

Follow up assessment – future regulatory status of *Phytophthora ramorum*

Assignment

This assessment is a follow-up on the assessment ‘A rapid assessment – future regulatory status of *Phytophthora ramorum*’ (SLU UA 2018.2.6-648) delivered on February 20, 2018. This document has been supplemented following additional questions from the Swedish Board of Agriculture and replaces the earlier submitted version.

Since 2002, *P. ramorum* has been regulated by a Commission Decision (2002/757/EC). The future regulatory status of this species is currently discussed and the designated annex working group (AWG) of COM has presented a proposal for potential future regulation of the pest (AWG, 2018).

The unit for risk assessment of plant pests at SLU has been given the assignment by the Swedish Board of Agriculture to evaluate the proposal and in particular to assess the following questions;

- 1) Regarding the motivation for the proposal concerning the risks associated with North American isolates to Europe. Is it only the risk of sexual reproduction that poses a threat? Page 2
- 2) Are there any evidence suggesting that North American isolates are already present in Europe? This is relevant in light of the proposal to list isolates of non-European origin as union quarantine pests and isolates of European origin as regulated non-quarantine pests. Page 3
- 3) Are the proposed requirements for wood in proportion to the risk the pathway pose, e.g. with regard to the information about the risk of spread in the AWG report? Page 3
- 4) Are the proposed requirements for bark in proportion to the risk the pathway poses? Page 4
- 5) Other comments? Page 4
- 6) What evidence constitutes the basis for the suggested heat treatment of 60 min at 55°C and what is the associated uncertainty? Appendix 1, Page 8

Assessment

1) Regarding the motivation for the proposal concerning the risks associated with North American isolates to Europe. Is it only the risk of sexual reproduction that poses a threat?

The AWG conclude in their report that there is no scientific evidence that introduction of the North American lineages of *P. ramorum* would lead to more damages in itself (AWG, 2018). Instead, the main risk identified is the risk of sexual reproduction which may give rise to more aggressive phenotypes that could have unpredictable consequences.

In our opinion it would be more relevant to not separate these two aspects but to consider both the risks associated with the introduction of new isolates *per se* and the risk of sexual reproduction together. Both events would lead to an increased genetic and phenotypic variation in the *P. ramorum* population, but to a different degree, and both events could lead to unpredictable consequences.

There are known differences between North American lineages and European lineages with regard to morphology, growth rate, aggressiveness and observed host-pathogen interactions (Elliott et al, 2011; Franceschini et al. 2014; O’Hanlon et al. 2017). Although studies suggest that the lineages share a common ancestor, the morphological, behavioural and genetic differences suggest previous adaptation to different environments (Goss et al. 2009; Brasier et al, 2017). However, there also appear to be a very large, if not a total, overlap in host range (but unknown if all known hosts have been tested against all lineages) and inoculation studies indicate no general pattern in damage potential on different hosts comparing the lineages (Canadian Food Inspection Agency, 2003). Thus, an introduction of isolates of the North American lineages would in itself, i.e. even without sexual reproduction, increase the diversity in the population in terms of growth pattern, aggressiveness and host interactions. Such new combinations of pathogen-environment-host interactions would increase the risk of unpredictable events.

It is nevertheless very difficult to further assess what the potential consequences of this increase in variation in the population of *P. ramorum* would be. It can for example be noted that the lineage NA1 commonly observed to be the least aggressive lineage in laboratory tests on *Rhododendron* spp. leaves is the lineage responsible for the sudden oak death in California and the forest infestation in Oregon (Grünwald et al. 2012).

Sexual reproduction could further lead to the development of new phenotypes better adapted to new hosts and different environments. But as also mentioned in the AWG report, the evidence available suggests that it is unlikely that sexual reproduction will occur. Thus, this risk is mainly based on the fact that *P. ramorum*, like other *Phytophthora* species, very often display unpredictable behaviour and in acknowledgement of the uncertainty associated with the still

limited knowledge we have regarding the evolution of this species (Goss et al. 2009).

In addition, there are also recent findings of *P. ramorum* in Vietnam (Jung et al. 2017). In 2016, isolates were obtained from six forest streams and included both A1 and A2 mating types. Preliminary analysis suggests that the isolates found are not likely to be from a known lineage (Forest Research, 2017). If confirmed, the threat to EU is thus not restricted to the European and North American lineages.

2) Are there any evidence suggesting that North American isolates are already present in Europe? This is relevant in light of the proposal to list isolates of non-European origin as union quarantine pests and isolates of European origin as regulated non-quarantine pests.

