lengthwise, and incubated in a moist chamber for 3 or 4 days. Colonization by *H. annosum* was determined by measuring the distance conidiophores were produced above and below the inoculation point.

RESULTS

Although the symptoms on pines and firs differed, no clear differences were observed in symptoms caused by the two isolate groups. Pines showed first a mottled chlorosis and then dying of the lower needles. Resin often began to exude in droplets from the stem up to 5 cm above the inoculum block. Later, the upper needles of many pine seedlings became chlorotic, and the upper stem began to shrivel. Phloem necrosis (observed by making a slight break in the bark with a thumbnail) rapidly extended up from the block. Pines were harvested when phloem necrosis involved one-fourth to one-third of the stem length. Little or no color change was observed on fir foliage. The first crown symptom on firs was a flaccidity of the needles upon bending, followed by a subtle shriveling of the smaller twigs and then the upper stem. Spread of phloem necrosis was much slower and more limited than in pines, and firs were harvested when any phloem necrosis was detected.

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The experiments were terminated when mortality had declined substantially and some isolates had killed all of one host. Three control seedlings (two pines and one fir) died in the first experiment and none in the second.

At the termination of experiment 1, all remaining seedlings were collected and incubated as above. In experiment 2, all seedlings showing early symptoms and a sample of the remaining apparently healthy seedlings were collected. Seedlings were considered infected either when killed or when *H. annosum* had colonized beyond the tissues in contact with inoculum. Infection and mortality generally followed similar trends (Table 2). In experiment 2, however, pine isolates killed about 50% of the firs and 90% of the pines they infected, while fir isolates killed only about half the trees they infected.

Rate of mortality (Fig. 1) in experiment 1 was greatest between 50 and 100 days after inoculation. Experiment 2 showed similarly shaped mortality curves except that the highest incidence was between 35 and 80 days.

In both experiments, isolates from one host generally caused greater mortality to that host than did isolates from the other host (Table 2), although some differences were not statistically significant. Pine isolates were consistently more virulent on pine than on fir, causing over four times more mortality on pine in both experiments. Fir isolates caused about 50% higher mortality on pine than on fir in experiment 1 and a similar mortality level on the two hosts in experiment 2.

Percentage data were arc sine-transformed (arc sine $x^{1/2}$ where x = the percentage) prior to analysis. Two-way analyses of variance for both experiments (Table 3) showed a significant difference between isolate groups in relative killing of the two hosts (interaction, $P < 0.01$). Comparisons between isolate groups on a given host were made with an unpaired *t*-test and a paired *t*-test was used to compare an isolate group on pine versus fir seedlings (Table 2). Overall, more pines were killed than firs. In experiment 1, even fir isolates killed significantly more pines than firs ($P < 0.01$). However, this difference was much more pronounced with pine isolates ($P < 0.01$). Also, fir isolates killed significantly ($P < 0.01$) more firs than did pine isolates, thus contributing to significant interaction. In experiment 2, fir isolates killed about the same number of pines and firs, but as in experiment 1, pine isolates killed more pines than firs ($P < 0.01$). The pine isolates also killed more pines than did the fir isolates ($P < 0.01$).

Differences were observed in the pattern of tissue colonization in the two species. With rare exceptions, colonization in firs was confined to narrow strips in the xylem, usually close to the pith. Often firs were colonized in this way over much of their length. In pines, advancing margins of colonization were generally confined to the phloem and cambial region, although in cases where colonization exceeded several centimeters, the xylem was usually colonized shortly behind the margin. This pattern held true irrespective of the inoculum source.

