

Preliminary results on the genetic structure of *Heterobasidion annosum* in white fir (*Abies concolor*) root decay centers.

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## INTRODUCTION

It is known that *Heterobasidion annosum* is a complex species comprised of at least three biological species, more precisely defined as intersterility groups (ISGs) (1,2). The S ISG is widely diffused in North America, Europe and probably Asia. Although with regional variations, S ISG isolates are commonly found associated with *Picea spp.*, *Abies spp.*, *Tsuga spp.*, *Pseudotsuga* and *Sequoiadendron*.

Our study focuses on the S ISG- white fir (*Abies concolor*) association in the two main mountain ranges of the Western Pacific United States; the Cascades and the Sierra Nevada. The rationale for this study lies in the paucity of information regarding the above mentioned pathosystem. Nevertheless *H. annosum* plays an important role in fir or fir-dominated forests. It has been reported (3) that as of 1980 up to 18% of 632000 acres surveyed were infested by this pathogen, with a total of 25% of fir mortality due to annosum. Our field observations have confirmed that *H. annosum* is not simply a butt rot in white fir. Instead it is also commonly found in the sapwood, thus causing vigor decline and/or mortality. The increase of the fir component due to "selective" logging practices and fire suppression only reinforces the need for a better understanding of the ecology and epidemiology of this pathogen. The epidemiology of *H. annosum* in fir is made more complex by the fact that, differently than in pine, wounds in live trees are thought to be viable infection courts.

Our study has the following objectives:

- 1. Determine the genetic structure of the *H. annosum* ISG S in true fir by clonal analysis and mating allele distribution.
- 2. Screen isolates for virulence through a combination of greenhouse and field inoculation studies.
- 3. Determine whether adjacent clones are related through di-mon mating events.
- 4. Develop fingerprinting techniques to facilitate the identification of clones, to characterize the genotypic composition of heterokaryons, and to provide a bank of genetic markers for future genetic studies.

This is a report on preliminary results regarding point 1.

## MATERIALS AND METHODS

**Field sampling and isolates collection.** We have sampled 14 decay centers in four National Forests in California. The B1, B2, B3, 2M, 4M, and MC centers are in the Eldorado National Forest; the S1-4 centers are in the Stanislaus; the P11 is in the Plumas and the L1-3 centers are located in the Lassen National Forest. All decay centers were characterized by dead and dying trees, as well as by typical signs and symptoms (e.g. white laminated decay and basidiocarps) of the pathogen. All study

plots were located in mixed conifer forest with a strong predominance of white fir (>70%) over Ponderosa or Jeffrey pine, Incense cedar, black oak and Douglas fir. Most study plots were located in mature (>80 years old) stands

In each plot most of the asymptomatic trees and all of the dying and dead (<15 years) trees or stumps were sampled. Standing trees were felled and for each stump a whole bole cross-section at ground level was collected and a variable number of roots were excavated, sectioned, and collected. Each collection point was carefully mapped.

Samples were immediately placed in sealed plastic bags and incubated for 7-10 days in the laboratory. Strongly decayed wood samples or basidiocarps were plated on Fomes select medium (4) within three days from collection. Conidiophores (*Spiniger* state) emerging from the sample were isolated on Fomes select medium according to the following strategies: each root<sup>SAMPLE</sup> was considered as infected by a single isolate and each decay patch on horizontal tree sections was regarded as a separate isolation. Whenever a decay patch was particularly large multiple isolations were performed. All isolates were subcultured once and stored at 10 °C on MEA.

**Clonal analysis.** Isolates from each study plot were paired on Hagem medium (5) in two phases. In the first phase all isolates collected from the same tree were paired in all possible combinations. In the second phase the different somatic clones determined by phase 1 for each tree within a study plot, were paired in all possible combinations. All pairings were duplicated at least once. The somatic compatibility reaction among the isolates was observed frequently (every 2-3 days). The first reading was usually recorded in two to three weeks. The second reading was recorded about two weeks after the first one.