No reports of observations of North American isolates in Europe were found when searching the scientific literature and other information sources. As mentioned in the AWG report, the uncertainty is associated with the apparent lack of systematic surveys for different lineages in most parts of Europe. Nevertheless, extensive surveys of *P. ramorum* have been done in the UK and Ireland (e.g. King et al. 2015). It is also in these two countries where the fourth lineage (EU2) has been discovered (van Poucke et al. 2012).

It could be noted, that the reports of findings of three isolates of the A2 mating type in Belgium, were not of north American origin, but instead belonged to the European lineage EU1 (Chandelier et al. 2014). These isolates also appear to have been eradicated (Vercauteren et al. 2011).

3) Are the proposed requirements for wood in proportion to the risk the pathway pose, e.g. with regard to the information about the risk of spread in the AWG report?

This question is two-fold; how large is the risk and are the requirements suggested in proportion to this risk.

The risk associated with the commodity wood

Our interpretation is that from the perspective of natural spread it is true as stated in the AWG-report that hosts where the infections are limited to the inner bark are considered to be dead ends. This is due to that the potential of such hosts to contribute to natural spread is practically zero compared to that of hosts with foliar infections. However, from the perspective of preventing international human assisted spread through trade of wood, relatively rare occasions should also be considered. Thus, since spread may occur from wood, e.g., formation of chlamydospores has been observed in the sapwood of *N. densiflorus* (Parke et al.

2007), the risks associated with this pathway should not be considered to be practically zero from a PRA perspective.

The proposed requirements

Based on the fact that chlamydospores, which are resistant long term survival structures, can be formed in wood it is not unreasonable to place some requirements on the commodity. The requirements in the proposal by the AWG are slightly different from the ones included in the current emergency measure and the reasons for these changes are not fully clear. The option of a PFA is the same but the alternative options are different. The proposed requirement gives the option of heat treatment (55°C, 60 min) or removal of 3 cm of sapwood. According to the emergency measure the options are instead debarking combined with squared wood or different drying/disinfection options. We agree with the AWG that it is reasonable to use heat treatment instead of different drying/disinfection options since there are no studies that have evaluated the latter option (see Appendix 1 regarding the efficiency of heat treatment). Why the option debarked and squared has been changed to removal of 3 cm of sapwood is however not clear.

4) Are the proposed requirements for bark in proportion to the risk the pathway pose?

The proposed new requirement for bark is less stringent than the current emergency measure where ‘susceptible bark’ originating in the United States of America was not permitted entry into the EU community but broader, since the list of plants included in the category ‘susceptible bark’ is now longer and the requirements are suggested to encompass all non-European countries.

To place requirements on bark seems justified in relation to the risk posed by this pathway. However, whether the risk justifies the inclusion of all non-European countries needs further justification.

5) Other comments?

- Regarding the motivation given in the AWG report that regulating EU1 and EU2 as QP would not be justifiable. The AWG conclude that “As the EU1 and EU2 lineages are present in a number of MSs and can no longer be eradicated in some parts of the EU, regulating the pathogen as IAI / QP in the internal market would not be justifiable.” We agree that it is probably not possible to eradicate *P. ramorum* from the areas where it has established in natural habitats. But, if eradication is not possible, measures may be taken with the purpose of containment (article 28(2), regulation 2016/2031). If the AWG consider that the available phytosanitary measures do not allow prevention of spread

from areas where *P. ramorum* has established in natural environments the evidence for this should also be presented.

Another possibility is that the AWG considers that *P. ramorum* should be regarded to be ‘widely distributed’ and subsequently not fulfilling the criteria to become a QP. It is however not clearly stated in the report if that is the case. The AWG conclude that “Considering the said above [i.e. a description of the current situation in the EU], *P. ramorum* still appears to be not widely distributed in the EU” but continue by stating that “the situation is likely to be underestimated: in part of the MSs the surveys including sampling are not intensive enough, survey methods are not always well-developed, or not all survey methods available are used, e.g. baiting the pores from water.”. This is likely to be the case, but this is also likely to be the case for almost all regulated pests. If the AWG consider *P. ramorum* to be widely distributed additional information should be given to support that. We think that it is important that the motivation for why it is not justifiable to regulate the European lineages of *P. ramorum* as QP is made clear.