TABLE 1. California isolates of *Heterobasidion annosum* from pine and fir that were used in greenhouse inoculations of pine and fir seedlings

Designation[a]	Host[b]	Tree[c]	Stump[c]	Location[d]
Isolates from pine				
†Mod 12P	PP	X		MNF
†Bog 3P	PP	X		BMSF
†Bog 7P	PP	X		BMSF
†Las 3P	PP	X		LNF
†Las 8P	PP	X		LNF
†Las 11P	PP	X		LNF
JL1	JP		X	SBNF
PP1	PP	X		SBNF
JP6	JP		X	SBNF
HB11	JP		X	SBNF
PP2	PP	X		SBNF
Y7	PP	X		YNP
JP7	JP		X	SBNF
Y2	PP	X		YNP
12-3-2	PP	X		SBNF
Isolates from fir				
†Mod 14F	WF	X		MNF
†Mod 15F	WF	X		MNF
†Las 1F	WF	X		LNF
†Las 7F	WF	X		LNF
†Las 17F	WF	X		LNF
†Las 21F	WF	X		LNF
35-3-3	WF	X		SBNF
36A4-21	WF	X		SBNF
28-4-2	WF	X		SBNF
††O Las	WF		X	LNF
††Hell	WF		X	EDNF
††S Las	WF		X	LNF
21-4-3	WF	X		SBNF
††304	WF	X		LNF
††321	WF	X		LNF

[a] Daggers († and ††) indicate isolates collected in 1981 or 1976–1978, respectively, by the authors. Other isolates were collected prior to 1978 by R. L. James.

[b] JP = *Pinus jeffreyi*, PP = *Pinus ponderosa*, and WF = white fir (*Abies concolor*).

[c] Diseased tree isolates were clearly pathogenic in nature. Isolates from stumps may have been causing disease in the trees before felling or may have infected the stumps after felling.

[d] MNF = Modoc National Forest, BMSF = Boggs Mountain State Forest, LNF = Lassen National Forest, SBNF = San Bernardino National Forest, YNP = Yosemite National Park, and EDNF = El Dorado National Forest.

DISCUSSION

These results demonstrate physiological specialization in populations of *H. annosum* from pine and fir. The highly

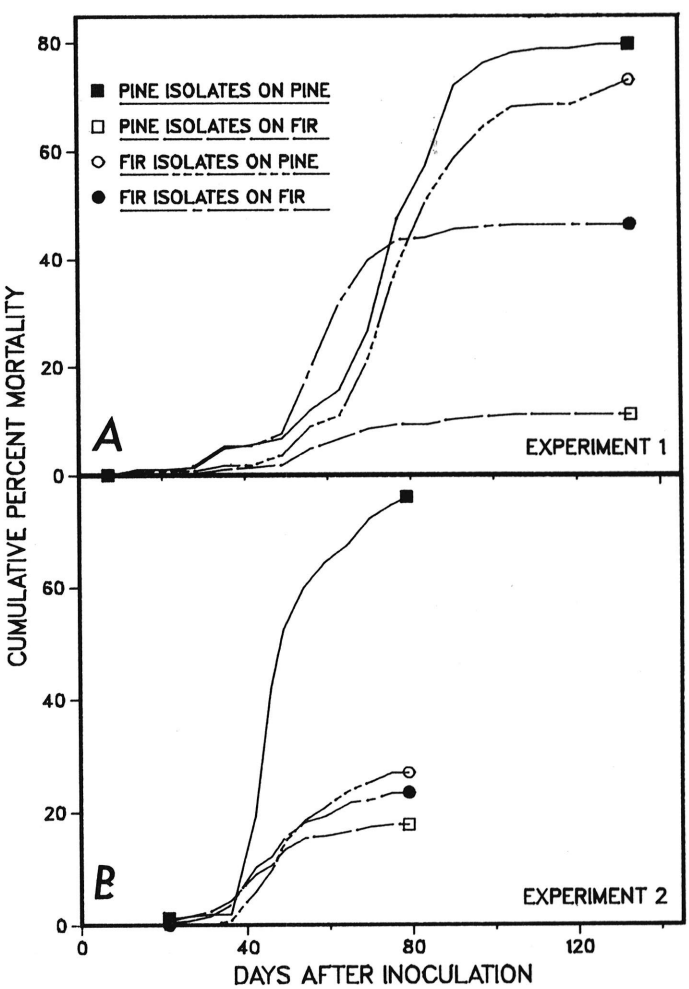

Fig. 1. Rates of mortality of fir and pine seedlings inoculated with isolates of *Heterobasidion annosum* from pine and fir in **A**, experiment 1 and **B**, experiment 2.

