

National regulatory control systems**PM 9/ 28 (1) *Heterobasidion irregulare*: procedures for official control****Specific scope**

EPPO Standards on national regulatory control systems are intended to be used by National Plant Protection Organizations (NPPOs) in their capacity as bodies responsible for official control of regulated pests. This Standard describes the procedures for official control with the aim of eradicating or

containing *Heterobasidion irregulare*, detailing the actions to undertake for this. Recommended measures could be useful to eradicate, contain or suppress some other pathogen species.

Approval and amendment

First approved in 2019–09.

1. Introduction

The root rot agent of conifers *Heterobasidion irregulare* (EPPO Code: HETEIR) is included in the EPPO A2 List of pests recommended for regulation. This fungal pathogen is native to North America, where it generally attacks pines (*Pinus* spp.), junipers (*Juniperus* spp.) and incense cedar (*Calocedrus decurrens*) (Garbelotto & Gonthier, 2013). The confirmed host list of the pathogen includes several genera of gymnosperms and a few genera of angiosperms. Most *Pinus* species are expected to be susceptible to the pathogen based on inoculation studies. The list of known host plants and pathogen preferences is given in Appendix 3.

Heterobasidion irregulare establishes in new forest stands mainly by means of airborne spores landing either on freshly cut stumps or on fresh wounds (primary infestation). Once stumps or trees are colonized, mycelial growth may allow the fungus to infest neighbouring trees through root contacts or grafts (secondary infestation) (reviewed by Redfern & Stenlid, 1998; Stenlid & Redfern, 1998; Garbelotto & Gonthier, 2013). After its introduction in a single site in Central Italy (Gonthier *et al.*, 2004; Garbelotto *et al.*, 2013), the fungus has spread to pine stands and urban parks over a 103 km stretch of Tyrrhenian coast near Rome, in association with significant mortality of *Pinus pinea* (Gonthier *et al.*, 2014). Based on population genetics data and on the observed invasion patterns in Italy, airborne spores of *H. irregulare* have been suggested to effectively infest new stands 15–20 km away from previously known infestations (Garbelotto *et al.*, 2013; Gonthier *et al.*, 2014). In infested stands in Central Italy, mycelial spread in root systems results in foci enlarging at an estimated rate of 40 cm per year (Gonthier *et al.*, 2014).

Practical guidelines to control *Heterobasidion* spp. have been previously reviewed (Holdenrieder & Greig, 1998; Korhonen *et al.*, 1998; Pratt *et al.*, 1998; Gonthier & Thor, 2013). The most cost-effective options rely on the protection of freshly cut stump surfaces with biological or chemical treatments and on timing logging operations (e.g. cutting or thinning) in periods of the year characterized by a low risk of stump infection. Stump removal may also be effective, although this operation cannot be recommended in all circumstances due to its costs and technical feasibility. Other control options include (1) the adoption of the widest possible spacing among trees in new plantations and reforestations in order to decrease the probability of root contact, (2) changes in tree species composition turning a susceptible forest into a more tolerant one and (3) promoting establishment of mixed forests. Management guidelines aimed at minimizing the risk of spread of *H. irregulare* in Europe and at reducing the damage caused by the fungus in infested sites have also been drafted (Gonthier *et al.*, 2014).

This Standard presents the basis for a national regulatory control system for the monitoring, eradication and containment of *H. irregulare* and describes:

- elements of the survey programme that should be conducted to detect an infestation or to delimit an infested area
- measures to eradicate recent infestations (incursions) by the fungus
- containment measures to apply (1) as part of eradication procedures and (2) in areas where the pest is present but its eradication is no longer considered feasible by the NPPO concerned, with the aim of preventing its further spread within a country or to neighbouring countries.

