Submissions for Diagnostic Protocols

1. General information

Submission number	2021-007
Title of Proposal	Diagnostic protocol for Heterobasidion annosum
Submitted by	IPPC Contracting Party New Zealand
Submission supported by	Ministry for primary industries

2. Contact information

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3. Summary of proposal

Summary of justification for the proposal	Heterobasidion annosum, a root rot pathogen, is a cause of major economic losses to the forestry industry throughout Europe, USA and Canada. Host commodities potentially harboring the pathogen include wood, bark, plants for planting and packaging material. Symptoms of H. annosum overlap with other diseases and definitive identification requires laboratory confirmation by morphological identification of fruiting bodies and molecular analysis. Heterobasidion annosum is spread via airborne spores and movement of contaminated plant material. Early detection and confirmation during a biosecurity outbreak is critical. The differentiation of H. annosum from other closely related species such as H. irregulare is very difficult and identification using morphological characteristics alone do not allow identification to species level. Detection is based on the examination of symptoms and the finding of fruiting bodies but must be complemented by molecular analysis to ensure the accurate identification of this pathogen. A diagnostic protocol that harmonises methods for the isolation, detection and identification of this pathogen would be beneficial especially for detection in exported/imported wood products.
Proposed priority	2
Comments	Heterobasidion annosum is an important forestry fungal pathogen and the proposed diagnostic protocol will be of benefit to many countries.

4. Literature review

Literature review	Within the genus Heterobasidion (Buchanan, 1988), H. annosum has the widest geographic distribution (Europe, North America, China, and Japan) and infects a large range of species (CABI 2021). Heterobasidion annosum sensu lato is a species complex of pathogenic wood decay fungi, consisting of strains with distinct host preferences, which cause root rot in conifer and hardwood species. Annual losses in the European Union alone are valued up to 790 million annually (Garbelotto and Gonthier, 2013), due to tree mortality, reduction in timber yield, and wood decay. Heterobasidion annosum spores can become airborne when daily temperatures rise above 0°C. Spores deposited on the forest soil are carried down by rainwater, may remain infective for several months, and can be spread from tree to tree by root contact or grafts. H. annosum

	may be introduced into disease-free areas in infected plants, wood packaging material and other wood products. Primary infections are by spores landing on stump surfaces or wounds, followed by secondary infections by growth of mycelium through root contacts or grafts. H. annosum appears to be more effective than other species in colonizing their hosts (Garbelotto and Gonthier, 2013). Trees die in clusters and mortality progresses over time. Symptoms include stunted growth, thinned tree crown, shorter needle retention, and dark wet staining of the wood. Characteristic root symptoms begin with resin-soaked wood that decays to a white-stringy rot. In some cases, a mycelial mat may form in advanced decayed wood. Other species within the genera, e.g. Heterobasidion irregulare and H. occidentale, have a partially overlapping host and geographic range, cause similar disease symptoms and produce similar fruiting bodies, making discrimination between them very difficult. It is possible to see fruit bodies directly emerging from infected roots. A definitive field diagnosis requires identification of fruiting bodies and lab confirmation using morphological and molecular methods. Morphological differences among basidiocarps produced by different species are present, but they are small and morphological characters often overlap. Species can also be determined by performing in vitro mating tests using known testers of each species (Schulze and Bahnweg, 1998). There are several DNA-based detection methods to differentiate among species (Diipars and Ruoiis, 2011, Lamarche et al. 2016, Schulze and Bahnweg, 1998, Shamoun et al. 2019). Considering the invasive behaviour of H. annosum it is essential to prevent further spread of this pathogen into areas free of this disease. Control focuses on prevention and containment with stump removal being one of the main control strategies. References: • Buchanan, P. A new species of Heterobasidion (Polyporaceae) from Australia. Mycotaxon 1988, 32, 325–337. • Ðíipars, V.; Ruòìis, D. Detection of Heterobasidion annosum in Scots pine trees using a polymerase chain reaction based method. Balt. For. 2011, 17, 2–7. • Garbelotto, M.; Gonthier, P. Biology, epidemiology, and control of Heterobasidion species worldwide. Annu. Rev. Phytopathol. 2013, 51, 39–59. • Lamarche, et al. Real-time PCR assays for the detection of the Heterobasidion annosum complex. For. Pathol. 2016. • Schulze, et al. Identification techniques for Armillaria spp. and Heterobasidion annosum root and butt rot diseases: A critical review." Archives of Phytopathology & Plant Protection 31;(1997): 141-163. • Shamoun et al. New Taxon-Specific Heterobasidion PCR Primers Detect and Differentiate North American Heterobasidion spp. in Various Substrates. Pathogens. 2019; 8:156.

5. Criteria for prioritization of Diagnostic Protocols

Criteria	Information provided by Submitter
1. Need for international harmonization of the diagnostic techniques for the pest (e.g. due to difficulties in diagnosis or disputes on methodology)	Identification of fungi causing root rot diseases can be challenging especially at the early stages of infection. Heterobasidion annosum is a species complex and strains within this complex can have certain host preferences. The taxonomy is complex and the differentiation of H. annosum from other closely related species such as H. irregulare can make identification very difficult. Symptoms and morphological characters overlap with other diseases and definitive identification requires laboratory confirmation by identification of fruiting bodies and molecular analysis. There are no publications or diagnostic protocols that provides all the key information in one place to assist with diagnostics.
2. The relevance of the diagnosis to the protection of plants	Heterobasidion annosum is a significant forest pathogen and causes one of the most important diseases on conifers worldwide. Accurate identification and early detection are critical to managing the risk posed by H. annosum. Control mainly focuses on prevention and containment.

Criteria	Information provided by Submitter
including measures to limit the impact of the pest.	A standardised protocol will be useful to certify timber, wood packaging and plants as Heterobasidion-free, thus, facilitating trade. Early detection and species-level diagnosis of Heterobasidion will help with biosecurity response management especially in countries free of the disease but also have closely related Heterobasidion species that may confound diagnosis. Early detection may help formulate better disease management strategies.
3. Importance of the plants protected on the global level (e.g. relevant to many countries or of major importance to a few countries).	Heterobasidion annosum has a wide geographic distribution (Europe, North America, China and Japan) and infects a large range of forest species. Annual losses in the European Union alone are valued up to 790 million annually, due to tree mortality, reduction in timber yield, and wood decay.
4. Volume / importance of trade of the commodity that is subjected to the diagnostic procedures (e.g. relevant to many countries or of major importance to a few countries).	Trade in potentially affected wood products, wood packaging and plants is relevant to many countries. This pathogen is of particular importance to Europe and North America.
5. Other criteria for topics as determined by CPM that are relevant to determining priorities	n/a
6. Th balance between pests of importance in different climatic zones (temperate, tropics etc) and commodity classes.	This invasive forest pathogen is mainly of concern in temperate climates and mostly attacks pines.
7. Number of labs undertaking the diagnosis.	There are many laboratories that would undertake this diagnosis especially in the forestry service. Also quarantine laboratories in countries that do not have this disease would be interested in this protocol.
8. Feasibility of production of a protocol, including availability of knowledge and expertise.	A protocol is feasible as there are publications available that separately cover key aspects of diagnostics that include symptoms, morphology and molecular methods. Many experts available throughout Europe and North America.