Biology, Ecology and Genetics of *Heterobasidion annosum*

Genetics and Population Structure of *Heterobasidion annosum* with Special Reference to Western North America[1]

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Abstract: Recent advances in the genetics and population biology of Heterobasidion annosum are reviewed. H. annosum is a heterothallic (outbreeding) basidiomycete with a unifactorial, multiallelic incompatibility system which regulates mating. H. annosum in western North America consists of two intersterility groups (biological species) which can be identified through mating tests and isozyme analysis. Intersterility groups in western North America appear to be host specific. Intersterility between groups is attributable to specific genes. Intergroup hybrids have been formed in the laboratory, but have not been identified from the field.

Heterobasidion annosum (Fr.) Bref. [formerly Fomes annosus (Fr.) Karst.] has been the subject of numerous investigations because of its significance as a root- and butt-rot pathogen of many commercially important conifers (Koenigs 1960; Hodges and others 1971). Most research on H. annosum has been devoted to various aspects of its pathology, impact, ecology, physiology, and biological and chemical control. The sexuality, genetics, and population structure of H. annosum have received little attention until recently. Knowledge concerning the genetics and population structure of the pathogen has the potential to significantly enhance our efforts to manage disease losses resulting from H. annosum. This paper reviews research conducted over the past 10 years on the life cycle, sexuality, host specificity, and population structure of H. annosum. Publications in this area are relatively few, but some significant advances have been made nonetheless.

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SEXUALITY AND LIFE CYCLE

Early efforts to examine the genetics of H. annosum were hampered by a lack of definitive knowledge regarding the sexuality and life cycle of the fungus. Recent studies have established, however, that H. annosum is a heterothallic (i.e., outbreeding) fungus (Korhonen 1978; Chase and Ullrich 1983) to which standard genetic techniques can be applied readily (Chase and Ullrich 1985). The major steps of the life cycle of H. annosum are now well established (figure 1). Individual basidiospores of the fungus, upon germination, give rise to homokaryotic mycelia that are characterized by simple septa (cell cross-walls) and multinucleate cells with identical haploid (n = single set of chromosomes) nuclei. Homokaryotic mycelia are self-sterile and under normal conditions do not differentiate to form a basidiocarp (fruiting body or conk). Mating must take place between two sexually compatible homokaryotic mycelia in order to form a mycelium capable of fruiting. Mating is initiated by anastomosis (fusion) of hyphae from different homokaryons. The mycelium arising from a compatible mating is termed a dikaryon. Individual cells of a dikaryon contain nuclei from both of the contributing homokaryons (n + n condition). During conjugate mitotic division of paired nuclei in the dikaryon clamped septa (also termed clamp connections) are formed, which are easily recognized under the light microscope (Raper 1966; Korhonen 1978; Chase and Ullrich 1983). A dikaryon will have simple septa as well as clamped septa. The percentage of clamped vs. simple septa varies among dikaryons.

There are two other species in the genus Heterobasidion. H. araucariae, a newly described species (Buchanan, 1988) from Australia, New Zealand, and possibly other areas of the Far East, was previously considered a variety or biological species of H. annosum (Chase and others 1985; Buchanan 1988). H. araucariae cannot be distinguished easily from H. annosum in culture, but it is homothallic (self-fertile) (Chase and others 1985). Thus, in contrast to H. annosum, monobasidiospore strains of H. araucariae, upon germination, give rise to clamped mycelia capable of fruiting to complete the life cycle without undergoing mating (Chase and others 1985). The