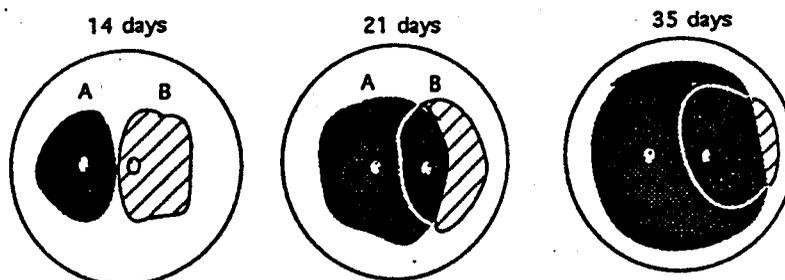
Random Amplified Polymorphic DNAs were also implemented on a small scale in order to verify clonal analysis (6).

## RESULTS AND DISCUSSION

We sampled 14 decay centers and we performed most of the somatic compatibility tests on isolates from 11 of them. From these 11 centers we sampled 375 trees, collected 1494 samples, performed 7000 isolations and obtained 452 isolates of *H. annosum*. In many instances viable isolates were obtained from the roots only. This was especially true of very old and decayed trees or stumps; some isolates were still viable in the distal portion of the roots up to an estimated 15 years after the initial infection.

We performed over 8000 somatic compatibility pairings. About 5% of the pairings were scored with uncertainty. We observed four major types of interactions among the isolates. In the first one, two genotypes bearing identical vegetative compatibility (v-c) alleles merge their colonies: there is no clear demarcation between the two colonies (except for sometimes a thin raised line) and the two colonies share the same growth patterns. In the second one, two incompatible genotypes are separated by an area with sparse mycelium (barrage). In the third one a thick raised line (often with a pseudosclerotial plate) is raised between two

different genotypes. In the fourth one a genotype acts "aggressively" towards the other one and grows over it (Fig. 1).



**Figure 1:** Exemplification of "aggressive behaviour" of isolate A on isolate B, in vitro. The A colony grows over B and covers up the original somatic interaction between the two.

The dominant nature of this phenomenon has been confirmed by subculturing areas of the overlapping mycelia and by subsequently pairing such subcultures with the two original "parents". Although such subsamples were collected well into the area originally occupied by the "subordinated" isolates, they were fully compatible only with the "aggressive" genotype. The importance of this observation is twofold. First, it indicates the presence of intraspecific predation (at least in vitro) in *H. annosum*. Second, it requires that the somatic compatibility pairings are checked much more often and much earlier than it is generally done. In fact, a plate in which one isolate has overgrown the other will look like a homogeneous plate if observed after a month or so. In actuality the plate shows not two compatible genotypes, but only the dominant genotype that has occupied the whole plate. Thus, a different method to look at fungal clones needs to be implemented to support the somatic compatibility data. We will use RAPDs and Southern Blots to resolve the ambiguity of some somatic crosses and to test the levels of sensitivity of this traditional method.

Results of our sampling and clonal analysis are condensed and summarized in tables 1 to 3.

The first observation (tables 1 and 2) is what appears as a single decay center is caused by the presence of several different genotypes of the pathogen. More than 50% of the genotypes detected in each plot were limited to a single tree. In all plots at least one larger genotype was detected affecting two or more trees. Maximal clonal expansion was in the 12-15 m range. Although a large pathogen diversity was detected in all plots, the fewer but larger genotypes impact forest stands more heavily. It was interesting to notice that isolates belonging to larger clones often exhibited the "aggressive" in vitro reaction described above.

Results summarized in table 3 indicate that multiple infections on the same host are a frequent occurrence. Multiple fungal clones were found in dead, dying and living trees. In many cases the pattern of clonal distribution in a single tree was rather complex (fig. 2).

This also explains why the presence of a genotype does not exclude other genotypes from the same area of the decay center. Overlapping clones can often be observed (fig.3), because spatial antagonism occurs at a finer scale, within a single tree.

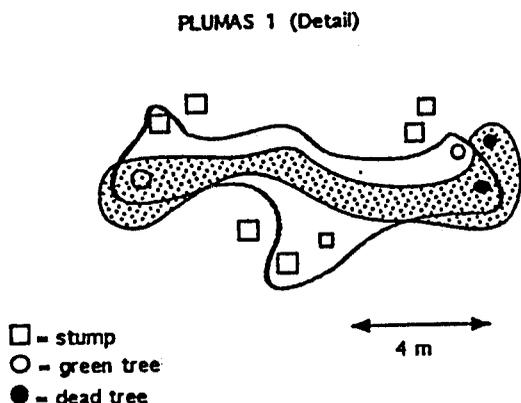


Figure 3: Two different clones of *Heterobasidion annosum* overlap in the same area of the decay center.