2. Monitoring of *H. irregulare*

In a country or area where *H. irregulare* is not known to be present, surveillance actions could be undertaken based on pest risk and consisting mainly of a detection survey (the detection survey method is described in Appendix 1). If *H. irregulare* is detected and identification confirmed, a delimiting survey should be carried out in order to delimit the infested area (the delimiting survey method is described in Appendix 2). Intensive surveillance should be carried out twice a year in this infested area until *H. irregulare* is eradicated.

The analyses of samples as well as the identification methods are described in the EPPO Diagnostic Standard for *H. irregulare* (EPPO, in press).

It is also recommended that actions to raise professional and public awareness are undertaken by the NPPO.

3. Demarcation of a regulated area

When an infestation is confirmed following surveys, the following should be demarcated: the infested area, an inner buffer zone of at least 20 km from the edges of the infested area in which intensive monitoring should be conducted, and outer buffer zone of at least 40 km from the edges of the inner buffer zone in which specific forest management prescriptions (detailed below) should be followed (see Section 5 for details). Any movement of potentially infested materials (any wood, cut branches and plants for planting) out of the infested area and the inner buffer zone should immediately be prohibited. The “regulated area” comprises the infested area and the buffer zones.

4. Eradication of *H. irregulare*

When an infestation is reported and confirmed following the surveys described in the monitoring section, eradication measures should be adopted if feasible. The opportunity of implementing eradication measures primarily depends on the size of the infested area: the larger the size the lower the feasibility of the eradication process and hence the chance of successfully eradicating the pathogen. The NPPO concerned should consider whether eradication is feasible and compare the risks and feasibility of measures to be taken with the costs of long-term containment measures.

The infested area should be demarcated through delimiting surveys as described in Appendix 2. In addition, within the infested area, surveys targeting trees or groups of trees showing symptoms of root rot and infested by *H. irregulare* should be conducted (Appendix 2).

In the infested area, the following operations should be implemented:

- prompt removal of trees confirmed to be infested by *H. irregulare* as well as the neighbouring trees (even apparently healthy) in a radius of at least 20 m (exact radius to be defined by the NPPO concerned depending

on the size of infested trees¹), including their root systems. Trees should be uprooted mechanically. Alternatively, trees should be felled and destumping should be carried out, including the removal of all roots down to a diameter of at least 2 cm.

- any stump present within the tree removal area should also be removed.
 - introduction of activities to raise professional and public awareness concerning the threat of *H. irregulare* and the measures adopted to prevent its introduction into, and spread within, the country and the EPPO region.
- During any of the above operations, care should be taken to avoid injuries to neighbouring trees.

In the infested area, the following operations should be prohibited:

- any silvicultural operation, including cutting and thinning, at least until the pathogen is officially declared eradicated.
- any replanting of any host plants (Appendix 3) in the proximity of infested and felled trees (i.e. within the expected radius of their root systems) for at least 10 years. Natural regeneration of any host plants should also be prevented there during the same period of time.

Two different prescriptions for wood disposal are recommended: (a) for symptomatic or felled infested trees and (b) for asymptomatic trees.

The wood deriving from symptomatic or felled infested trees, including their stumps and roots

- can be transported out of the infested area and inner buffer zone and from infested area to the buffer zone, only if it is heat-treated so that the wood-core temperature is maintained at 56°C for 30 min, according to the EPPO Standard PM 10/6 *Heat treatment of wood to control insects and wood borne nematodes* (EPPO, 2009).
- if not treated using this procedure, the wood, including bark, should be destroyed completely by burning at or near the place where the trees were felled in the infested area, or;
- can be used for industrial purposes within the infested area or chipped and transported, under official control, to an approved processing facility. If the chips are not to be used immediately for industrial purposes, they should be heat-treated (to at least 56°C for at least 30 min).

The wood deriving from the lower portions of stems (trunks) of asymptomatic trees (within 2.5 m of the tree collar), including their stumps and roots, should be treated as described above for symptomatic or felled infested trees. The wood deriving from the upper portion of stems (trunks) (above 2.5 m from the tree collar) of asymptomatic trees, including branches, can be freely transported out of the infested area, provided that this operation is done immediately after felling.

