A First Generation *Heterobasidion* Hybrid Discovered in *Larix lyalli* in Montana.

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On September 25, 2010, a wood sample was collected from an entirely decayed root ball of an alpine larch (*Larix lyallii* Parl.), 10 cm in DBH, recently downed, but still green. No attempts were made to determine whether the decay progressed into the stem. The discovery occurred in a stand in the Bitterroot Mountains, south of Darby, Montana (elev. 8300 ft.; Lat. 45.893528°, Long. -114.278322°). Several adjacent alpine larches were either dead or displayed thin crowns, and an old *Heterobasidion* basidiocarp was found on the decayed root ball of a neighboring dead tree, suggesting the presence of a root disease pocket. The stand is mature and composed of alpine larch, whitebark pine (*Pinus albicaulis* Engelm.*),* and a few subalpine firs (*Abies lasiocarpa* (Hooker) Nuttall), but only larches were symptomatic. No stumps were visible, and the site is in a designated wilderness area characterized by minimal forest management. Wood chips displaying a white rot with bleached speckles were plated on 2% malt agar, and cultures displaying the typical *Heterobasidion* anamorph (*Spiniger meineckellus*) were visible after 7 days. DNA was extracted from two distinct cultures, and the sequences of three nuclear loci, namely the Internal Transcribed Spacer, the Elongation Factor 1-alpha, and the Glyceraldehyde 3-Phosphate Dehydrogenase, were analyzed. The sequence of the mitochondrial ATPase was also sequenced. All loci were amplified using the primers indicated in Linzer et al. (2). Sequences of all three nuclear loci (GB accessions KF811480-2) unequivocally indicated both isolates to be first generation hybrids between *H. irregulare* (Underw.) Garbel. & Otrosina and *H. occidentale* Otrosina & Garbel. Cumulatively, sequences were heterozygous at over 40 positions in all three loci, and for the presence of two indels (one in ITS, one in EF 1-alpha). Polymorphisms and indels indicated alleles from both species were present in these heterokaryotic (ploidy n+n) isolates. The mitochondrial ATPase (GB accessions KF811483-4) indicated instead the cytoplasm belonged to *H. occidentale* suggesting that species was the first to be established in the infected tree and was either dikaryotized by a basidiopsore of the other species, or subject to nuclear re-assortment through di-mon mating with a genotype of *H. irregulare.* This is the first report of a *Heterobasidion* sp. in *L. lyalli*, and it is the second report of a natural *Heterobasidion* hybrid in North America (1). This finding indicates Alpine larch may be a host for both *Heterobasidion* species, as described for pine stumps in California (1). Thus, this conifer may have provided a substrate for the hybridization and interspecific gene introgression documented to have occurred before stumps were generated in high frequency by modern forestry practices (2).

1- Garbelotto, M., Ratcliff, A., Bruns, T. D., Cobb, F. W., Jr., and Otrosina, W. J. 1996. Use of taxon specific competitive priming PCR to study host specificity, hybridization, and intergroup gene flow. Phytopathology 86: 543-551.

2- - Linzer R, Otrosina W, Gonthier P, Bruhn J, Laflamme G, Bussieres G, Garbelotto M (2008). Inferences on the phylogeography of the fungal pathogen *Heterobasidion annosum*, including evidence of interspecific horizontal genetic transfer and human-mediated, long-range dispersal. Mol Phylogenet Evol 46(3): 844-862.

Plant Disease "First Look" paper • http://dx.doi.org/10.1094/PDIS-12-13-1211-PDN • posted 02/18/2014