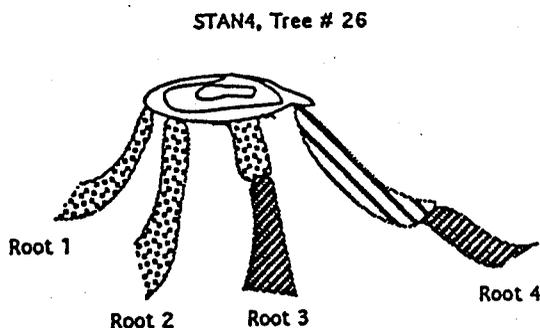


Figure 2: Complexity of clones of *Heterobasidion annosum* in a tree already infected when cut. Different shadings refer to different clones. All three clones were present in other trees.

No strong evidence of a possible role played by insect vectors in secondary spread of *H. annosum* has been gained yet. In most cases clonal expansion could be explained by vegetative growth. In the ST1 plot two isolates were found on two trees at about 10 m from other trees infected by the same clone. Even in this case though, root continuity between such trees could not be excluded.

These preliminary results highlight the importance of primary infections in the epidemiology of Annosum root disease and put forward interesting questions regarding the level of relatedness of the numerous genotypes present in relatively limited areas. It would be particularly interesting to determine whether the smaller clones were generated by basidiospores of the larger -and supposedly older- genotypes, or whether some of the clones have shared some nuclei through di-mon mating.

Finally, the presence of several clones in living trees is well in line with the hypothesis that *H. annosum* can infect live white fir trees through wounds. It also poses the question of a possible role played by insects in the primary infection process.

Plot	Area	# of trees	Basal Area	# of isolat.	Max clonal spread
B1	512	58	139.7	69	10 m
B2	400	49	139.7	13	3 m
B3	750	69	139.7	33	8 m
2M	900	41	104.4	26	15 m
4M	1050	101	119	52	nd
MC	235	33	nd	71	nd
STAN1	960	42	119	53	(18) 10
STAN2	192	20	101	25	4 m
STAN3	196	18	91.8	6	1 m
STAN4	193	30	119	62	12 m
PLUM1	664	96	78.7	42	12 m

Table 1. Maximum clonal spread of *H. annosum*  
nd= not determined. Area in sq. m. Basal area in sq.m/ha.

Plot	# clones per plot	# of trees with H.a.	Basal Area	# of trees/clone	
				Mean	Range
B1	nd	22	139.7	-	(1-4)
B2	5	6	139.7	1.2	1-2
B3	nd	11	139.7	-	(1-4)
2M	6	7	104.4	1.66	1-3
4M	nd	17	119	-	-
MC	nd	25	nd	-	-
STAN1	30	11	119	1.16	1-6
STAN2	6	7	101	1.33	1-2
STAN3	6	5	91.8	1	1-1
STAN4	26	20	119	2.7	1-10
PLUM1	8	15	78.7	2	1-4

Table 2: Number of trees infected by a single clone of *H. annosum*.  
nd= not determined. Basal area in sq.m/ha.

Plot	# clones/tree		# clones/green tree		# clones/dead tree	
	Mean	Range	Mean	Range	Mean	Range
B1	nd	nd	nd	nd	nd	nd
B2	1	1-1	1	1-1	1	1-1
B3	nd	nd	nd	nd	nd	nd
2M	1.4	1-3	1	1-1	1.75	1-3
4M	nd	nd	nd	nd	nd	nd
MC	2.5	1-5	2.4	2-3	2.6	1-5
STAN1	3	1-7	4.2	1-7	1.6	1-2
STAN2	2.63	2-4	4	4	2	2
STAN3	1.2	1-2	1.25	1-2	1	1-1
STAN4	1.58	1-3	1.3	1-3	2	1-3
PLUM1	1.3	1-3	1.25	1-2	1.37	1-3

Table 3. Number of clones of *H.annosum* per tree.  
nd= not determined.

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