¹Each 1 cm of the trunk diameter corresponds to 80 cm diameter of tree root system (clear cut diameter). For example, a tree with a 1 m diameter trunk requires a 40 m radius clear cut area.

Heterobasidion irregulare can be considered eradicated when the following conditions are fulfilled:

- absence of any symptoms for 10 years and
- absence of spores of the pathogen (using woody spore traps as described in Appendix 1) both at the edges of the infested area and in the proximity (150 m outside of the border of the infested area) of places where infested trees were present in sampling performed twice a year over a period of at least 10 years.

These procedures also apply to urban areas.

5. Containment of *H. irregulare*

As part of eradication measures (see above) and in the case of an established population of the pathogen, when eradication is considered no longer feasible, the following recommendations for the containment of *H. irregulare* should be applied.

An inner buffer zone² should be delimited, including all host trees located within at least 20 km of the edges of an infested area. Intensive monitoring should be implemented every 2 years in this buffer zone using woody spore traps as described in Appendices 1 and 2 at 150 m (control), 2 km, 10 km and 20 km from the edges of the infested area. When *H. irregulare* spores are detected during surveys in this buffer zone, new delimiting surveys (Appendix 2) should be carried out, the infested area and buffer zone should be moved outwards, and eradication and containment measures adjusted accordingly.

An outer buffer zone³ should be delimited, including all host trees located within at least 40 km of the edges of the inner buffer zone. In this buffer zone, specific forest management prescriptions (detailed below) should be followed, symptomatic trees should be reported to the NPPO and sampled as described in Appendix 1, and a local educational program should be put into place to raise professional and public awareness of the increased risk of disease spread.

In the regulated area (the infested area and the two buffer zones), the following containment and suppression measures should be implemented:

- any silvicultural operation, including cutting and thinning, should be performed only in periods of the year characterized by low risk of stump infestation by spores of *H. irregulare*. The NPPO should indicate the most suitable period for silvicultural operations with the aim of minimizing the risk of spore infestation.
- any stump surface should be treated immediately after felling (ideally within 3 minutes of cutting), for example with a solution of urea at 30% concentration or with a suspension of spores of the fungal antagonist *Phlebiopsis*

gigantea, with an application dose of 1 L of solution or suspension per square metre of stump surface corresponding to a 1 mm thick layer of treatment. The NPPO should indicate the most suitable stump treatment subject to the necessary regulatory approvals.

- during the above operations, care should be taken to avoid injuries to neighbouring trees.

In the infested area and inner buffer zone, the following containment and suppression measures should also be adopted:

- any movement of potentially infested materials (any wood, cut branches and plants for planting) out of the infested area and the inner buffer zone and from infested area to buffer zone should be prohibited;
- prioritize removing the trees infested by *H. irregulare* or suspected trees displaying symptoms typically caused by *H. irregulare* as described in the EPPO Diagnostic Standard for *H. irregulare* (EPPO, in press). These trees should be mechanically uprooted. Alternatively, trees should be felled and destumping should be carried out, including the removal of all roots down to a diameter of 2 cm. If the above two options cannot be achieved, stumps should be ground to a depth of at least 60 cm below the tree collar.
- stumps deriving from the cutting of healthy-looking trees may be left on site, provided that their surfaces are treated as previously described.
- machinery used in the above operations should be cleaned of all wood debris before leaving a forest stand.

The wood obtained from asymptomatic trees and from symptomatic trees that tested negative for *H. irregulare* in the outer buffer zone can be freely moved.

