# DRAFT ANNEX TO ISPM 27: *AUSTROPUCCINIA PSIDII* (2006-018)

## Summary of comments

| Name | Summary |
|---|---|
| Cameroon | Examen achevé |
| Cuba | No hay comentarios al PD |
| EPPO Σ | Finalised by the EPPO Secretariat on behalf of its 51 Member Countries. |
| European Union | Comments finalised by the European Commission on behalf of the EU and its 28 Member States on 29/09/2017. |
| Samoa | no further comments |
| South Africa | No comments from the National Plant Protection Organisation of South Africa. |

| # | Para | Text | Comment | SC's response |
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| 1 | G | (General Comment) | **Cameroon**<br>This diagnostic protocol is comprehensive, detailed and extensively illustrated. It provides a tool for the identification of this fungus. It will assist NPPOs in their activities<br>*Category : TECHNICAL* | **Noted** |
| 2 | G | (General Comment) | **Myanmar**<br>This disease is absent in Myanmar.<br>*Category : SUBSTANTIVE* | **Noted** |
| 3 | G | (General Comment) | **Peru**<br>We agree with the DRAFT ANNEX to ISPM 27 – Puccinia psidii Winter (2006-018)<br>*Category : TECHNICAL* | **Noted** |
| 4 | G | (General Comment) | **United States of America**<br>The United States has no comments on this draft standard.<br>*Category : SUBSTANTIVE* | **Noted** |
| 5 | G | (General Comment) | **Canada**<br>Canada supports the draft annex to ISPM 27 on Puccinia psidii.<br><br>Minor editorial comments are presented.<br>*Category : SUBSTANTIVE* | **Noted** |

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| 6 | G | (General Comment) | **Guyana**<br>Guyana has no objection to this Annex<br>*Category : SUBSTANTIVE* | **Noted** |
| 7 | G | (General Comment) | **Nicaragua**<br>This protocol should take into account the specificity of Puccinia for the identification at the level of species, every species has its own specificity, which indicates that for each species primers should be designed for its identification.<br>*Category : TECHNICAL* | **Considered, but not incorporated:**<br>This protocol described methods for identification to species level. The PCR primers recommended are species-specific. |
| 8 | G | (General Comment) | **Nicaragua**<br>Each laboratory analysis has its own specification appropriate for the method used. These instructions have specific environmental conditions, which have been validated or verified with the same analytical processes to make sure that reliable results are obtained. Paragraph 93 is linked to paragraph 39. Nicaragua agrees with what is indicated in both paragraphs. It is proposed that the Standards Committee extends the testing for the identification of the Puccinia species through molecular methods.<br>*Category : TECHNICAL* | **Considered, but not incorporated:**<br>Molecular methods for identification of *Austropuccinia psidii* are presented. It is up to the individual laboratory to optimise and validate methods to their own laboratory. The scope of this DP is for *Austropuccinia psidii.* |
| 9 | G | (General Comment) | **Panama**<br>Panama has no comments on this document.<br>*Category : EDITORIAL* | **Noted** |
| 10 | G | (General Comment) | **Tajikistan**<br>I support the document as it is and I have no comments<br>*Category : SUBSTANTIVE* | **Noted** |
| 11 | G | (General Comment) | **PPPO**<br>I do agree with the draft. Have not comments to make<br>*Category : EDITORIAL* | **Noted** |
| 12 | G | (General Comment) | **New Zealand**<br>Have no comments to make on the draft<br>*Category : SUBSTANTIVE* | **Noted** |
| 13 | G | (General Comment) | **Bahamas**<br>The vast distribution of P. psidii, its means of movement and dispersal and immense social and economic impacts, poses a phytosanitary risk for the region. The Bahamas therefore supports the adoption of this diagnostic protocol.<br>*Category : SUBSTANTIVE* | **Noted** |

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| 14 | G | (General Comment) | **Thailand**<br>agree with the proposed draft DP for Puccinia psidii<br>*Category : SUBSTANTIVE* | **Noted** |
| 15 | G | (General Comment) | **Lao People's Democratic Republic**<br>Lao PDR agreed with this drafted ISPM.<br>*Category : SUBSTANTIVE* | **Noted** |
| 16 | G | (General Comment) | **Honduras**<br>HONDURAS NO TIENE COMENTARIOS<br>*Category : TECHNICAL* | **Noted** |
| 17 | G | (General Comment) | **Lao People's Democratic Republic**<br>Lao PDR has no comment on draft annex to ISPM 27<br>*Category : SUBSTANTIVE* | **Noted** |
| 18 | G | (General Comment) | **Colombia**<br>The Colombian Agricultural and Livestock Institute (ICA), as the National Plant Protection Organization of Colombia, reviewed and analyzed the draft in question, finding that the proposed diagnostic protocol meets the requirements and is updated according to existing scientific evidence.<br>*Category : TECHNICAL* | **Noted** |
| 19 | G | (General Comment) | **Algeria**<br>Clarification is needed on:<br>the number of samples to be analyzed in the laboratory (representative sample)<br>the control if it must be carried out obligatorily on all the parts of the plant.<br>The protocol of analysis is valid for the asymptomatic samples.<br>*Category : TECHNICAL* | **Considered, but not incorporated:**<br>The number of samples has been specified in section 3.2. The type of sample required for testing has been specified for each method. The scope of the protocol is for testing symptomatic material. Suspicious lesions should be tested as described.<br>It is not recommended to test asymptomatic material or parts of a plant that are not showing symptoms. |
| 20 | 1 | **DRAFT ANNEX to ISPM 27 – ~~*Puccinia*~~ *Austropuccinia psidii* Winter (2006-018)** | **China**<br>The Taxonomic state of Puccinia psidii has been changed. See LUDWIG BEENKEN (2017) Austropuccinia: a new genus name for the myrtle rust Puccinia psidii placed within the redefined family Sphaerophragmiaceae (Pucciniales)<br>*Category : SUBSTANTIVE* | **Incorporated** |
| 21 | 17 | 2016-07 ~~To~~To Technical Panel on Diagnostic Protocols (TPDP) meeting | **China**<br>Editorial mistake.<br>*Category : EDITORIAL* | **Considered but not incorporated** |
| 22 | 37 | As a result of the description of a second rust pathogen from eucalyptus in a recent publication (Maier *et al.*, 2016), the diagnostic protocol was strengthened to ensure distinction from that rust. | **Nicaragua**<br>The team of IPSA of Nicaragua for the revision of ISPM 27 (Puccinia psidii) considers that: It is suggested to maintain what was raised by Maier et al., 2016. | **Noted**<br>This has been well described in section 4 – Identification. |

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| | | | *Category : TECHNICAL* | |
| 23 | 38 | A real-time PCR has recently been published (Baskarathevan *et al.*, 2016) and is included in the protocol. | **Nicaragua**<br>Nicaragua is in agreement with the inclusion.<br>*Category : TECHNICAL* | **Noted** |
| 24 | 39 | The protocol does not discriminate between genotypes of the rust. Identifying and distinguishing *P. psidii* pathogenic genotypes is still a research question, and is not robust or reproducible enough for diagnostic purposes yet. | **Nicaragua**<br>Nicaragua agrees to continue to perform studies on identification of genotypes and to validate the protocol.<br>*Category : TECHNICAL* | **Noted** |
| 25 | 40 | Name of *Puccinia psidii* has been changed to *Austropuccinia psidii* Beenken 2017 after the approval of the draft DP for consultation by the SC (Beenken, L., 2017. *Austropuccinia*: a new genus name for the myrtle rust *Puccinia psidii* placed within the redefined family Sphaerophragmiaceae (Pucciniales). Phytotaxa, 297(1): 53-61). | **Nicaragua**<br>Nicaragua suggests to take into account the reclassification of the pathogen and include it in the protocol.<br>*Category : TECHNICAL* | **Incorporated** |
| 26 | 53 | ~~*P.*~~ *Puccinia psidii* is considered to be a threat to plants of the family Myrtaceae worldwide (Coutinho *et al.*, 1998). Since the rust spread out of its native region, its host range has expanded rapidly (Maier *et al.*, 2016). As of September 2014, the global host list comprised more than 300 species from 73 genera in this family (Giblin and Carnegie, 2014), but it is likely that the majority of the thousands of Myrtaceae species have the potential to be infected (Carnegie and Lidbetter, 2012; Morin *et al.*, 2012). *P. psidii* is not known to infect host plants that are not Myrtaceae. There is evidence of physiological specialization within *P. psidii* (Graça *et al.*, 2013), which may have quarantine implications (Roux *et al.*, 2016). | **Canada**<br>Sentence to start with the genus name and not abbreviation.<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>IPPC's style guide allows sentences to start with a genus abbreviation and the genus name was mentioned before. |
| 27 | 54 | ~~*P.*~~ *Puccinia psidii* is an obligate biotroph with an autoecious, but incomplete, life cycle, producing urediniospores, teliospores and basidiospores on an infected host (Glen *et al.*, 2007). Under natural conditions, *P. psidii* can reproduce quickly and simply through asexual reproduction whereby urediniospores are produced in pustules known as uredinia. These spores are dispersed to leaves on the same plant or to other hosts, which in turn are infected and on which the pathogen produces pustules with more urediniospores. In some circumstances, the uredinia may switch to producing teliospores, which can germinate *in situ* to produce basidiospores. Teliospores may also be produced by another type of spore producing body, telia. Teliospore and basidiospore production were initially considered rare stages of the life cycle, but in some regions are often observed along with urediniospore production within a single sorus (Pegg *et al.*, 2014). While the | **Canada**<br>Sentence to start with genus name and not abbreviation.<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>IPPC's style guide allows sentences to start with a genus abbreviation and the genus name was mentioned before. |