- How efficient a regulation of *P. ramorum* as a RNQP will be to prevent further spread through trade of plants within the EU is very much dependent on which tolerance level that will be applied. Is the goal to prohibit spread through trade of plants within the union (i.e. tolerance level 0) or only to restrict spread (i.e. a higher tolerance level)? If the goal is to prohibit spread through trade of plants (tolerance level 0) the requirements need to be more stringent than the current emergency measures. It is evident from the large numbers of observations of *P. ramorum* from plants in trade that the current measures in place do not prohibit further spread. It should be noted that a tolerance level of 0 will be a challenge for the nurseries currently surrounded with areas where *P. ramorum* occurs in the natural environment due to the risk of reinfestations.
- In relation to the section on detection and identification in the AWG report. There are also a molecular based method, a combination of four ASO-PCR assays, which can distinguish the different lineages from each other (Gagnon et al. 2014). The method is reported to provide a rapid identification of the four lineages.
- The research on *P. ramorum* has been intense during the last years, there are for example currently twice as many publications as there were in 2009 when the RAPRA PRA was performed. The uncertainty associated with the earlier assessments could potentially be decreased should the vast number of research articles published lately be reviewed.

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Prepared by the Unit for Risk Assessment of Plant Pests Swedish University of Agricultural Sciences

Niklas Björklund, Dept. of Ecology, Swedish University of Agricultural Sciences, P.O. Box 7044, S-750 07 Uppsala, Sweden. Delivery address: Ullsväg 16, E-mail: Niklas.Bjorklund@slu.se

Johanna Boberg, Dept. of Forest Mycology and Plant Pathology, PO Box 7026, SE-750 07 Uppsala, Sweden. Visiting address: Almas allé 5, E-mail: Johanna.Boberg@slu.se

Appendix 1

Question

What evidence constitutes the basis for the suggested heat treatment of 60 min at 55 °C and what is the associated degree of uncertainty?

Assessment

The pest risk management report from EPPO (2013) and the RAPRA PRA (Sansford et al. 2009) on *Phytophthora ramorum* together base their conclusions regarding the efficiency of heat treatment on several studies where the survival of *P. ramorum* under different temperature conditions were tested. In addition to these there are some additional studies published on this topic after the report from EPPO was published. The main results from all studies are described below.

In summary, these studies show that survival may depend on (1) the different growth structures produced by *P. ramorum*, (2) the substrate on which it grows and (3) the type of heat treatment tested, e.g. wet or dry heat (Table 1 and Figure 1). *P. ramorum* may be present in wood as hyphae, sporangia and/or chlamydospores. Treatments that eliminate hyphae and sporangia may not be adequate to kill chlamydospores that are more robust structures (Tooley et al. 2008). For example, while hyphae were found to be killed after 2.5 min at 50°C when held on agar plates (Browning et al. 2008), a high recovery rate after 1 week at 55°C was found from inoculated California Bay Laurel (*Umbellularia californica*) leaves, presumably from surviving chlamydospores (Harnik et al. 2004).

Only one study has investigated the survival of *P. ramorum* in wood after heat treatment under shorter time periods. In Tubajika et al. (2008) artificially and naturally infected wood of tanoak (*Notholithocarpus densiflorus*) was heat-treated at temperatures 50, 56, 60 and 65°C for 30, 45 and 60 minutes with a constant humidity of 20%. *P. ramorum* was reisolated from 1 sample (number of replicates not known) after exposure to 56°C for 30 min and after 45 min at 50°C.

Unfortunately, there was a very low level of detection of the pathogen in the control samples, which makes the interpretation of the experiment very uncertain and it can only be concluded that at least under certain circumstances *P. ramorum* survives these conditions. Swain et al. (2006) also incubated inoculated wood (*Quercus agrifolia*) at 55°C but these were treated for 2 full weeks, which resulted in no recovery.

Two studies were performed on inoculated *Rhododendron* spp. leaf disks (incl. chlamydospores). Funahashi and Parke (2018) showed that a 0.1% recovery was achieved after 20 minutes at 50°C through a modelling approach. Schweigkofler et al. (2014) found a clear difference between heat treatment during wet and dry conditions. Incubation in wet heat (water bath) eliminated the pathogen after 30

min at 50°C. Dry heat was less effective and a 10% survival was found after 120 min at 50°C. With dry heat a lethal effect was obtained after 30 min at 60°C. However, in a similar study where inoculated agar plugs were used (incl. chlamydospores), no difference between wet and dry heat was observed and no survival was found after 30 min at 50°C (Ditta et al. 2015). Yet again, *P. ramorum* colonies on agar were not killed after 30 min at 55°C in the study performed by Swain et al. (2006). Extending the time to 60 min did however efficiently kill the pathogen. Finally, Tooley et al. (2008) report that free chlamydospores in sand held at 40°C for 1 day showed “nearly zero recovery” while inoculated *Rhododendron* sp. leaves required 2 days at 40°C to eliminate recovery.