Any wood of host species deriving from the infested area and the inner buffer zone:

- can be freely transported out of the infested area to the inner buffer zone and out of inner buffer zone, under official control, provided that it is heat-treated in an approved processing facility, so that the wood-core temperature is maintained at 56°C for 30 min, according to the EPPO Standard PM 10/6 *Heat treatment of wood to control insects and wood borne nematodes* (EPPO, 2009), or
- can be used within the infested area or within the inner buffer zone or chipped and left on site, or chipped and transported, under official control, to an approved processing facility within the infested area and within the inner buffer zone, or
- can be disposed of, under official control, in an approved landfill, or destroyed completely by burning at or near the place where the trees were felled in the infested area and the inner buffer zone.

These procedures also apply to urban areas.

The NPPO should carry out activities to raise professional and public awareness concerning the threat of *H. irregulare* and the measures adopted to prevent its introduction into and spread within the country and the EPPO region, and the conditions regarding movement of host plants of *H. irregulare* from the established regulated area.

²The width of the inner buffer zone is based on the evidence of spore dissemination capacities, which is 20 km.

³The width of the outer buffer zone is the double of spore dissemination capacities, which is 20 km as a precautionary measure.

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Appendix 1 – Survey in an area where *Heterobasidion irregulare* is not known to occur

Purpose

In order to verify that a country or an area is free from *H. irregulare* (and its hybrids), regular detection surveys should be carried out.

Strategy

Two situations could be distinguished: when symptoms of the disease are present (e.g. decline of annual shoot growth, shedding of old needles resulting in the so-called lion-tailing phenomenon, thinner crown, death of single trees or group of trees in clusters [Garbelotto & Gonthier, 2013; Gonthier & Thor, 2013], see also the EPPO Diagnostic Standard for *H. irregulare* (EPPO, in press) and when they are no symptoms. However, there are no pest-specific symptoms.

Independent of symptom presence, surveys should be performed by using spore traps. Such trapping should be focused on the areas of high pest risk:

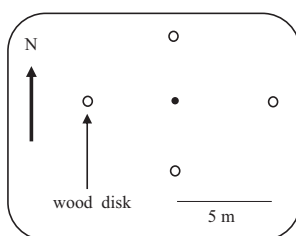
- destination points of imported risky materials
- stands of most susceptible hosts
- areas where the native species *H. annosum* is present (Giordano *et al.*, 2018)
- areas of newly reported host mortality.

Both active (e.g. burkard or rotary) and passive (e.g. Petri dish, woody disks) spore trapping could be used. Active methods are more appropriate to sample larger areas but are not yet as standardized as some of the passive methods. So far, the most commonly used passive method is a modified version of the wood disk exposure method (Gonthier *et al.*, 2007). Both *Pinus* spp. and *Picea abies* wood may be used for trapping spores.

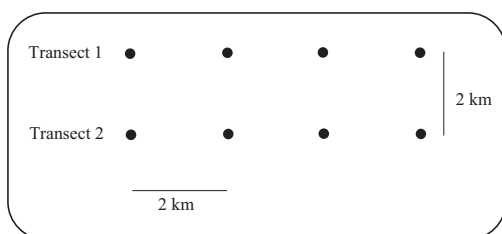
Preparation and placement of woody spores traps

Four wood disks, approximately 12 cm in diameter and 0.5 cm in thickness, obtained from freshly cut healthy trees should be sprayed, after bark removal, with 65% ethanol and exposed inside open Petri dishes containing sterile pieces of filter paper dampened with sterile water for 24 h at each sampling point. The four disks should be placed 5 m from the centre of each sampling point along the four cardinal directions. Sampling points should be established along transects at an optimal distance of 2 km from each other. For a survey, at least four sampling points should be established along each 8 km transect. The number and location of transects would depend on resources and risks. Distances larger than 2 km between sampling points may result in gaps in detection. Three closed Petri dishes should be included as controls at each collection time to check for possible contamination caused by either *Heterobasidion* spp. isolates already present in the wood disks used as traps or airborne spores that landed on the wood during trap preparation.