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| | | production of all three types of spores in a host is considered to be a strategy for survival in adverse conditions, the role of teliospores and basidiospores in the life cycle of *P. psidii* has not been understood (Morin *et al.*, 2012; Giblin, 2013). Spermagonia and aecia have never been observed. | | |
| 28 | 55 | ~~*P.*~~ *Puccinia psidii* prefers wet tropic and subtropic regions where moist conditions and warm temperatures prevail, but a spread to cool regions has been reported (Kriticos *et al.*, 2013) and the optimum temperature for survival of the fungus is unknown. Disease development is favoured following periods of rainfall or in high humidity or fog. Extended periods of leaf wetness promote urediniospore germination and infection of the host. Urediniospores must encounter a host plant during stages of active growth or flush, which can occur throughout the year depending on the host species and climatic conditions (Pegg *et al.*, 2014). | **Canada**<br>Sentence to start with genus name and not abbreviation.<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>IPPC's style guide allows sentences to start with a genus abbreviation and the genus name was mentioned before. |
| 29 | 58 | **Name:** Austropuccinia~~*Puccinia*~~ *psidii* G. Winter, 1884 | **Australia**<br>Austropuccinia: a new genus name for the myrtle rust Puccinia psidii placed within the redefined family Sphaerophragmiaceae (Pucciniales) Beenken, L. (2017). Phytotaxa 297(1): 53-61<br>*Category : SUBSTANTIVE* | **Incorporated** |
| 30 | 58 | **Name:** *Austopuccinia* ~~*Puccinia*~~ *psidii* G. Winter, 1884 | **China**<br>Name has been changed.<br>*Category : EDITORIAL* | **Incorporated** |
| 31 | 59 | Synonyms:<br>*Austropuccinia psidii* (G. Winter) Beenken, 2017<br>Basionym: *Puccinia psidii* G. Winter, 1884.<br>≡*Bullaria psidii* (G. Winter) Arthur & Mains, 1922<br>≡*Dicaeoma psidii* (G. Winter) Kuntze, 1898.<br>= *Aecidium glaziovii* Henn., 1897<br>= *Caeoma eugeniarum* Link, 1825<br>= *Puccinia actinostemonis* H.S. Jacks. & Holw., 1931<br>= *Puccinia barbacensis* Rangel, 1916<br>= *Puccinia brittoi Rangel*, 1916<br>= *Puccinia camargoi* Puttemans, 1930<br>= *Puccinia cambucae* Puttemans, 1916<br>= *Puccinia eugeniae* Rangel, 1916<br>= *Puccinia grumixamae* Rangel, 1917<br>= *Puccinia jambolani* Rangel, 1912<br>= *Puccinia jambosae* Henn., 1902 | **China**<br>The Taxonomic state of Puccinia psidii has been changed. See LUDWIG BEENKEN (2017) Austropuccinia: a new genus name for the myrtle rust Puccinia psidii placed within the redefined family Sphaerophragmiaceae (Pucciniales), Farr, D.F., & Rossman, A.Y. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved July 25, 2017, from https://nt.ars-grin.gov/fungaldatabases/. There are missing data about Synonyms according to International Code of Nomenclature for algae, fungi, and plants (Melbourne Code).<br>*Category : SUBSTANTIVE* | **Incorporated.**<br>The synonyms are all there, but are in chronological order, not alphabetical order. |

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| | | = *Uredo eugeniarum* Henn., 1895<br>= *Uredo flavidula* G. Winter, 1885<br>= *Uredo goeldiana* Henn., 1903<br>= *Uredo myrtacearum* Pazschke, 1890<br>= *Uredo myrciae* Mayor, 1913<br>= *Uredo neurophila* Speg., 1884<br>= *Uredo pitangae* Speg., 1899 (published as "pitanga")<br>= *Uredo puttemansii* Henn., 1902<br>= *Uredo rangelii* Simpson, Thomas & Grgurinovic, 2006<br>= *Uredo rochaei* Puttemans, 1906<br>= *Uredo seclusa* Jacks. & Holw., 1931<br>= *Uredo subneurophila* Speg., 1884 | | |
| 32 | 85 | **Taxonomic position:** Eukaryota, Fungi, Basidiomycota, Pucciniomycotina, Pucciniomycetes, Pucciniales~~,~~ Sphaerophragmiaceae, Austropuccinia ~~Pucciniaceae, *Puccinia*~~ | **China**<br>The Taxonomic state of Puccinia psidii has been changed.<br>*Category : SUBSTANTIVE* | **Incorporated** |
| 33 | 90 | All plants in the family Myrtaceae should be considered potentially susceptible to infection by *P. psidii*, and rust infecting any plants in this family should be investigated to rule out *P. psidii* infection. ~~Early detection is very important to minimize spread of the disease.~~ | **European Union**<br>Sentence not really relevant in this paragraph.<br>*Category : SUBSTANTIVE* | **Incorporated** |
| 34 | 90 | All plants in the family Myrtaceae should be considered potentially susceptible to infection by *P. psidii*, and rust infecting any plants in this family should be investigated to rule out *P. psidii* infection. ~~Early detection is very important to minimize spread of the disease.~~ | **EPPO**<br>Sentence not really relevant in this paragraph.<br>*Category : SUBSTANTIVE* | **Incorporated** |
| 35 | 93 | In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated. | **European Union**<br>Redundant with footnote 1 (paragraph 118)? If this is the case, delete paragraph 93 or footnote 1.<br>*Category : EDITORIAL* | **Modified**<br>The text in the main body of the document and the footnote has been adjusted to avoid repetition while still including all relevant information. |
| 36 | 93 | In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original | **European Union**<br>Better wording (see for example DP Bactrocera dorsalis). | **Incorporated** |

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| | | level of sensitivity, specificity ~~and/or~~ and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated. | *Category : EDITORIAL* | |
| 37 | 93 | ~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated. | **Nicaragua**<br>Each laboratory analysis has its own specification appropriate for the method used. These instructions have specific environmental conditions, which have been validated or verified with the same analytical processes to make sure that reliable results are obtained. Paragraph 93 is linked to paragraph 39. Nicaragua agrees with what is indicated in both paragraphs. It is proposed that the Standards Committee extends the testing for the identification of the Puccinia species through molecular methods.<br>*Category : TECHNICAL* | **Considered, but not incorporated:**<br>Molecular methods for identification of *Austropuccinia psidii* are presented. It is up to the individual laboratory to optimise and validate methods to their own laboratory. The scope of this DP is for *Austropuccinia psidii.* |
| 38 | 93 | In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity ~~and/or~~ and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated. | **EPPO**<br>Better wording (see for example DP Bactrocera dorsalis).<br>*Category : EDITORIAL* | **Incorporated** |
| 39 | 93 | In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of | **EPPO**<br>Redundant with footnote 1 (paragraph 118)? If this is the case, delete paragraph 93 or footnote 1.<br>*Category : EDITORIAL* | **Modified**<br>The text in the main body of the document and the footnote has been adjusted to avoid repetition while still including all relevant information. |

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| | | individual laboratories, provided that they are adequately validated. | | |
| 40 | 93 | In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and/or reproducibility achieved. ~~The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable.~~ Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated. | **Uruguay**<br>Text deleted to avoid repetition with text in the footnote<br>*Category : TECHNICAL* | **Incorporated**<br>The text in the main body of the document and the footnote has been adjusted to avoid repetition while still including all relevant information. |
| 41 | 95 | Symptoms of infection by *P. psidii* range from minor leaf spots to severe foliage and stem blight, and in some species, flowers and fruits are infected (Figures 2–4). Since ~~establishing~~ it has established in Australia in 2010, the rapid ~~increase~~ expansion of the fungus' host range has resulted in a wide range of symptoms being observed which vary depending on the host species, the level of host susceptibility within a host species and the age of the host tissue. Symptoms become more obvious during rust epidemics. | **Singapore**<br>Proposed revision for better sentence structuring.<br>*Category : EDITORIAL* | **Incorporated** |
| 42 | 96 | The primary symptom is the appearance of yellow pustules (uredinia) on the upper and lower leaf surfaces of Myrtaceae hosts, with ~~more tending to be found~~ a higher prevalence on the lower leaf surfaces (abaxial). Pustules can also be found on stems, fruits and flowers. | **Singapore**<br>Proposed revision for better sentencing.<br>*Category : EDITORIAL* | **Incorporated** |
| 43 | 97 | The first symptoms and signs of infection are often chlorotic flecks and young sori on leaves, shoots and fruits, which appear two to four days after infection. These early symptoms are similar to those caused by many other pests and ~~disorders so are not enough for a diagnostic~~disorders.vHence, ~~but lesions~~ identification based on symptoms alone may not be ~~used~~ sufficient for diagnosis. Further confirmation for *P. psidii* infection can be carried out using molecular ~~testing if there is any reason to suspect it is~~ diagnostic methods. ~~*P. psidii.*~~ | **Singapore**<br>Proposed revision to better structure the sentence.<br>*Category : EDITORIAL* | **Incorporated** |
| 44 | 101 | **3.2 Sampling and sample preparation** | **Uruguay**<br>For symptomless plants, it would be very useful to include the minimum number of leaves, flowers, etc required for P. psidii detection<br>*Category : TECHNICAL* | **Considered, but not incorporated.** To our knowledge, this information does not exist. We do not suggest that symptomless material should be collected, only samples with suspicious lesions. |