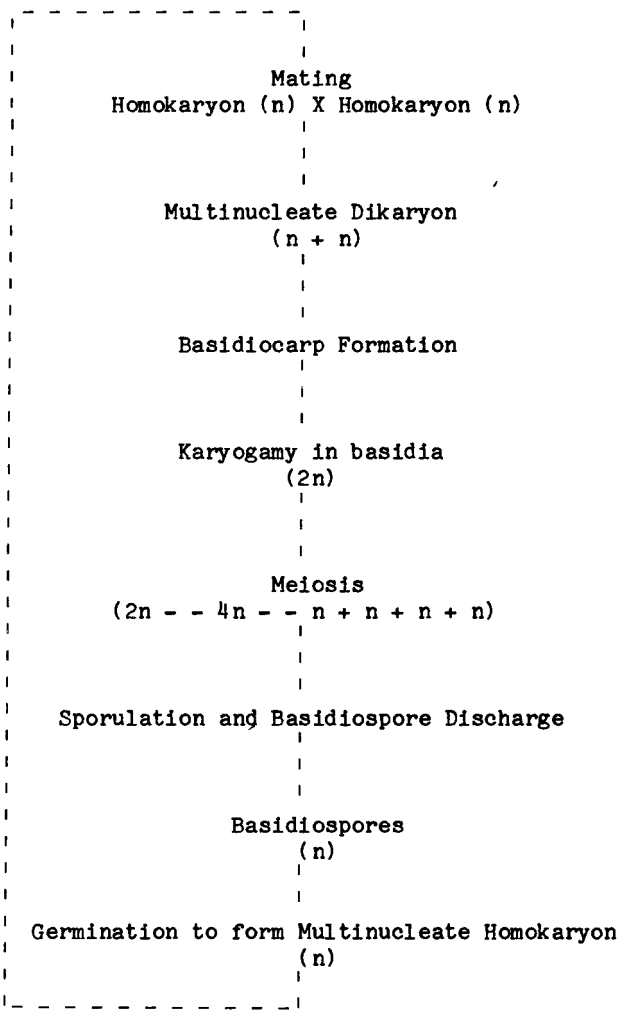

Figure 1--Life Cycle of Heterobasidion annosum. The chromosome complement of nuclei is indicated as n = haploid, 2n = diploid. Conidia are produced on both homokaryotic and dikaryotic mycelia. Dikaryons yield conidia that may carry one or the other or both of the parental type nuclei. Basidiospores are binucleate as a result of an additional mitotic division following meiosis in the basidium.

third species, H. insulare, is readily distinguished from the other two Heterobasidion species on morphological differences in basidiocarps; little is known concerning its sexuality or life cycle (Buchanan 1988). It has been isolated from throughout much of the Far East (Buchanan 1988).

GENETICS OF HETEROBASIDION ANNOSUM

Mycelial interactions within H. annosum fall under two general categories, sexual and vegetative. Sexual interactions (i.e., mating) involve anastomosis (cell fusion), plasmogamy (cytoplasmic fusion), heterokaryosis (occurrence of dissimilar nuclei in a common cytoplasm), and dikaryosis (pairing of compatible nuclei). Formation of fruiting bodies, meiosis, and formation and discharge of basidiospores are dependent on successful mating. Consequently, important evolutionary processes such as genetic recombination and gene flow are also affected. Sexual interactions occur between homokaryons, or between dikaryons and homokaryons, with dikaryons acting as unilateral nuclear donors. Vegetative interactions involve only dikaryons. Typically, genetically dissimilar dikaryons exhibit mutually antagonistic reactions upon confrontation. Vegetative mycelial interactions thus represent self vs. non-self recognition and consequently may affect intra-specific competition and resource partitioning of individual substrates.

Incompatibility and Intersterility Systems

The ability of paired homokaryons to mate is governed by two distinct genetic systems that act in a coordinated manner: the intersterility and incompatibility systems. Intersterility genes delimit subpopulations or intersterility groups (ISGs) within H. annosum. ISGs have also been termed biological or sibling species because they are usually indistinguishable by the traditional morphological criteria used to separate species. Interfertile strains mate and form dikaryons, whereas intersterile strains do not. Generally speaking, interfertile strains belong to the same group, and intersterile strains belong to different groups. The incompatibility system affects the ability of homokaryons within an ISG to mate. Strains that are compatible can mate and attain the dikaryotic state. Homokaryons that are incompatible fail to form a dikaryon. Thus, intersterility genes define the limits of the population or ISG within which interbreeding and concomitant gene flow and genetic recombination can occur, whereas the incompatibility factor with its many mating-type alleles regulates the degree of inbreeding and outbreeding that can occur within the ISG.

Intersterility genes are epistatic to incompatibility genes, because strains must be interfertile in order for compatibility to be expressed. Conversely, strains in different ISGs have different incompatibility alleles but fail to mate because they are intersterile.