TABLE 2. Percent infection and mortality of ponderosa pine and white fir seedlings inoculated with isolates of *Heterobasidion annosum* from pine and fir

Pine isolates (PI)	Fir isolates (FI)	Infection[v]				Mortality			
		Pine inoculated with		Fir inoculated with		Pine inoculated with		Fir inoculated with	
		PI	FI	PI	FI	PI	FI	PI	FI
Experiment 1[w]									
JL1	35-3-3	88	93	0	78	85	90	0	74
PP1	36A4-21	77	100	40	87	67	79	37	77
JP6	28-4-2	87	73	19	57	83	73	11	54
HB11	O Las	41	72	0	31	41	72	0	24
PP2	Hell	70	31	0	0	67	31	0	0
Y7	S Las	97	87	0	57	90	83	0	57
JP7	21-4-3	100	61	70	63	100	53	37	50
Y2	304	97	73	7	54	93	63	7	38
12-3-2	321	90	97	15	73	90	97	12	60
$\bar{x}$[y]		83 c	76 b	17 ac	56 ab	80 d	71 e	12 df	48 ef
Experiment 2[x]									
JL1	35-3-3	100	75[z]	14	89	100	39	11	61
PP1	36A4-21	96	57[z]	32	68	86	14	7	11
JP6	28-4-2	61[z]	32[z]	7	21	32	7	0	0
Mod 12P	Mod 14F	100	21[z]	50	21	89	21	36	7
Bog 3P	Mod 15F	89[z]	75[z]	14	96	71	61	11	61
Bog 7P	Las 1F	54[z]	100[z]	0	93	32	64	0	43
Las 8P	Las 7F	93	100[z]	36	75[z]	86	25	18	18
Las 8P	Las 17F	100	0[z]	61	0	96	0	36	0
Las 11P	Las 21F	100	11[z]	43	14	100	11	39	11
$\bar{x}$[y]		88 gH	52 H	29 g	53	77 ij	27 i	17 j	23

[v] Seedlings were considered infected either if dead or *H. annosum* had colonized beyond the inocuation point at the end of the experiment.
[w] Each isolate was inoculated into 27–30 seedlings of each host.
[x] Each isolate was inoculated into 28 seedlings of each host.
[y] Pairs of means, within infection or mortality groups followed by the same lowercase letters are significantly different, $P<0.01$, and the single pair followed by capital Hs are significantly different, $P<0.05$. Unpaired *t*-tests were used to compare isolate group means and paired *t*-tests were used to compare hosts inoculated with a given isolate group. Arc sine transformation of percentages was performed prior to analysis.
[z] Based on a sample of apparently healthy seedlings at the end of the experiment.

TABLE 3. Results of analyses of variance for mortality[a] of ponderosa pine and white fir seedlings inoculated with isolates of *Heterobasidion annosum* from pine and fir

	Experiment 1			Experiment 2		
	Mean square	F	P	Mean square	F	P
Host species	11,236	41	0.01	5,232	15.8	0.01
Isolate group	1,229	4.5	0.05	2,567	7.7	0.01
Interaction	2,276	8.3	0.01	3,681	11.1	0.01
Error	275			332		

[a] Percent mortality was arc sine-transformed prior to analysis.

significant interaction term indicates that the relative virulence of the two isolate groups depends on the host species. Korhonen (14) reported the existence of intersterile groups in Finland, which differ in the frequency of their isolation from different hosts. This suggests that a similar phenomenon may occur with Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). Preliminary matings of single-basidiospore isolates from California indicate that clamp formation is infrequent and that these isolates are less amenable to Korhonen's (14) technique (*unpublished*). Nevertheless, further collections and pairings may yield information on possible mating barriers.