| # | Para | Text | Comment | SC's response |
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| 45 | 101 | **3.2 Sampling and sample preparation** | **Argentina**<br>In the case of symptomless plants, it would be very useful to include the minimum number of leaves, flowers,etc required for P. psidii detection.<br>*Category : TECHNICAL* | **Considered, but not incorporated.**<br>To our knowledge, this information does not exist. We do not suggest that symptomless material should be collected, only samples with suspicious lesions. |
| 46 | 102 | Samples of suspected rust or suspicious lesions on plant stems, flowers, fruits, shoots or leaves should be collected. Where possible, triplicate samples from each infected host species should be collected, with each sample comprising several lesions. The likelihood of obtaining enough material for DNA extraction and of finding features for a robust morphological diagnosis is increased with an increased number of collected lesions. In the event of an older infection, woody twigs and branches with swellings or galls or other evidence of infection should be sampled. | **European Union**<br>Disinfection of tools with bleach requires "draining". Is this rinsing with water? Implies that also water is taken to the field in sufficient quantities to rinse the disinfected tools. According to the instructions, paper towelling also needs to be taken to the field. Usually alcohol based cleaning/disinfection is used, also allows flaming of metal parts. If not rinsed than tools will corrode due to bleach.<br>*Category : TECHNICAL* | **Considered, but not incorporated.**<br>A disinfection kit is generally taken into the field. This includes concentrated bleach, water, paper towelling, plastic tubs. The bleach is diluted to 5% in a plastic tub and the tools paced in the tub. By draining, it just means that the bleach is allowed to run off and drain back into the tub. The secateurs are then blotted dry with paper towelling. The tools can be washed properly after the collecting trip to remove the bleach. Flaming is not always possible in the field – if it is hot and dry, for instance, it could cause a bushfire hazard. It could be too windy. The described method has been used in Australia, New Zealand and New Caledonia. |
| 47 | 102 | Samples of suspected rust or suspicious lesions on plant stems, flowers, fruits, shoots or leaves should be collected. Where possible, triplicate samples from each infected host species should be collected, with each sample comprising several lesions. The likelihood of obtaining enough material for DNA extraction and of finding features for a robust morphological diagnosis is increased with an increased number of collected lesions. In the event of an older infection, woody twigs and branches with swellings or galls or other evidence of infection should be sampled. | **European Union**<br>It would be appropriate to provide a definition of "sample" as one person may see it as an individual leaf (with several lesions possible) and others may collect 10 leaves in one sample. In case of the latter, then triplicate samples seems much more than needed.<br>*Category : TECHNICAL* | **Considered, but not incorporated.**<br>It will depend on the circumstances. Where possible, the more material, the better, as it will be easier to collect spores for DNA extraction. But if only a few lesions are present, then they should all be sampled. If 10 leaves are collected, then that can be considered 10 samples, or three samples of 3-4 leaves. This should not be too prescriptive as it will depend on the situation in the field. |
| 48 | 102 | Samples of suspected rust or suspicious lesions on plant stems, flowers, fruits, shoots or leaves should be collected. Where possible, triplicate samples from each infected host species should be collected, with each sample comprising several lesions. The likelihood of obtaining enough material for DNA extraction and of finding features for a robust morphological diagnosis is increased with an increased number of collected lesions. In the event of an older infection, woody twigs and branches with swellings or galls or other evidence of infection should be sampled. | **EPPO**<br>It would be appropriate to provide a definition of "sample" as one person may see it as an individual leaf (with several lesions possible) and others may collect 10 leaves in one sample. In case of the latter, then triplicate samples seems much more than needed.<br>*Category : TECHNICAL* | **Considered, but not incorporated.**<br>It will depend on the circumstances. Where possible, the more material, the better, as it will be easier to collect spores for DNA extraction. But if only a few lesions are present, then they should all be sampled. If 10 leaves are collected, then that can be considered 10 samples, or three samples of 3-4 leaves. This should not be too prescriptive as it will depend on the situation in the field. |
| 49 | 102 | Samples of suspected rust or suspicious lesions on plant stems, flowers, fruits, shoots or leaves should be collected. Where possible, triplicate samples from each infected host species should be collected, with each | **Philippines**<br>Specify size and weight of samples to be collected<br>*Category : TECHNICAL* | **Considered, but not incorporated.**<br>It will depend on the circumstances. Where possible, the more material, |

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| | | sample comprising several lesions. The likelihood of obtaining enough material for DNA extraction and of finding features for a robust morphological diagnosis is increased with an increased number of collected lesions. In the event of an older infection, woody twigs and branches with swellings or galls or other evidence of infection should be sampled. | | the better, as it will be easier to collect spores for DNA extraction. But if only a few lesions are present, then they should all be sampled. If 10 leaves are collected, then that can be considered 10 samples, or three samples of 3-4 leaves. This should not be too prescriptive as it will depend on the situation in the field. |
| 50 | 103 | Direct handling of the plant material should be avoided to prevent spread of the disease, as rust spores are readily dispersed by wind and carried on clothing and other equipment, and can survive for up to three months. A new pair of disposable gloves should be worn for each sampling. A paper bag should be placed around the sample before snipping the infected part from the plant. Collecting tools should be thoroughly dipped and lightly shaken in bleach (a solution of sodium hypochlorite (NaOCl) diluted to at least 5% active chlorine) for about 1 min, followed by draining and drying with paper towelling before and after each sampling. Each sample should be sealed in a single paper bag and labelled. | **European Union**<br>Transport at 4°C is recommended. This is fine, but an exact temperature is hard to implement, especially during mail transport. A temperature range, or a less specific condition (e.g. "cooled") might be considered.<br>*Category : TECHNICAL* | **Modified.**<br>"storage and transport at cool temperatures (e.g. 4 °C) is recommended ….." |
| 51 | 103 | Direct handling of the plant material should be avoided to prevent spread of the disease, as rust spores are readily dispersed by wind and carried on clothing and other equipment, and can survive for up to three months. A new pair of disposable gloves should be worn for each sampling. A paper bag should be placed around the sample before snipping the infected part from the plant. Collecting tools should be thoroughly dipped and lightly shaken in bleach (a solution of sodium hypochlorite (NaOCl) diluted to at least 5% active chlorine) for about 1 min, followed by draining and drying with paper towelling before and after each sampling. Each sample should be sealed in a single paper bag and labelled. | **EPPO**<br>Transport at 4°C is recommended. This is fine, but an exact temperature is hard to implement, especially during mail transport. A temperature range, or a less specific condition (e.g. "cooled") might be considered.<br>*Category : TECHNICAL* | **Modified.**<br>"storage and transport at cool temperatures (e.g. 4 °C) is recommended ….." |
| 52 | 103 | Direct handling of the plant material should be avoided to prevent spread of the disease, as rust spores are readily dispersed by wind and carried on clothing and other equipment, and can survive for up to three months. A new pair of disposable gloves should be worn for each sampling. A paper bag should be placed around the sample before snipping the infected part from the plant. Collecting tools should be thoroughly dipped and lightly shaken in bleach (a solution of sodium hypochlorite (NaOCl) diluted to at least 5% active chlorine) for about 1 min, followed by draining and drying with paper towelling before and after each sampling. Each sample should be sealed in a single paper bag and labelled. | **EPPO**<br>Disinfection of tools with bleach requires "draining". Is this rinsing with water? Implies that also water is taken to the field in sufficient quantities to rinse the disinfected tools. According to the instructions, paper toweling also needs to be taken to the field. Usually alcohol based cleaning/disinfection is used, also allows flaming of metal parts. If not rinsed than tools will corrode due to bleach.<br><br>*Category : TECHNICAL* | **Considered, but not incorporated.**<br>A disinfection kit is generally taken into the field. This includes concentrated bleach, water, paper towelling, plastic tubs. The bleach is diluted to 5% in a plastic tub and the tools paced in the tub. By draining, it just means that the bleach is allowed to run off and drain back into the tub. The secateurs are then blotted dry with paper towelling. The tools can be washed properly after the collecting trip to remove the bleach. Flaming is not always possible in the field – if it is hot and dry, for instance, it could cause a |