Both compatibility and interfertility in H. annosum are based on the ability of homokaryons to form a dikaryon when paired in culture (Chase and Ullrich 1983; Korhonen 1978). In H. annosum the two homokaryons being tested are inoculated about 0.5 cm apart on 1.25 percent malt extract agar in standard 9-cm Petri dishes and allowed to incubate at 23 C or at room temperature on a laboratory bench. Pairings are incubated for 10 days and then a subculture is made from the junction line of the pairing to fresh 1.25 percent malt extract agar. Subcultures are incubated 5 to 10 days

before they are scored for the presence or absence of clamp connections. Clamp connections are easily observed through the inverted Petri dish (standard compound microscope, 150x magnification). Strains that form a dikaryon are interfertile and compatible. Negative mating reactions can be interpreted as either intersterile or incompatible only within the context of the experimental design and background of the strains utilized.

Genetics of the Incompatibility System

Incompatibility systems have been described in higher fungi for a number of species and groups (Raper 1966) and have been studied extensively in Schizophyllum commune (Raper 1966). Heterobasidion annosum is characterized by a bipolar (=unifactorial) system. Incompatibility is governed by a single incompatibility (mating-type) factor designated A. Many different mating-type alleles or variants of the A factor exist throughout H. annosum and can be designated as a numerical series (i.e., A1, A2, A3, etc.). Any two strains are capable of dikaryon formation when paired as long as they carry different mating-type alleles. Homokaryons carrying identical mating-type alleles cannot mate to form a dikaryon. Thus every dikaryon, upon fruiting, yields two mating-type alleles among its progeny homokaryons in a standard 1:1 Mendelian ratio. One of the consequences of this is that, among the progeny homokaryons from a single dikaryon, there will be a reduced level of compatibility (50 percent) and thus a reduction in inbreeding (Burnett 1965). Regulation of the degree of inbreeding appears to be the primary role of mating-type alleles in the basidiomycetes (Burnett 1965; Raper 1966; Ullrich 1977).

The number of mating-type alleles in the worldwide population of H. annosum is very high. For instance, forty different alleles were identified in a study of H. annosum from 31 unique dikaryons in four Pinus resinosa plantations in Vermont (Chase and Ullrich 1983). Other studies (Korhonen 1978; Stenlid 1985) have also documented the existence of large numbers of mating-type alleles in small samples. Although no attempt has yet been made to estimate the worldwide number of mating-type alleles, it is certainly safe to assume that there are hundreds of alleles in existence. As a consequence, outbreeding is highly favored in H. annosum, and a high potential for gene flow and genetic recombination exists within interbreeding populations of the fungus.

One of the useful features of mating type alleles is that they can be used as naturally occurring markers of the distribution of individual dikaryons in the field (Chase and Ullrich 1983; Stenlid 1985). Dikaryons of H. annosum with identical sets of incompatibility alleles have been found only in proximity to one another, either on the same stump or tree, or on adjacent trees or stumps, usually within 10-20 m. This is consistent with the concept of vegetative spread of H. annosum through root contacts of neighboring trees. The maximum extent for spread of clones seems to be much less for H. annosum than for species in the Armillaria mellea complex (Chase and Ullrich 1983; Piri and others 1989; Stenlid 1985). Piri and others (1989) showed that the average number of trees occupied by a clone of H. annosum is two, although some clones were shown to infect as many as 16 trees. Clones of A. mellea have been shown to be extensive, especially in the western United States. Anderson and others (1979) suggested that individual clones may extend as much as 500 m and infect large numbers of trees within these limits. There have been no large-scale studies utilizing genetic methods to assess clonal spread of H. annosum in western North America. Such studies would be very useful in assessing the spread of the fungus in individual disease centers. Evidence for clonal spread was seen in a small study of a western red cedar/grand fir plot in British Columbia (Chase 1985).