Based on the seasonal availability of spore inoculum of *Heterobasidion* spp. (Gonthier *et al.*, 2005; Garbelotto *et al.*,



Layout of a sampling point



Layout of sampling points in an example with two transects

2010), exposure of spore traps should normally be conducted once every 2 years, from late autumn to spring in Mediterranean climates, including central and southern Italy, and in late summer or early autumn in more continental climates when the mean daily temperature is between 5 and 20°C. The NPPO should be in charge of indicating the most suitable period of the year for spore surveys. Exposure of spore traps should be carried out in the absence of wind and rain.

In situations where symptoms of root rot are visible on host trees and infestation by *H. irregulare* is

suspected, sampling should be performed on symptomatic trees. These trees should be sampled by drilling three holes (about 10 cm deep below the bark and at 120° from one another) at the base of the trunk and by pooling all generated wood chips (Guglielmo *et al.*, 2010). The recommended drill bit should be 4 mm diameter and should be approximately 43 cm long. The bit should be made of chrome vanadium steel with a cobalt/tungsten carbide tip and should be used in a drill with a maximum speed of 1400 rev/min. Wood chips obtained during the drilling process are collected in 9-cm diameter plastic Petri dishes or in tubes and used as a sample for the detection of the pathogen as described in EPPO Diagnostic Standard for *H. irregulare* (EPPO, in press).

Drill bits should be cleaned and disinfected between trees with a 0.5% (w/v) sodium hypochlorite solution, rinsed with sterilized water and wiped with ethanol 95% (v/v). It is recommended that after sampling, thiophanate-methyl fungicide 38.5% (w/v) is applied on holes to prevent fungal infections on trees. Drilling may also be used to sample timber or wood in service.

Heterobasidion fruiting bodies and/or wood samples collected from outer sapwood at the level of roots or tree collar may also be collected for analyses. Testing of samples is performed as described in EPPO Diagnostic Standard for *H. irregulare* (EPPO, in press).

Surveys and sampling of symptomatic (suspected) trees may be performed in any period of the year. Surveys with woody spore traps exposed in the proximity of symptomatic and suspected trees may also be carried out as described above.

Professional and public awareness

Activities to raise awareness should especially target those trading plants and plant products, and agencies and stakeholders working with plantations of host plants (e.g. municipalities), parks, nurseries, shelterbelts, host tree forests, etc. This is very important for early detection and reduced spread of *H. irregulare*. Public awareness activities can be achieved, for example, via the Internet and through workshops involving growers, gardeners, schoolchildren, tree pruning company employees, plant pathologists, etc.

Appendix 2 – Delimiting surveys in an area where *H. irregulare* has been detected

Purpose

If *H. irregulare* is found during a detection survey, an intensive delimiting survey to establish the full extent of the infestation should be carried out immediately. The purpose of this action is to determine the geographical limits of the infested area (or areas) and then to define the regulated area.

Strategy

If *H. irregulare* is found during a detection survey as previously described (Appendix 1), a delimiting survey covering an area of 2 km radius surrounding the infested point/site should be carried out. Surveys should be conducted by using woody spore traps (Appendix 1). Four sampling points should be established along the four cardinal directions at 2 km distance from the infested point/site. To serve as positive controls, four sampling points should be established at each sampling period along the four cardinal directions at about 150 m distance from the infested point/site (Gonthier *et al.*, 2012). Depending on the size of the infested area, the number of sampling points should be increased accordingly. If further positive results are obtained, the edges of the delimiting survey should be moved outwards (by 2 km from the additional infested points/sites) until no further positive results are recorded.

Further surveys within the delimiting survey area should be conducted to identify trees or groups of trees showing symptoms of root rot and infested by *H. irregulare*.