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| | | | | bushfire hazard. It could be too windy. The described method has been used in Australia, New Zealand and New Caledonia. |
| 53 | 106 | In the diagnostic laboratory, the sample should be allowed to dry inside the paper bag at room temperature. All plant material should be preserved as air-dried samples in sealed paper bags to minimize the growth of saprophytic organisms. | **Kenya**<br>respiration<br>*Category : TECHNICAL* | **Modified.**<br>Included temperatures (20-25 °C). Note we think this is the same issue as described by Singapore. |
| 54 | 106 | ~~In the diagnostic laboratory, the sample should be allowed to dry inside the paper bag at room temperature. All plant material should be preserved as air-dried samples in sealed paper bags to minimize the growth of saprophytic organisms.~~ | **Singapore**<br>In tropical countries with high humidity, drying inside a paper bag at room temperature is not possible as the sample will rot faster with more saprophytes.<br>*Category : SUBSTANTIVE* | **Modified.**<br>Included temperatures (20-25 °C). |
| 55 | 108 | Because rust spores have a high potential for dispersal, samples should be handled with care during the diagnostic process. A large sheet of paper can be placed under the work area and changed between samples. Use of a laminar flow cabinet is not advisable as this may disperse the spores. At the end of the work period, all packaging, gloves, benchcoating, paper, tissues, etc. should be considered contaminated, double-bagged and disposed of as required for quarantineable waste (e.g. by autoclaving or incineration). Equipment should be surface-sterilized with 70% ethanol. | **European Union**<br>Change of gloves and disinfection of equipment (scalpel, forceps, etc.) between samples should also be advised (given that you advice other similar measures).<br>*Category : TECHNICAL* | **Incorporated.**<br>Gloves should be changed and equipment (scalpels, forceps, etc.) disinfected between samples. |
| 56 | 108 | Because rust spores have a high potential for dispersal, samples should be handled with care during the diagnostic process. A large sheet of paper can be placed under the work area and changed between samples. Use of a laminar flow cabinet is not advisable as this may disperse the spores. At the end of the work period, all packaging, gloves, benchcoating, paper, tissues, etc. should be considered contaminated, double-bagged and disposed of as required for quarantineable waste (e.g. by autoclaving or incineration). Equipment should be surface-sterilized with 70% ethanol. | **European Union**<br>Does it need to be ethanol or can IMS or similar also be used?<br>*Category : TECHNICAL* | **Modified.**<br>Sentence rephrased to "or similar disinfectant known to be effective against rust spores". We have not included IMS as we do not know if it is effective against myrtle rust spores. |
| 57 | 108 | Because rust spores have a high potential for dispersal, samples should be handled with care during the diagnostic process. A large sheet of paper can be placed under the work area and changed between samples. Use of a laminar flow cabinet is not advisable as this may disperse the spores. At the end of the work period, all packaging, gloves, benchcoating, paper, tissues, etc. should be considered contaminated, double-bagged and disposed of as | **EPPO**<br>Does it need to be ethanol or can IMS or similar also be used?<br>*Category : TECHNICAL* | **Modified.**<br>Sentence rephrased to "or similar disinfectant known to be effective against rust spores". We have not included IMS as we do not know if it is effective against myrtle rust spores. |

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| | | required for quarantineable waste (e.g. by autoclaving or incineration). Equipment should be surface-sterilized with 70% ethanol~~.~~. | | |
| 58 | 108 | Because rust spores have a high potential for dispersal, samples should be handled with care during the diagnostic process. A large sheet of paper can be placed under the work area and changed between samples. Use of a laminar flow cabinet is not advisable as this may disperse the spores. At the end of the work period, all packaging, gloves, benchcoating, paper, tissues, etc. should be considered contaminated, double-bagged and disposed of as required for quarantineable waste (e.g. by autoclaving or incineration). Equipment should be surface-sterilized with 70% ethanol. | **EPPO**<br>Change of gloves and disinfection of equipment (scalpel, forceps, etc.) between samples should also be advised (given that you advice other similar measures).<br>*Category : TECHNICAL* | **Incorporated.**<br>Gloves should be changed and equipment (scalpels, forceps, etc.) disinfected between samples. |
| 59 | 110 | The samples should be examined under a dissecting microscope for rust sori. Rust spores should be picked up carefully with a disposable scalpel or needle, mounted in a drop of lactic acid on a microscope slide and covered with a coverslip. The slide should be heated gently to remove air bubbles, then observed under a compound light microscope fitted with high quality optics, including a 100× oil immersion objective. Urediniospores and teliospores of *P. psidii* are readily distinguishable by light microscopy (Figure 5). | **European Union**<br>Is it critical that it is mounted in lactic acid? Other labs may use other mounting media such as water or lactoglycerol<br>*Category : TECHNICAL* | **Modified:**<br>to 'clear mountant such as lactic acid' |
| 60 | 110 | The samples should be examined under a dissecting microscope for rust sori. Rust spores should be picked up carefully with a disposable scalpel or needle, mounted in a drop of lactic acid on a microscope slide and covered with a coverslip. The slide should be heated gently to remove air bubbles, then observed under a compound light microscope fitted with high quality optics, including a 100× oil immersion objective. Urediniospores and teliospores of *P. psidii* are readily distinguishable by light microscopy (Figure 5). | **European Union**<br>Is a 100x oil immersion objective a necessity for identification of this rust? In the descriptions below one cannot see any features that require a 100x lens to see them, that cannot been seen with a 40x lens.<br>*Category : TECHNICAL* | **Incorporated.**<br>'including a 100x oil immersion objective' has been removed |
| 61 | 110 | The samples should be examined under a dissecting microscope for rust sori. Rust spores should be picked up carefully with a disposable scalpel or needle, mounted in a drop of lactic acid on a microscope slide and covered with a coverslip. The slide should be heated gently to remove air bubbles, then observed under a compound light microscope fitted with high quality optics, including a 100× oil immersion objective. Urediniospores and | **EPPO**<br>Is a 100x oil immersion objective a necessity for identification of this rust? In the descriptions below one cannot see any features that require a 100x lens to see them, that cannot been seen with a 40x lens.<br>*Category : TECHNICAL* | **Incorporated.**<br>'including a 100x oil immersion objective' has been removed |

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| | | teliospores of *P. psidii* are readily distinguishable by light microscopy (Figure 5). | | |
| 62 | 110 | The samples should be examined under a dissecting microscope for rust sori. Rust spores should be picked up carefully with a disposable scalpel or needle, mounted in a drop of lactic acid on a microscope slide and covered with a coverslip. The slide should be heated gently to remove air bubbles, then observed under a compound light microscope fitted with high quality optics, including a 100× oil immersion objective. Urediniospores and teliospores of *P. psidii* are readily distinguishable by light microscopy (Figure 5). | **EPPO**<br>Is it critical that it is mounted in lactic acid? Other labs may use other mounting media such as water or lactoglycerol<br>*Category : TECHNICAL* | **Modified:**<br>to 'clear mountant such as lactic acid' |
| 63 | 110 | The samples should be examined under a dissecting microscope for rust sori. Rust spores should be picked up carefully with a disposable scalpel or needle, mounted in a drop of lactic acid on a microscope slide and covered with a coverslip. The slide should be heated gently to remove air bubbles, then observed under a compound light microscope fitted with high quality optics~~, including a 100× oil immersion objective~~. Urediniospores and teliospores of *P. psidii* are readily distinguishable by light microscopy (Figure 5). | **China**<br>It is not necessary to use the microscope with 100× oil immersion objective for observation of urediniospores and teliospores. See （da S. Machado 2015）：Urediniospores 18–23 µm diam. 20–26×15–22 µm, wall 1.5–2.5 µm thick. Teliospores 27–43×16–24 µm, wall 0.7–1.0 µm, pedicel 9–13 µm long （epitype specimen）.<br>*Category : TECHNICAL* | **Incorporated.** |
| 64 | 115 | **3.4.1 Preparation of plant material** | **Singapore**<br>More appropriate definition of this section title<br>*Category : EDITORIAL* | **Incorporated** |
| 65 | 116 | DNA for PCR analysis can be extracted from individual sori or small pieces (10–100 mm2) of infected plant tissue excised from the sample if sori are not yet erumpent. If spores are abundant, they should preferentially be used by placing them into a microcentrifuge tube using a clean brush. | **European Union**<br>How is contamination via the brush avoided? New brush for each sample? Bleach? EtOH will not be sufficient to remove DNA. How is the mortar and pestle decontaminated between samples?<br>*Category : TECHNICAL* | **Modified.**<br>With addition of ' clean brush for each sample.'. Yes, a new brush for each would be preferred as we are talking small paint brushes which are inexpensive. However, the brushes can be disinfected, washed and dried for use on another day. Soapy water is fine. It is not DNA that is being removed, it is spores. Examining the brush under low magnification would show if the spores are still present or have been removed. The mortar and pestles should be washed in soapy water, rinsed with ethanol and autoclaved prior to and after use. Guidance provided in para 117. |
| 66 | 116 | DNA for PCR analysis can be extracted from individual sori or small pieces (10–100 mm2) of infected plant tissue excised from the sample if sori are not yet erumpent. If spores are abundant, they should preferentially be used by placing them into a microcentrifuge tube using a clean brush. | **EPPO**<br>How is contamination via the brush avoided? New brush for each sample? Bleach? EtOH will not be sufficient to remove DNA. How is the mortar and pestle decontaminated between samples? | **Modified.**<br>With addition of ' clean brush for each sample.'. Yes, a new brush for each would be preferred as we are talking small paint brushes which are inexpensive. However, the brushes can be disinfected, washed |