Vegetative Interactions

A vegetative compatibility test has recently been developed for H. annosum (Stenlid 1985). Individual dikaryons are paired in culture on Hagem agar, and those showing complete intermingling of mycelia at the junction zone are interpreted as being the same dikaryotic clone (i.e., vegetatively compatible). Dikaryons that form a zone of inhibited mycelial growth or mutual antagonism at the junction zone are interpreted to be genetically different clones (i.e., vegetatively incompatible). Vegetative compatibility tests are useful for several reasons. First, they provide a much more efficient way to conduct clonal distribution studies, because they allow complete sampling of a stand unrestricted by the availability of fruiting bodies or the need to induce fruiting of isolated dikaryons in the laboratory. Secondly, work load is reduced by not having to isolate homokaryons in order to test for identity of mating-type alleles. Last but not least, vegetative compatibility tests are also valuable because they can distinguish between dikaryons that are truly identical clones and those that simply have identical mating-type alleles (Stenlid 1985).

The genetic basis for vegetative incompatibility has not been elucidated in H. annosum or any other Basidiomycete. Since sibling-related dikaryons with identical mating-type alleles display vegetative incompatibility (Stenlid 1985), it is apparent that the incompatibility factor is not directly involved. Presumably some kind of polygenic system is indicated, since the strength of vegetative incompatibility reactions may vary over a wide range. The consequence of the existence of vegetative incompatibility in H. annosum is that a substrate may be occupied by a number of dikaryons each within a different space. The degree to

which different dikaryons within a substrate undergo competition or cooperativity with one another is unknown.

Intersterility in H. annosum

In his studies on the breeding biology of H. annosum, Korhonen (1978) identified two intersterility groups in the Finnish population. He designated these the 'S' and 'P' intersterility groups. The 'S' group was isolated primarily from butt-rotted Norway spruce [Picea abies (L.) Karst.] and from seedlings of Scotch pine (Pinus sylvestris L.) growing adjacent to infected Norway spruce stumps. The 'P' group was isolated from a much broader range of host trees, primarily saplings and mature trees of Scotch pine, juniper (Juniperus communis L.), birch (Betula sp.), and alder (Alnus incana (L.) Moench.), but also including mature butt-rotted P. abies. Nearly all pairings (97 percent) between 'S' and 'P' group homokaryons failed to give rise to dikaryons. In contrast, virtually all pairings within a group gave rise to dikaryons except for the occasional cases in which homokaryons carried identical incompatibility mating-type alleles.

Korhonen (1978) extended his study to include dikaryons from a worldwide collection. Dikaryons were paired with the homokaryon testers (di-mon pairings; Raper 1966), because homokaryotic testers are capable of being dikaryotized only by dikaryons of the same group. The results showed that both the 'S' and 'P' groups were found throughout the world on a wide variety of hosts, but the conclusion was that "pine species are typically attacked by the 'P' intersterility group of H. annosum" (Korhonen 1978). Korhonen (1978) also described a third intersterility group, members of which failed to dikaryotize either 'S' or 'P' group testers. He designated these the '0' group, but subsequent studies (Chase and others 1985) have shown these to be the homothallic form since redescribed as H. araucariae (Buchanan 1988). Korhonen and others (1988) have described a new intersterility group ('F' group) from silver fir in the Appenine Mountains of Italy, which is interfertile with Finnish 'S' group strains but is intersterile with 'S' group strains from the Italian Alps.

Intersterility Groups in North America

The 'S' and 'P' groups are distributed throughout western North America, but so far only the 'P' group has been found in eastern North America (Chase 1989, Chase and Ullrich 1989a; Chase 1985). Homokaryotic North American strains were identified as belonging to the 'S' and 'P' groups on the basis of their reactions with Finnish tester strains (Chase and Ullrich 1989a: Chase 1985); however, the two groups were found to be partially interfertile. In some cases, the patterns of mating reactions suggested that intersterility was under the control of Mendelian-like determinants (i.e., genes). For instance, all the homokaryons isolated from an Alaskan dikaryon mated with 'S' testers from Finland but failed to mate with 'P' testers from Finland. However, half of the Alaskan homokaryons mated with Vermont strains (which mate with Finnish 'P' strains and not with Finnish 'S' strains), but the remainder did not. As many as 50 percent of pairings between eastern North American 'P' group homokaryons and western North American 'S' group isolates are interfertile and result in dikaryon formation. Partial interfertility exists between western North American 'S' group and the western North American 'P' groups but is not as pronounced. Dikaryons were formed in 57 out of 320 (18 percent) of pairings in preliminary experiments (T.E. Chase, unpublished data).