The heat treatment of 60 min at 55°C suggested by the AWG appear to be largely based on the study by Tubajika et al. (2007) showing that *P. ramorum* in wood may survive a treatment of 56°C for 30 min and the study reported by Swain et al. (2006) showing that a treatment of 60 min at 55°C was sufficient to eliminate the pathogen. However, the study by Swain et al. (2006) appear to be wrongly cited in the previous assessments since the experiment testing 60 min at 55°C was performed on cultures on agar plates and not on wood. However, the same article does include an experiment performed on wood but these samples were held for two weeks at 55°C. Thus, it seems as if there are currently no studies on wood material reporting lethal thresholds. For wood it is only known that a two week treatment at 55°C leads to complete mortality (Swain et al. 2006) and “preliminary observations” that *P. ramorum* may survive a treatment of 56°C for 30 min (Tubajika et al. 2007). The uncertainty associated with the efficiency of the suggested heat treatment (60°C, 60 min) is thereby likely to have been underestimated in the previous assessment, i.e. in EPP0 (2013).

Considering all the cited studies (Table 1 and Figure 1), our interpretation is that (1) temperatures below 40°C would require unreasonable long exposure times to be effective, (2) depending on the substrate, treatment conditions etc. the length of exposure at 50-60°C to eliminate *P. ramorum* generally seems to vary between a few minutes up to one hour. There is however one worrying exception and that is the study on leaves of *Umbellularia californica* where *P. ramorum* could still be reisolated after 1 week (Harnik et al. 2004). Why this result deviates from the other studies is unclear and such a long heat treatment would not be practically feasible. Based on studies on all types of substrates a less stringent heat treatment (56°C / 30 min) does not seem to be generally sufficient. The uncertainty of this conclusion is assessed to be low. A heat treatment with a higher temperatures (60°C/30 min), or longer exposure times (55°C/60 min) appear to eliminate the pathogen for almost all tested substrates under the tested circumstances. The uncertainty of this conclusion is assessed to be high due to the limitations of the studies on wood and the extreme heat tolerance during certain circumstances indicated in one study.

Conclusion

The stringency of the heat treatment suggested by the AWG seems to be based largely on the preliminary observations by Tubajika et al. (2008) where a single sample where *P. ramorum* was reisolated after exposure to 56°C for 30 min and on data from an experiment by Swain et al. (2006) that they mistakenly thought were done on wood. Thus, the support from laboratory studies on wood for the suggested level of stringency of heat treatment is poor.

Our attempt here to determine a suitable stringency of heat treatments based on the currently available laboratory experiments turned out to be very difficult. Mainly due to the limitations of the studies performed on wood and the uncertainty associated with relying on studies performed on other substrates. The risk associated with the pathway wood is assessed to be low (Sansford, 2009; EPPO, 2013). To decrease it further the summary of laboratory studies above provides some guidance on the expected risk reduction that can be obtained depending on the stringency of the measures.

Table 1. Summary of studies conducted on heat treatment to eliminate *Phytophthora ramorum* including lethal thresholds for temperature and time combinations

Morphological structure	Substrate	Treatment conditions	Temperature (°C)	Time (h)	Reference
Chlamyospores	Sand	Heat	40	24	Tooley et al. 2008
Chlamyospores	Agar plates	Heat	40	24	Turner et al. 2005
Hyphae, chlamyospores	Leaves of <i>Rhododendron</i> spp.	In moist soil	40	48	Tooley et al. 2008
Hyphae only	Membrane	Heated agar	50	0.04	Browning et al. 2008
Hyphae, Chlamyospores	Leaves of <i>Rhododendron</i> sp.	Water bath	50	0.33	Funahashi & Parke 2018
Hyphae, Chlamyospores	Leaves of <i>Rhododendron</i> sp.	Water bath	50	0.5	Schweigkofler et al. 2014
Hyphae, sporangia, chlamyospores	Agar plugs	Water bath	50	0.5	Ditta et al. 2015
Hyphae, sporangia, chlamyospores	Agar plugs	Dry heat	50	0.5	Ditta et al. 2016
Hyphae	Cultures	Dry heat	50	1	Turner et al. 2008 ¹
?	Agar plates	Heat	55	1	Swain et al. 2006
Hyphae, sporangia, chlamyospores	Leaves of <i>U. californica</i>	Heat	55	336	Harnik et al. 2004
?	Wood chips and cankers (<i>Q. agrifolia</i>), Leaves (<i>U. californica</i>)	Heat	55	<336 ²	Swain et al. 2006
Hyphae, Chlamyospores	Leaves of <i>Rhododendron</i> sp.	Dry heat (RH 8.5% at 60°C)	60	0.5	Schweigkofler et al. 2014
Hyphae	Cultures	Dry heat	60	0.5	Turner et al. 2008 ¹