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| | | | *Category : TECHNICAL* | and dried for use on another day. Soapy water is fine. It is not DNA that is being removed, it is spores. Examining the brush under low magnification would show if the spores are still present or have been removed. The mortar and pestles should be washed in soapy water, rinsed with ethanol and autoclaved prior to and after use. Guidance provided in para 117. |
| 67 | 117 | The sample is placed into a tube or mortar bowl. Different grinding methods can be used such as mortar and pestle with liquid nitrogen, plastic pestles and microcentrifuge tubes with liquid nitrogen, or a TissueLyser (Qiagen[1]). The spore wall is very strong and all methods for DNA extraction and purification depend on its adequate disruption. For the TissueLyser, the addition of two 3 mm tungsten carbide beads, the pre-freezing of tubes in liquid nitrogen for 1 min, and two 2 min sessions in the TissueLyser at 30 ~~r.p.m.~~ Hz is sufficient for adequate grinding. For other methods, adequate grinding can be checked by microscopic examination of the ground material: if >50% of the urediniospores have lost their contents and are hyaline rather than yellow, grinding is sufficient. | **European Union**<br>Technical correction.<br>*Category : TECHNICAL* | **Incorporated** |
| 68 | 117 | The sample is placed into a tube or mortar bowl. Different grinding methods can be used such as mortar and pestle with liquid nitrogen, plastic pestles and microcentrifuge tubes with liquid nitrogen, or a TissueLyser (Qiagen[1]). The spore wall is very strong and all methods for DNA extraction and purification depend on its adequate disruption. For the TissueLyser, the addition of two 3 mm tungsten carbide beads, the pre-freezing of tubes in liquid nitrogen for 1 min, and two 2 min sessions in the TissueLyser at 30 ~~r.p.m~~Hz. is sufficient for adequate grinding. For other methods, adequate grinding can be checked by microscopic examination of the ground material: if >50% of the urediniospores have lost their contents and are hyaline rather than yellow, grinding is sufficient. | **EPPO**<br>technical correction<br>*Category : TECHNICAL* | **Incorporated** |
| 69 | 118 | In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity ~~and/or~~ and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be | **European Union**<br>Better wording (see for example DP Bactrocera dorsalis).<br>*Category : EDITORIAL* | **Modified**<br>The text in the main body of the document and the footnote has been adjusted to avoid repetition while still including all relevant information. |

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| | | adjusted to the standards of individual laboratories, provided that they are adequately validated. | | |
| 70 | 118 | In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity ~~and/or~~ and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated. | **EPPO**<br>Better wording (see for example DP Bactrocera dorsalis).<br>*Category : EDITORIAL* | **Modified**<br>The text in the main body of the document and the footnote has been adjusted to avoid repetition while still including all relevant information. |
| 71 | 118 | ~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and/or reproducibility achieved.~~ The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. ~~Laboratory procedures presented in~~ This information is given for the ~~protocols may be adjusted to~~ convenience of users of this protocol and does not constitute an endorsement by the ~~standards~~ CPM of ~~individual laboratories~~the chemical, ~~provided that~~ reagent and or equipment named. Equivalent products may be used if they ~~are adequately validated~~can be shown to lead to the same results. | **Uruguay**<br>Text deleted to avoid repetition with paragraph 93. Text added according text agreed for the footnotes.<br>*Category : TECHNICAL* | **Modified**<br>The text in the main body of the document and the footnote has been adjusted to avoid repetition while still including all relevant information. |
| 72 | 121 | **3.4.3 Conventional PCR using primers that amplify fungal, rust or *P. psidii* DNA** | **European Union**<br>This section should be placed after the species specific tests.<br>Also it is unclear whether it is being used as a diagnostic tool or an identification tool. If it is diagnostic does it add anything when there are two species specific tests in the protocol. If it is being used as an identification tool, it should be moved to section 4.2.<br><br>*Category : TECHNICAL* | **Modified.**<br>This method is both a detection test and an identification tool. It does not require special primers or probes so is listed first. However, during the delimiting phase when many samples could be coming into the lab, the real-time species-specific test may be more practical once identity is confirmed.<br>The following has been added: The PCR products should be sequenced (in-house or sent to a sequencing facility) and the sequence data compared with the reference data described in Section 4.2. |
| 73 | 121 | **3.4.3 Conventional PCR using primers that amplify fungal, rust or *P. psidii* DNA** | **EPPO**<br>This section should be placed after the species specific tests.<br>Also it is unclear whether it is being used as a diagnostic tool or an identification tool. If it is diagnostic does it add anything when there are two species specific tests in the protocol. If it is being used as an identification tool, it should be moved to section 4.2<br><br>*Category : TECHNICAL* | **Modified.**<br>This method is both a detection test and an identification tool. It does not require special primers or probes so is listed first. However, during the delimiting phase when many samples could be coming into the lab, the real-time species-specific test may be more practical once identity is confirmed.<br>The following has been added: The PCR products should be sequenced (in-house or sent to a sequencing facility) and the |

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| | | | | sequence data compared with the reference data described in Section 4.2. |
| 74 | 121 | **3.4.3 Conventional PCR ~~using primers that amplify fungal, rust or *P. psidii* DNA~~** | **Singapore**<br>To simplify the title<br>*Category : EDITORIAL* | **Modified.** Changed to Conventional PCR and sequencing |
| 75 | 121 | **3.4.3 Conventional PCR using primers that amplify fungal, rust or *P. psidii* DNA** | **Philippines**<br>request for proof of specificity, reproducibility and repeatability of this method.<br>*Category : TECHNICAL* | **Considered, but not incorporated.**<br>These primers are commonly used in laboratories worldwide working on rust fungi and are well published. |
| 76 | 145 | ~~*β-tubulin*~~ | **China**<br>Due to the obligately biotrophic nature of Austropuccinia psidii and the poor condition of its DNA, it is difficult to amplify the fragment of β-tubulin and elongation factor 1α. An epitype has been designated for Austropuccinia psidii. A morphological description and DNA characterization are provided from the epitype, obtained from the same location and host of the lectotype, also designated. Thus it is better to amplify the fragment of rDNA, such as 28S, 18S and ITS.<br>Beenken, L., Zoller, S. & Berndt, R. (2012) Rust fungi on Annonaceae II: the genus Dasyspora Berk. & M. A. Curtis. Mycologia 104:659–681.<br>Beenken, L. & Wood, A.W. (2015) Puccorchidium and Sphenorchidium, two new genera of Pucciniales on Annonaceae related to Puccinia psidii and the genus Dasyspora. Mycological Progress 14: 49.<br><br>*Category : SUBSTANTIVE* | **Considered, but not incorporated.**<br>One of the coauthors of this protocol, Morag Glen, is also a co-author of the epitype paper (Machado et al 2015). She has written the molecular methodology for this IPPC. The aim of designating an epitype is to have both morphological and molecular data that fixes the type specimen for the species and can be used as the definitive reference. The paper describing the epitype gives Genbank references for ITS, β-tubulin and elongation factor 1α. It does not give reference sequences for 28S and 18S. Therefore, we recommend the same regions as are published for the epitype. In practice, the ITS alone is definitive. |
| 77 | 154 | ~~*elongation factor 1α*~~ | **China**<br>Due to the obligately biotrophic nature of Austropuccinia psidii and the poor condition of its DNA, it is difficult to amplify the fragment of β-tubulin and elongation factor 1α. An epitype has been designated for Austropuccinia psidii. A morphological description and DNA characterization are provided from the epitype, obtained from the same location and host of the lectotype, also designated. Thus it is better to amplify the fragment of rDNA, such as 28S, 18S and ITS.<br>Beenken, L., Zoller, S. & Berndt, R. (2012) Rust fungi on Annonaceae II: the genus Dasyspora Berk. & M. A. Curtis. Mycologia 104:659–681.<br>Beenken, L. & Wood, A.W. (2015) Puccorchidium and Sphenorchidium, two new | **Considered, but not incorporated.**<br>One of the coauthors of this protocol, Morag Glen, is also a co-author of the epitype paper (Machado et al 2015). She has written the molecular methodology for this IPPC. The aim of designating an epitype is to have both morphological and molecular data that fixes the type specimen for the species and can be used as the definitive reference. The paper describing the epitype gives Genbank references for ITS, β-tubulin and elongation factor 1α. It does not give reference sequences for 28S and 18S. Therefore, we recommend the same regions as are published for the epitype. In practice, the ITS alone is definitive. |