Genetics of Intersterility in H. annosum

The ability to mate some western North American 'S' group isolates with eastern North American 'P' group isolates provided the opportunity to examine the inheritance of intersterility determinants, since the strains being paired possessed diametrically opposed specificities for Finnish 'S' and 'P' strains (Chase and Ullrich 1989b). Progeny from these crosses were analyzed and segregation for intersterility genes was observed (Chase and Ullrich 1989b) allowing the formulation of a testable genetic model of how these genes interact as well as the construction of a simple genetic linkage map.

Five intersterility genes, each with two alternate alleles, have been identified (Chase and Ullrich 1989b) thus far and have been designated S^+/S^-, P^+/P^-, $V1^+/V1^-$, $V2^+/V2^-$, and $V3^+/V3^-$. Under standard laboratory conditions, dikaryon formation can occur between any two strains having a "positive" (+) allele in common at one or more of the five loci. For example, a strain with a $V1^-V2V3^+S^+P^-$ can form a dikaryon when paired with a strain carrying a $V1^-V2^+V3^+S^-P^+$ genotype, because $V3^+$ is common to both strains. Conversely, a strain with a $V1^-V2^+$ $V3^-S^-P^+$ cannot form a dikaryon in pairings with a strain carrying a $V1^-V2^-V3^+S^+P^-$. Homoallelism for "negative" (-) alleles does not allow interfertility. As stated previously, dikaryon formation is also dependent on two strains having different mating-type alleles. Intersterility genes identified in various groups of H. annosum are shown in Table 1.

Under periods of prolonged incubation, intersterile pairings may give rise to clamped mycelia. Genetic analysis of such "illegitimate" matings suggests they could arise from pairing of parasexually derived recombinant nuclei that are interfertile and compatible with one of the original parental nuclei (Chase 1985). The extent to which "illegitimate" mating occurs in nature is unknown, but it is doubtful that it plays an

Table 1-- Representative intersterility genotypes of Heterobasidion annosum.

Intersterility group	Intersterility gene V1	V2	V3	S	P
Finland					
'S' group	-/-[1]	-/-	-/-	+/+[2]	-/-
'P' group	-/-	-/-	-/-	-/-	+/+
North America					
Eastern 'P' group	?[3]	+/+	+/+	-/-	+/+
Western 'S' group	+[4]	-/-	+/-[5]	+/+	-/-
'P' group	+	+/+	+/-	-/-	+/-

[1] -/-, all dikaryons examined are homozygous for - allele at locus.

[2] +/+, all dikaryons examined are homozygous for + allele at locus.

[3] Existence of $V1^{+}$ in Eastern North American 'P' group not confirmed.

[4] $V1^{+}$ exists in population, but no collection has been fully analyzed for heterozygosity.

[5] +/-, evidence has been found for heterozygosity at locus in dikaryons of population. Individual dikaryons may be heterozygous or homozygous for either allele.

important role in allowing gene flow between intersterility groups. This conclusion is supported by recent isozyme surveys indicating a lack of evidence for gene flow between North American 'S' and 'P' groups (Otrosina and others 1989).

POPULATION STRUCTURE AND HOST SPECIFICITY RELATIONSHIPS OF H. ANNOSUM IN WESTERN NORTH AMERICA

A detailed picture of the population structure of H. annosum in western North America is beginning to emerge. Data from intersterility studies have delineated two biological species (Chase 1985; Chase and Ullrich 1989a), and data from isozyme studies have confirmed initial conclusions (Otrosina and others 1989). Most important for forest managers is the clear indication these studies have given that the two biological species of H. annosum have major differences in host specificity.

Chase and Ullrich (1989a) determined the intersterility genotypes of dikaryons isolated from various host species in North America. Dikaryons of the 'S' group were associated with infection and mortality in several host species including white fir, grand fir, western hemlock, and western red cedar. Dikaryons of the 'P' group were associated with infection and mortality in red pine and loblolly pine (both from eastern United States), and ponderosa pine in the western United States. Host specificity was not apparent in isolates from stumps. For instance, isolates from spruce stumps in New Hampshire were of the 'P' group, and isolates of the 'S' group were sampled from both pine and fir stumps in California. These data suggested that pathogenic activity (i.e., infection of host trees) is specific, whereas saprotrophic colonization of stumps is not.