¹ Data included as cited in Sansford et al. (2009).² Only one exposure time was used, i.e. two weeks, thus the lethal threshold could not be determined.

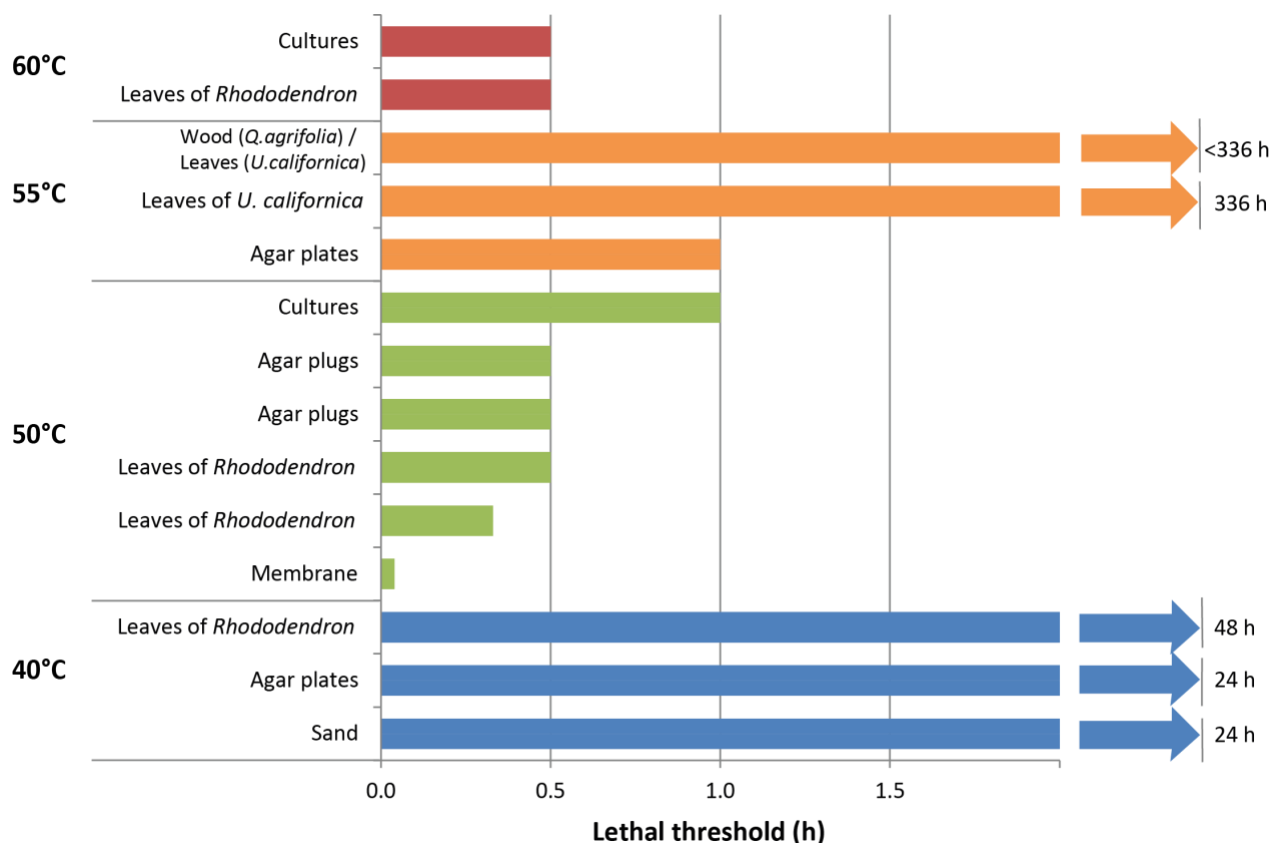


Figure 1. Illustration of the lethal threshold for *P. ramorum* in hours for different temperatures and substrates tested based on the data provided in Table 1.

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