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| | | | genera of Pucciniales on Annonaceae related to Puccinia psidii and the genus Dasyspora. Mycological Progress 14: 49.<br><br>*Category : SUBSTANTIVE* | |
| 78 | 172 | 2.0 mM | **Philippines**<br>what is mM? or should it be µM?<br>*Category : SUBSTANTIVE* | **Considered, but not incorporated.**<br>milliMolar is correct. |
| 79 | 188 | 94 °C for 2 min | **European Union**<br>Can this time be stated if the mastermix composition is not named as this may vary depending which reagents are used.<br>*Category : TECHNICAL* | **Considered, but not incorporated.**<br>The Master Mix composition is specified in para 167 - 183. Yes, the denaturation temperature are the requirements for these primers. |
| 80 | 202 | 95 °C for 3 min | **EPPO**<br>Can this time be stated if the mastermix composition is not named as this may vary depending which reagents are used.<br>*Category : TECHNICAL* | **Considered, but not incorporated.**<br>The Master Mix composition is specified in para 167 - 183. Yes, the denaturation temperature are the requirements for these primers. |
| 81 | 226 | This method is ~~fast and~~ fast, sensitive and is suitable for high throughput and for detection of the pathogen in its early infection stages, but it requires species-specific primers and a dual-labelled probe. Species specificity has been demonstrated against other *Puccinia* species, including *P. coronata*, *P. graminis*, *P. hemerocallidis*, *P. hordei*, *P. myrsiphylla*, *P. oxalidis* and *P. striiformis* (Baskarathevan *et al.*, 2016). The TaqMan real-time PCR can detect as little as 0.011 pg of *P. psidii* genomic DNA (Baskarathevan *et al.*, 2016). An internal control based on amplification of the host cytochrome oxidase (*cox1*) gene (Weller *et al.*, 2000) should be included unless the PCR is performed on fungal material. When the internal control is included, an additional PCR reagent "COX BLOCK" (dNature Diagnostics & Research Ltd[1]) is required to delay the amplification of the *cox* gene, thereby preventing the internal control from outcompeting the detection of *P. psidii* DNA. | **European Union**<br>Better wording.<br>*Category : EDITORIAL* | **Incorporated** |
| 82 | 226 | This method is fast and sensitive and is suitable for high throughput and for detection of the pathogen in its early infection stages, but it requires species-specific primers and a dual-labelled probe. Species specificity has been demonstrated against other *Puccinia* species, including *P. coronata*, *P. graminis*, *P. hemerocallidis*, *P. hordei*, *P. myrsiphylla*, *P. oxalidis* and *P. striiformis* (Baskarathevan *et al.*, 2016). The TaqMan real-time PCR can detect as little as 0.011 pg of *P. psidii* genomic DNA (Baskarathevan *et al.*, 2016). An internal control based on | **European Union**<br>Comment 1 In the original paper specificity was not tested against the other rust species stated as being found on Myrtaceae in this protocol, Puccinia cygnorum, Phakopsora juelii, Phakopsora myrtacearum, Uredo seclusa and Uredo xanthestemonis. The original paper states that in silica analysis was carried out but has any other validation been carried out since the original paper? Does there need to be an note in this paragraph to say that verification is needed against these | **Modified.**<br><br>1 – The DNA sequences of these species were checked in silico and the primers do not match. In addition, Myrtle rust is so phylogenetically remote from these other species that cross amplification is unlikely. It is up to the individual lab when they verify the test to what species they use for verification. |

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| | | amplification of the host cytochrome oxidase (*cox1*) gene (Weller *et al.*, 2000) should be included unless the PCR is performed on fungal material. When the internal control is included, an additional PCR reagent "COX BLOCK" (dNature Diagnostics & Research Ltd[1]) is required in the reaction to delay the amplification of the *cox* gene, thereby preventing the internal control from outcompeting the detection of *P. psidii* DNA. | rusts in the lab before using the first time or is as in our lab is that just carried out as standard?<br><br>Comment 2 PCR reagent "COX BLOCK" (dNature Diagnostics & Research Ltd1) this reagent could not be found on the website so it needs a number.<br><br>Comment 3 The paper states this can be run as a duplex or single plex. If competition is a problem it could alternatively be ran it a single plex.<br>*Category : TECHNICAL* | 2 – This reagent has been added to the company website.<br><br>3 – The wording has been modified to include this as an option. |
| 83 | 226 | This method is fast and sensitive and is suitable for high throughput and for detection of the pathogen in its early infection stages, but it requires species-specific primers and a dual-labelled probe. Species specificity has been demonstrated against other *Puccinia* species, including *P. coronata*, *P. graminis*, *P. hemerocallidis*, *P. hordei*, *P. myrsiphylla*, *P. oxalidis* and *P. striiformis* (Baskarathevan *et al.*, 2016). The TaqMan real-time PCR can detect as little as 0.011 pg of *P. psidii* genomic DNA (Baskarathevan *et al.*, 2016). An internal control based on amplification of the host cytochrome oxidase (*cox1*) gene (Weller *et al.*, 2000) should be included unless the PCR is performed on fungal material. When the internal control is included, an additional PCR reagent "COX BLOCK" (dNature Diagnostics & Research Ltd[1]) is required in the reaction to delay the amplification of the *cox* gene, thereby preventing the internal control from outcompeting the detection of *P. psidii* DNA. | **EPPO**<br>Comment 1 In the original paper specificity was not tested against the other rust species stated as being found on Myrtaceae in this protocol, Puccinia cygnorum, Phakopsora juelii, Phakopsora myrtacearum, Uredo seclusa and Uredo xanthestemonis. The original paper states that in silica analysis was carried out but has any other validation been carried out since the original paper? Does there need to be an note in this paragraph to say that verification is needed against these rusts in the lab before using the first time or is as in our lab is that just carried out as standard?<br><br>Comment 2 PCR reagent "COX BLOCK" (dNature Diagnostics & Research Ltd1) this reagent could not be found on the website so it needs a number.<br><br>Comment 3 The paper states this can be run as a duplex or single plex. If competition is a problem it could alternatively be ran it a single plex.<br>*Category : TECHNICAL* | **Modified.**<br><br>1 – The DNA sequences of these species were checked in silico and the primers do not match. In addition, Myrtle rust is so phylogenetically remote from these other species that cross amplification is unlikely. It is up to the individual lab when they verify the test to what species they use for verification.<br><br>2 – This reagent has been added to the company website.<br><br>3 – The wording has been modified to include this as an option. |
| 84 | 226 | This method is fast and sensitive and is suitable for high throughput and for detection of the pathogen in its early infection stages, but it requires species-specific primers and a dual-labelled probe. Species specificity has been demonstrated against other *Puccinia* species, including *P. coronata*, *P. graminis*, *P. hemerocallidis*, *P. hordei*, *P. myrsiphylla*, *P. oxalidis* and *P. striiformis* (Baskarathevan *et al.*, 2016). The TaqMan real-time PCR can detect as little as 0.011 pg of *P. psidii* genomic DNA | **EPPO**<br>change<br>This method is fast and sensitive and is suitable....<br>to<br><br>This method is fast, sensitive and is suitable<br>*Category : EDITORIAL* | **Incorporated** |

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| | | (Baskarathevan *et al.*, 2016). An internal control based on amplification of the host cytochrome oxidase (*cox1*) gene (Weller *et al.*, 2000) should be included unless the PCR is performed on fungal material. When the internal control is included, an additional PCR reagent "COX BLOCK" (dNature Diagnostics & Research Ltd[1]) is required to delay the amplification of the *cox* gene, thereby preventing the internal control from outcompeting the detection of *P. psidii* DNA. | | |
| 85 | 227 | The method is repeatable and reproducible with a coefficient of variation when repeated (for cycle threshold (Ct)) between 0.8 and 1.6. Three combinations of primers/probes were developed – two targeting the rDNA ITS and one targeting *β-tubulin* – but only the most sensitive primer combination is outlined here. | **Philippines**<br>Provide records of validation as attachment<br>*Category : TECHNICAL* | **Modified.**<br>The validation data is specified in the paper and the reference has been added. |
| 86 | 256 | CAL Fluor Red 610-TGC TTA CGC TGG ATG GAA TGC CCT-BHQ2 | **European Union**<br>This is not the dye listed in Weller et al, either needs to be the same dye or note that it is amended from Weller et al.<br>*Category : TECHNICAL* | **Incorporated.**<br>For the duplex PCR the dye was changed because the wavelengths were too similar to the the target probe dye. So it was noted that it was amended. |
| 87 | 256 | CAL Fluor Red 610-TGC TTA CGC TGG ATG GAA TGC CCT-BHQ2 | **EPPO**<br>This is not the dye listed in Weller et al, either needs to be the same dye or note that it is amended from Weller et al.<br>*Category : EDITORIAL* | **Incorporated.**<br>For the duplex PCR the dye was changed because the wavelengths were too similar to the the target probe dye. So it was noted that it was amended. |
| 88 | 309 | PCR products can be visualized on a transilluminator after electrophoresis on agarose gel and staining with a compatible DNA-binding dye such as ~~ethidium bromide.~~ gel red. | **Philippines**<br>ethidium bromide is carcinogenic, gel red is a good replacement.<br>*Category : TECHNICAL* | **Modified**.<br>Gel red has been provided as an alternative example. |
| 89 | 353 | ~~95~~ 94 °C for 3 min | **Singapore**<br>Incorrect temperature cited from Langrell et al 2008 paper.<br>*Category : EDITORIAL* | **Modified.**<br>A sentence has been included to show that these have been amended from Langrell et al. 2008. Morag Glen was a co-author on the Langrell paper and has since amended the methdology slightly and wrote this section. |
| 90 | 357 | ~~95~~ 94 °C for 1 min | **Singapore**<br>Incorrect temperature cited from Langrell et al 2008 paper.<br>*Category : EDITORIAL* | **Modified.**<br>A sentence has been included to show that these have been amended from Langrell et al. 2008. Morag Glen was a co-author on the Langrell paper and has since amended the methdology slightly and wrote this section. |
| 91 | 359 | ~~57~~ 55 °C for 1 min | **Singapore**<br>Incorrect temperature cited from Langrell et al 2008 paper.<br>*Category : EDITORIAL* | **Modified.**<br>A sentence has been included to show that these have been amended from Langrell et al. 2008. Morag Glen was a co-author on the Langrell paper and has |