Subsequently, a more detailed cooperative study involving the USDA Forest Service Pacific Southwest Forest and Range Experiment Station and the University of California at Berkeley has been undertaken. In this study, sets of mono-basidiospore isolates were established from fruiting bodies collected from stumps of true fir (Abies) and hard pines throughout much of the Sierra Nevada, southeastern Oregon, and from the San Bernardino Mountains. Both 'S' and 'P' group isolates were identified from ponderosa and Jeffrey pine stumps. True fir stumps yielded only 'S' group isolates; however, nearly all fir stumps sampled were left from trees infected before felling, as evidenced by characteristic decay patterns. Samples from fallen giant sequoia and true fir stumps in the vicinity of fallen giant sequoia yielded only 'S' group isolates.

Isozymes were analyzed for dikaryons isolated from the context tissue of the same conks used as sources for monobasidiospore isolates (Otrosina and others 1989). A strict concordance between isozyme and intersterility data was seen, indicating the existence of two genetically isolated populations of H. annosum. Subsequent sampling of recently killed or dying trees in the Sierra Nevada and the San Bernardino Mountains is consistent with the hypothesized concordance between intersterility groups and host range of H. annosum isolates (W.J. Otrosina, T. Chase, and F.W. Cobb, unpublished data). All isolates from naturally infected ponderosa and Jeffrey pine trees have the isozyme profile associated with the 'P' group. All isolates from true fir displayed the 'S' group isozyme profile. All isolates from naturally infected incense cedar, manzanita, juniper, Coulter pine, and Pinyon had the 'P' group isozyme profile, but only a few isolates from each of these hosts were examined.

Although the concordance between inter-sterility group and host range has been consistent thus far, it would be desirable to obtain information from controlled inoculation studies to support these conclusions. Worrall and others (1983) conducted inoculation trials on pine and

fir seedlings in the greenhouse with isolates from ponderosa pine and true fir. The intersterility groups of the isolates were unknown, but differences in "host specialization" between the two groups of isolates were demonstrated. Recently completed seedling inoculation experiments utilizing well characterized 'S' and 'P' isolates and nine different host species also suggest differences in host specificity between the groups (F.W. Cobb, T.E. Chase, and W.J. Otrosina, unpublished data).

In addition to controlled inoculation studies, we will need more survey data from a variety of sampling situations and localities. Data for outplanted seedlings as well as mixed conifer stands, and a variety of other ecological situations are needed. For instance, are both groups able to attack outplanted seedlings of fir and pine? And does the isolation of the 'P' group from incense cedar reflect host specificity or rather the proximity of incense cedar to pine in mixed stands? Another important question is to determine whether host specificity is maintained under adverse conditions such as drought and bark beetle attack.

Answering these questions will strengthen our understanding of the population structure and perhaps indicate possibilities for stand manipulation and biological control.

Lack of Evidence for Inter-Group Hybrids

Despite the existence of genes allowing hybridization, there is no evidence as yet for the existence of natural hybrids between the 'S' and 'P' groups in North America. The reasons for this are not apparent. One possibility is that such hybrids might be at a selective disadvantage because they carry genes allowing host recognition and defense systems of both pine and fir to operate against them. Another possibility is that, in nature, hybrids might have a reduced ability to compete, a reduced capability for fruiting, or a reduced spore viability. An alternative possibility is that sampling to date has simply failed to identify "hybrid zones" of H. annosum which may have a limited geographic or unusual host distribution.