Appendix 3 – Host plant list and host preference for *Heterobasidion irregulare* (EPPO Global Database, 2018; last accessed 02/28/2018)

Host plant	Pathogen preference	Level of uncertainty of pathogen preference*
<i>Abies</i> spp. (EPPO Code: 1ABIG)	Minor	L
<i>Abies balsamea</i> (EPPO Code: ABIBA)	Major	H
<i>Arbutus menziesii</i> (EPPO Code: ARDME)	Incidental	L
<i>Arctostaphylos manzanita</i> (EPPO Code: ARYMA)	Incidental	L
<i>Calocedrus</i> spp. (EPPO Code: 1CCDG)	Minor	H
<i>Calocedrus decurrens</i> (EPPO Code: CCDDE)	Major	L
<i>Chamaecyparis thyoides</i> (EPPO Code: CHCTH)	Incidental	L
<i>Erica arborea</i> (EPPO Code: EIAAR)	Incidental	L
<i>Juniperus</i> spp. (EPPO Code: 1IUPG)	Minor	H
<i>Juniperus occidentalis</i> (EPPO Code: IUPOC)	Major	L

(continued)

Appendix 3 (continued)

Host plant	Pathogen preference	Level of uncertainty of pathogen preference*
<i>Juniperus virginiana</i> (EPPO Code: IUPVI)	Minor	M
<i>Larix</i> spp. (EPPO Code: 1LAXG)	Minor	H
<i>Larix laricina</i> (EPPO Code: LAXLA)	Minor	H
<i>Larix lyallii</i> (EPPO Code: LAXLY)	Minor	H
<i>Larix occidentalis</i> (EPPO Code: LAXOC)	Minor	H
<i>Picea</i> spp. (EPPO Code: 1PIEG)	Minor	H
<i>Picea abies</i> (EPPO Code: PIEAB)	Minor	H
<i>Picea glauca</i> (EPPO Code: PIEGA)	Minor	H
<i>Picea sitchensis</i> (EPPO Code: PIESI)	Major	M
<i>Pinus</i> spp. (EPPO Code: 1PIUG)	Minor	H
<i>Pinus banksiana</i> (EPPO Code: PIUBN)	Major	L
<i>Pinus brutia</i> (EPPO Code: PIUBR)	Major	M
<i>Pinus coulteri</i> (EPPO Code: PIUCO)	Minor	M
<i>Pinus edulis</i> (EPPO Code: PIUED)	Minor	M
<i>Pinus elliotii</i> (EPPO Code: PIUEL)	Major	L
<i>Pinus halepensis</i> (EPPO Code: PIUHA)	Major	M
<i>Pinus jeffreyi</i> (EPPO Code: PIUJE)	Major	L
<i>Pinus lambertiana</i> (EPPO Code: PIULA)	Minor	L
<i>Pinus monophylla</i> (EPPO Code: PIUMP)	Minor	M
<i>Pinus palustris</i> (EPPO Code: PIUPA)	Major	M
<i>Pinus pinaster</i> (EPPO Code: PIUPL)	Major	M
<i>Pinus pinea</i> (EPPO Code: PIUPN)	Major	L
<i>Pinus ponderosa</i> (EPPO Code: PIUPO)	Major	L
<i>Pinus radiata</i> (EPPO Code: PIURA)	Minor	M
<i>Pinus resinosa</i> (EPPO Code: PIURE)	Major	L
<i>Pinus strobus</i> (EPPO Code: PIUST)	Major	H
<i>Pinus sylvestris</i> (EPPO Code: PIUSI)	Major	M
<i>Pinus taeda</i> (EPPO Code: PIUTD)	Major	L
<i>Prunus serotina</i> (EPPO Code: PRNSO)	Incidental	L
<i>Pseudotsuga</i> spp. (EPPO Code: 1PSTG)	Minor	L
<i>Pseudotsuga menziesii</i> (EPPO Code: PSTME)	Minor	L
<i>Quercus</i> spp. (EPPO Code: 1QUEG)	Incidental	L
<i>Thuja plicata</i> (EPPO Code: THUPL)	Minor	L
<i>Tsuga</i> spp. (EPPO Code: 1TSUG)	Incidental	L
<i>Tsuga canadensis</i> (EPPO Code: TSUCA)	Incidental	L

*L, low; M, medium; H, high.