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| | | | | since amended the methdology slightly and wrote this section. |
| 92 | 363 | 72 °C for ~~7~~ 10 min | **Singapore**<br>Incorrect temperature cited from Langrell et al 2008 paper.<br>*Category : EDITORIAL* | **Modified.**<br>A sentence has been included to show that these have been amended from Langrell et al. 2008. Morag Glen was a co-author on the Langrell paper and has since amended the methdology slightly and wrote this section. |
| 93 | 373 | ~~For~~ To reliably consider the ~~test result~~ obtained ~~to be considered reliable~~test results, appropriate controls – which ~~will depend~~ depends on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the target pest or target nucleic acid. For PCR, a positive nucleic acid control and a negative amplification control (no template control) are the minimum controls that should be used. | **Ghana**<br><br>*Category : EDITORIAL* | **Considered, but not incorporated.**<br>This alters the meaning of sentence away from its original intent. |
| 94 | 373 | For the test result ~~obtained~~ to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the DNA sequence of the target ~~pest or target nucleic acid~~pest. For PCR, a positive nucleic acid control and a negative amplification control (no template control) are the minimum controls that should be used. | **Philippines**<br><br>*Category : SUBSTANTIVE* | **Incorporated** |
| 95 | 374 | **Positive nucleic acid control.** This control is used to monitor the efficiency of the test method (apart from the extraction), and specifically the amplification. Pre-prepared (stored) genomic DNA, whole genome amplified DNA or a synthetic control (e.g. cloned PCR product) may be used. Any fungal DNA will be a suitable positive control for the ITS1-F/ITS4 primers, and any rust DNA for the ITS1-F/Rust1 primers. The other primer pairs (PpsiBtubF/PpsiBtubR, PPEFF/PPEFR, PpsiITS1F/PpsiITS1R, Ppsi1/Ppsi6 and Ppsi2/Ppsi4) require *P. psidii* DNA (genomic DNA or suitable plasmid or amplicon) as a positive control. In the absence of a positive control, it may be possible to confirm the presence of *P. psidii*, but not its absence. | **Philippines**<br>identify NPPO who are willing to provide genomic DNA to be used as positive control.<br>*Category : SUBSTANTIVE* | **Incorporated.** |
| 96 | 375 | The efficiency of the extraction method is confirmed with amplification of the rDNA ITS using the primers ITS1-F/ITS4. | **European Union**<br>Does this require a heading to make it clear it is being used as an extraction control?<br>*Category : EDITORIAL* | **Incorporated.**<br>Title changed so that it is consistent with other IPPC DPs. |
| 97 | 375 | The efficiency of the extraction method is confirmed with amplification of the rDNA ITS using the primers ITS1-F/ITS4~~.~~. | **EPPO**<br>Does this require a heading to make it clear it is being used as an extraction control?<br>*Category : EDITORIAL* | **Incorporated.**<br>Title changed so that it is consistent with other IPPC DPs. |

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| 98 | 376 | **Negative amplification control (no template control).** This control is necessary to rule out false ~~positives~~ positives, due to contamination during preparation of the reaction mixture. PCR-grade water that was used to prepare the reaction mixture is added in place of template DNA at the amplification stage. | **Ghana**<br><br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>The comma changes the sentence and is not required. |
| 99 | 377 | **Negative extraction control.** This control is used to monitor ~~both~~ contamination both during nucleic acid extraction and cross-reaction with the host tissue. The control comprises nucleic acid that is extracted from uninfected host tissue and subsequently amplified. Alternatively, extraction blanks may be processed with the samples to be tested if sufficient uninfected host tissue is not available. | **Philippines**<br><br>*Category : EDITORIAL* | **Incorporated** |
| 100 | 380 | Baskarathevan *et al.* (2016) were able to detect 0.011 pg of *P. psidii* DNA at a Ct of 35, which represents less than one genome copy for an expected genome size of 100–150 mega base pairs. The infected plant samples had a Ct ranging from 17 to 35, depending to some extent on the severity of infection. Samples with a Ct of 35 or less can be regarded as positive, provided controls (negative amplification controls) are negative. | **European Union**<br>Does a Ct threshold need to be given here? In EPPO Protocols Ct threshold are only provided for situations where the Ct is unusual.<br><br>For other cases we have the following standard text:<br>Verification of the controls<br>• The PIC and PAC ( as well as IC and IPC as applicable) amplification curves should be exponential.<br>• NIC and NAC should give no amplification<br><br>When these conditions are met:<br>• A test will be considered positive if it produces an exponential amplification curve.<br>• A test will be considered negative, if it does not produce an amplification curve or if it produces a curve which is not exponential.<br>• Additionally for SYBR® Green based real-time PCR tests: the TM value should be as expected.<br>• Tests should be repeated if any contradictory or unclear results are obtained.<br><br>NIC = Negative isolation control<br>PIC = Positive isolation control<br>NAC = Negative amplification control<br>PAC= Positive amplification control<br>IC= Internal Control<br>IPC= Internal positive controls<br><br>The instructions to authors state that: 'if the need for a Ct cut-off value has been identified | **Incorporated**.<br>The last sentence regarding Ct cut off values have been removed. |

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| | | | during the validation of the test this should be stated and authors are encouraged to give a range of Ct values observed for true positive samples.<br>The following sentence should appear at the start of this section as a standard text when a Ct cut-off value is mentioned. The Ct value given below is as established in [name of the laboratory] As a Ct cut-off value is equipment, material and chemistry dependent it needs to be verified in each laboratory when implementing the test.<br><br>So does the range given mean anything between Ct 35 and Ct 40 is considered negative?).<br>*Category : TECHNICAL* | |
| 101 | 380 | Baskarathevan *et al*. (2016) were able to detect 0.011 pg of *P. psidii* DNA at a Ct of 35, which represents less than one genome copy for an expected genome size of 100–150 mega base pairs. The infected plant samples had a Ct ranging from 17 to 35, depending to some extent on the severity of infection. Samples with a Ct of 35 or less can be regarded as positive, provided controls (negative amplification controls) are negative. | **EPPO**<br>Does a Ct threshold need to be given here?<br>In EPPO Protocols Ct threshold are only provided for situations where the Ct is unusual.<br><br>For other cases we have the following standard text:<br>Verification of the controls<br>• The PIC and PAC ( as well as IC and IPC as applicable) amplification curves should be exponential.<br>• NIC and NAC should give no amplification<br><br>When these conditions are met:<br>• A test will be considered positive if it produces an exponential amplification curve.<br>• A test will be considered negative, if it does not produce an amplification curve or if it produces a curve which is not exponential.<br>• Additionally for SYBR® Green based real-time PCR tests: the TM value should be as expected.<br>• Tests should be repeated if any contradictory or unclear results are obtained.<br><br>NIC = Negative isolation control<br>PIC = Positive isolation control<br>NAC = Negative amplification control<br>PAC= Positive amplification control<br>IC= Internal Control<br>IPC= Internal positive controls | **Incorporated**.<br>The last sentence regarding Ct cut off values have been removed. |