CONCLUSIONS

The sexuality and life cycle of Heterobasidion annosum have been clarified, and utilization of techniques from fungal genetics and biochemistry is beginning to yield a detailed picture of the population structure of the fungus. Heterobasidion annosum is a heterothallic fungus with a well-defined sexual stage allowing mating and genetic recombination. In the western United States and Canada, H. annosum consists of at least two intersterility groups (ISGs) or biological species that are reproductively and genetically isolated from each other. The two ISGs have important differences in host range that have the potential to be exploited in making management decisions and developing biological controls. Further research should be allocated to continued sampling of populations in a variety of ecological situations and to means of silviculturally manipulating stands to reduce losses. Basic research should continue to focus on the nature of the intersterility genes as well as the genetic and biochemical nature of the host-pathogen specificity interaction. Information from both applied and basic research should be useful in generating realistic predictive models for disease dynamics and losses.

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Biology, Ecology, and Epidemiology of *Heterobasidion annosum*[1]

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Abstract: Pertinent literature on the biological aspects of annosus root disease is reviewed. Key features of the life cycle of Heterobasidion annosum such as stump infection, stump colonization, host-parasite relations, and interactions of various physical and biological factors are discussed in relation to forest stands in the western United States. This review suggests our knowledge of the pathology of this disease is limited on most affected tree species. Further research on various aspects of the life cycle of this fungus is essential to minimizing losses to this disease.

The literature on Heterobasidion annosum (Fr.) Bref. before 1970 contains over 1,000 titles dealing with various aspects of the fungus and the disease it causes (Hodges and others 1971; Koenigs 1960). A preliminary literature review conducted by Otrosina estimates at least 850 titles dealing with H. annosum have been published during the period 1970 to 1988. This paper is not intended to be a review and will not attempt to address all aspects of the biology of H. annosum; it will highlight pertinent research on the biological and ecological relationships as they pertain to the pathology of pine and true fir in the western coastal states of the United States. The disease as it relates to other hosts in other geographic areas is discussed elsewhere in these proceedings.

STUMP SUSCEPTIBILITY AND INFECTION

The most crucial stage in the disease cycle of H. annosum is the entry or invasion of the fungus into the stand where it can then move from tree to tree through roots. The primary mode of stand entry in pines is through freshly cut stump surfaces (Begs 1963) (fig. 1). We believe that stump infection is important in fir as well, though there are other modes of entry in Abies (fig. 2). Stump surfaces act as ideal semi-selective media for germination of deposited basidiospores of the fungus. Susceptibility of stump surfaces of various hosts has been studied by a number of investigators (Rishbeth 1951; Yde-Andersen 1962; Cobb and Barber 1968). The general consensus of these studies is that stumps of most susceptible species can remain receptive to invasion by the fungus for up to 45 days, depending upon season and host species. On the other hand, Cobb and Schmidt (1964) found eastern white pine (Pinus strobus L.) stumps to be highly susceptible for only a few days after felling. In the western United States, ponderosa pine (Pinus ponderosa Laws.) stumps may remain susceptible for at least 4 weeks, although susceptibility drops markedly after 1 to 2 weeks (Cobb and Barber 1968). Susceptibility of stumps also was greater in the autumn than in the spring. This was attributed to higher resin content of cut stump surfaces in the spring-felled trees than in those felled in the fall. Not all susceptible tree species are colonized through stump surfaces at the same rates or conditions as ponderosa pine. For example, spore inoculation studies of incense cedar (Calocedrus decurrens (Torr.) Florin) suggest substantially more resistance to colonization under the same conditions that allow ponderosa pine stumps to be colonized (Hunt and others 1974).

Temperature is an important factor in determining successful infection of stump surfaces by H. annosum. The optimal temperature for growth of the fungus in pure culture is 23-26 C for a wide variety of host and geographic origins of isolates (Cowling and Kelman 1964). The fungus is also capable of considerable growth at lower temperatures, averaging 21 percent of optimum at 8 C. In general, the fungus does not grow at temperatures above 32 C. The upper temperature limit has important implications regarding stump infection in certain climates. Ross (1969) found basidiospores and conidia of the fungus to be inactivated after 60 minutes at temperatures above 45 C. There is an apparent difference in temperature effects between mycelia and spores. Actively growing mycelia can become inactive or killed at temperatures above 35 C (Gooding 1964). In California, stumps in ponderosa pine stands may be exposed to temperatures greater than 35 C during the summer months. For example, Cobb and Barber (1968) noted ambient temperatures greater than 33 C during a study involving artificial inoculation of stumps, but they detected stump

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