Although fungal isolates and host species showed highly significant differential interaction in both experiments, the results of the two experiments suggest alternative interpretations of the nature of the interaction. The results of experiment 1 indicate that pine and fir isolates were almost equally able to infect and kill pine, but fir isolates were significantly more virulent to fir than to pine. While the pine isolates behaved consistently in the two experiments, the fir isolates in the second experiment were less virulent overall than before. As a result, in experiment 2 the pine isolates were much more virulent than were fir isolates to pine, but the two groups were not significantly different in virulence on fir. Thus, in experiment 1 the differential host was fir and in experiment 2 it was pine.

It seems unlikely that use of different fir isolates between experiments can fully explain this discrepancy since the isolates common to both experiments followed this trend. Local weather records indicate that average temperatures during the first 2 mo of experiment 2 were about 20 C as compared with 16 C for experiment 1. This corresponds with the more rapid progress of disease in experiment 2. Kuhlman (15), working with isolates from the southeastern United States, found that high soil temperatures (20–25 C) led to earlier mortality, but higher temperatures (30–35 C) resulted in significantly less mortality. Perhaps in our experiments the higher temperature selectively suppressed the aggressiveness of the fir isolates, which in nature cause disease at higher elevations under presumably cooler temperatures.

A clear understanding of the nature of differences between isolate groups in relative virulence on different hosts is important in assessing the implications of these results. Korhonen's isolation data (14) suggest that pine is a differential host: whereas his S group was practically restricted to spruce, the P group, in addition to infecting mature pine, was also associated with a butt rot in spruce. The potential for spread from pine to other species must also be considered in light of work by Greig (10). A severely infested Scots pine stand in England was underplanted with a number of species, including white fir. After 8 yr, up to 50% mortality from *H. annosum* and up to 15% in white fir had occurred in the understory as a whole. Gibbs (8) cites other instances of spruce killed after replacing pine. In California, we have observed that incense cedar (*Libocedrus decurrens* Torri) is commonly infected within spreading centers of *H. annosum* in ponderosa pine.

The heavy mortality of pines inoculated with pine isolates may be partially a function of the apparently greater susceptibility of living pine tissues. The mottled chlorosis in the upper needles of pine, far in advance of colonization, suggests that a toxin may be involved. Similarly suggestive symptoms were observed by Kuhlman (16) and a toxic metabolite is produced in vitro by *H. annosum* (1,12), but preliminary attempts to detect fommanosin in vivo have failed (C. Bassett, *personal communication*).

Mortality data and tissue colonization patterns reflect observations in nature: in pine, the fungus attacks the sapwood and cambium, girdling and killing trees, whereas fir and spruce usually sustain prolonged root and heartrot (3,8,21). The preferential colonization of the cambial region of pine and the inner wood of fir observed here was also noted in field inoculations by Gibbs (8) of pine and spruce, respectively. Together with the much slower progress of phloem necrosis of fir in this study, these observations suggest that fir has more resistant outer sapwood, cambium, and phloem than does pine. Thus, fir may have shown greater overall resistance than pine partly because no heartwood was present in the seedlings as it generally is in most natural fir hosts. In California, *H. annosum* often kills pine seedlings and saplings, but not young firs.

It is worth noting that pine isolates from stumps were similar in virulence to other pine isolates, while two fir stump isolates (named "O Las" and "Hell") caused mortality more like that caused by pine isolates. This can be explained if we assume, according to the hypothesis discussed above, that the "pine type" can also attack fir (presumably especially fir stumps) and be collected as a fir isolate. However, it must also be noted that isolates from pine stumps were from pure pine stands in the San Bernardino National Forest in California where trees were removed because they were dead or dying.

The absence of heartwood in the seedlings used here, the frequently greater resistance of mature trees to some diseases (9,19), and evidence that host specialization of some diseases may be more pronounced with mature trees (11,17) suggest that final evaluation of the significance of these results to forest management should involve additional long-term work in the field. In the absence of such information, forest managers should be encouraged to replant severely infested stands with several species on a trial basis where practical.

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