| # | Para | Text | Comment | SC's response |
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| | | | The instructions to authors state that: 'if the need for a Ct cut-off value has been identified during the validation of the test this should be stated and authors are encouraged to give a range of Ct values observed for true positive samples.<br>The following sentence should appear at the start of this section as a standard text when a Ct cut-off value is mentioned. The Ct value given below is as established in [name of the laboratory] As a Ct cut-off value is equipment, material and chemistry dependent it needs to be verified in each laboratory when implementing the test.<br><br>So does the range given mean anything between Ct 35 and Ct 40 is considered negative?).<br>*Category : TECHNICAL* | |
| 102 | 388 | **Table 7.** Morphological characters of the six currently accepted rust species that infect Myrtaceae<br><br>To distinguish the Austropuccinia psidii from other rust fungi, the host related and taxonomic related species should been taken into account. Such as *Phakopsora rossmaniae*, *Physopella jueli*, *Physopella xanthostemonis*, *Puccinia cygnorum*, *Puccinia rompelii*, *Puccinia sanguinolenta*, *Melampsora* spp, *Dasyspora* spp, *Puccorchidium* and *Sphenorchidium* spp. | **China**<br>See Simpson et al. (2006). Uredinales species pathogenic on species of Myrtaceae. Australasian Plant Pathology. 35 (5) 549–562. Beenken, L. & Wood, A.W. (2015) Puccorchidium and Sphenorchidium, two new genera of Pucciniales on Annonaceae related to Pucciniapsidii and the genus Dasyspora. Mycological Progress 14: 49.<br><br>*Category : TECHNICAL* | **Considered but not incorporated.**<br>Simpson's species are no longer accepted. Maier et al. 2016 gives the currently accepted species on Myrtaceae and was used as the source. |
| 103 | 431 | The following key (Maier *et al.*, 2016) can be used to distinguish the two described rust fungi on eucalyptus: | **European Union**<br>The key by Maier in 4.1. is not consistent with the description by Pegg in 4.1.1 in terms of the colour of the uredinia (bright yellow to orange versus yellowish brown). Which should be followed?<br>*Category : SUBSTANTIVE* | **Modified.**<br>Para 437 to clarify Maier is discussing the host eucalyptus; Pegg is considering a wide range of host genera. The morphological characters can vary considerably across host genera. |
| 104 | 431 | The following key (Maier *et al.*, 2016) can be used to distinguish the two described rust fungi on eucalyptus: | **EPPO**<br>The key by Maier in 4.1. is not consistent with the description by Pegg in 4.1.1 in terms of the color of the uredinia (bright yellow to orange versus yellowish brown). Which should be followed?<br>*Category : SUBSTANTIVE* | **Modified.**<br>Para 437 to clarify Maier is discussing the host eucalyptus; Pegg is considering a wide range of host genera. The morphological characters can vary considerably across host genera. |
| 105 | 443 | **4.2 Molecular identification** | **European Union**<br>There is some replication between these two sections (GenBank accession codes). Can this be avoided?<br>*Category : TECHNICAL* | **Incorporated.** |

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| 106 | 443 | **4.2 Molecular identification** | **European Union**<br>Comment 1<br>Intraspecific variation among the P. psidii sequences in GenBank is often just due to non-specific basecalling (e.g. an R is reported for a G, but this is not an error). It seems there is no real intraspecific variation, at least not in the rDNA ITS region that have been checked by BE experts. So the sequences should likely be identical and the margin of error allowed (>98%) is actually to accommodate sequencing errors? If 1% errors are allowed, it should be better to list ≥99% instead of >98% as with the latter almost 2% margin is allowed.<br><br>Comment 2<br>What to do if you get a positive result via qPCR but the material is deteriorated and no urediniospores are present? Confirmation via sequencing is not an option in that case.<br>*Category : TECHNICAL* | **Modified.**<br>1. Revised para 445.<br><br>2. The recommendation is to go back and collect fresh sample as indicated in the flow diagram. Confirmatory identification should be based on a reference specimen and sequence comparison with the epitype, not a positive qPCR result. The qPCR comes into its own when processing lots of samples after initial detection. It is not really the best method for confirming a first detection. |
| 107 | 443 | **4.2 Molecular identification** | **EPPO**<br>Comment 1<br>intraspecific variation among the P. psidii sequences in GenBank is often just due to non-specific basecalling (e.g. an R is reported for a G, but this is not an error). It seems there is no real intraspecific variation, at least not in the rDNA ITS region that have been checked by BE experts. So the sequences should likely be identical and the margin of error allowed (>98%) is actually to accommodate sequencing errors? If 1% errors are allowed, it should be better to list ≥99% instead of >98% as with the latter almost 2% margin is allowed.<br><br>Comment 2<br>What to do if you get a positive result via qPCR but the material is deteriorated and no urediniospores are present? Confirmation via sequencing is not an option in that case<br>*Category : TECHNICAL* | **Modified.**<br>3. Revised para 445.<br><br>The recommendation is to go back and collect fresh sample as indicated in the flow diagram. Confirmatory identification should be based on a reference specimen and sequence comparison with the epitype, not a positive qPCR result. The qPCR comes into its own when processing lots of samples after initial detection. It is not really the best method for confirming a first detection. |
| 108 | 443 | **4.2 Molecular identification** | **EPPO**<br>There is some replication between these two sections (GenBank accession codes). Can this be avoided?<br>*Category : TECHNICAL* | **Incorporated** |

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| 109 | 445 | For a definitive identification, the preferred method is to extract DNA from rust spores, amplify the selected region or regions, and compare the sequence data of the fungal barcoding region, the rDNA ITS region, with the sequence or sequences obtained from the epitype and voucher specimens available in GenBank (da S. Machado *et al.*, 2015, Rodas *et al.*, 2015). DNA sequencing of secondary regions such as *β-tubulin* and *elongation factor* 1α genes and the rDNA large sub-unit (LSU) region provides support for initial diagnoses. All regions have very low intraspecific variation (<1%), and they have barcode gaps of 10% (ITS), 17% (*β-tubulin*) or 20% (*elongation factor* 1α). | **Philippines**<br>For a definitive identification, the preferred method is to extract DNA from rust spores, amplify the selected region or regions (indicate the section where this process has been described)<br>*Category : SUBSTANTIVE* | **Incorporated.** |
| 110 | 451 | In cases where other contracting parties may be affected by the results of the diagnosis, in particular in cases of non-compliance (ISPM 13 *Guidelines for the notification of non-compliance and emergency action*) and where *Puccinia psidii* is found in an area for the first time, the following records and evidence and additional material should be kept for at least one year in a manner that ensures traceability~~:~~: Add the topic and sentence "Sample storage. After the samples have been detected as positive they should be kept in refrigerator with 4oC. Once expired storage, samples should been sterilized to prevent the spread of pest" | **China**<br>The storage of samples will be propitious to expert review.<br>*Category : TECHNICAL* | **Modified.**<br>**Added in section 5:** After the samples have been detected as positive, the positive DNA should be labelled and stored frozen at -20 or -80 C. The remaining infected plant material should be pressed and dried, packaged and labelled, and stored as appropriate for herbarium specimens. Microscopic slides should be sealed and stored with the plant specimens.<br>Comment – storage at 4 °C is not appropriate for either DNA or plant material. The DNA should be frozen. The plant material should be processed and kept as a herbarium specimen. |
| 111 | 464 | ~~**6. Contact Points for Further Information**~~ | **Viet Nam**<br><br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This is in line with the IPPC format for diagnostic protocols. |
| 112 | 464 | **6. Contact Points for Further Information** | **Viet Nam**<br>Move to Appendix 1<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This is in line with the IPPC format for diagnostic protocols. |
| 113 | 465 | ~~Further information on this protocol can be obtained from:~~ | **Viet Nam**<br>move to Appendix 1<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This is in line with the IPPC format for diagnostic protocols. |
| 114 | 466 | ~~Agriculture Victoria, Department of Economic Development, Jobs, Transport and Resources, Australia, AgriBio Centre for AgriBioscience, La Trobe University, 5 Ring Road, Bundoora, Victoria 3083, Australia (Jacqueline Edwards; e-mail: jacky.edwards@ecodev.vic.gov.au).~~ | **Viet Nam**<br>move to Appendix 1<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This is in line with the IPPC format for diagnostic protocols. |
| 115 | 467 | ~~School of Land and Food, University of Tasmania, Private Bag 98, Hobart, Tasmania 7001, Australia (Morag Glen; e-mail: Morag.Glen@utas.edu.au).~~ | **Viet Nam**<br>move to Appendix 1<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This is in line with the IPPC format for diagnostic protocols. |

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| 116 | 468 | ~~Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Laboratoire de la Santé des Végétaux [Plant Health Laboratory], Unité de mycologie [Mycology Unit], Domaine de Pixérécourt – Bâtiment E, C.S. 40009, 54220 Malzéville, France (Jacqueline Hubert; e-mail: jacqueline.hubert@anses.fr).~~ | **Viet Nam**<br>move to Appendix 1<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This is in line with the IPPC format for diagnostic protocols. |
| 117 | 469 | ~~General Research and Biotechnology Unit, Nigeria Agriculture Quarantine Service, Post-Entry Quarantine Station, Moor Plantation (NCRI Compound), Apata, Ibadan, Oyo State, Nigeria (Kazeem Shakiru Adewale; e-mail: kazeems2001@yahoo.com).~~ | **Viet Nam**<br>move to Appendix 1<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This is in line with the IPPC format for diagnostic protocols. |
| 118 | 470 | ~~United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Regulations, Permits and Manuals, 4700 River Rd. Unit 133, Riverdale, MD 20737, United States of America (José R. Hernández; e-mail: Jose.R.Hernandez@aphis.usda.gov).~~ | **Viet Nam**<br>move to Appendix 1<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This is in line with the IPPC format for diagnostic protocols. |
| 119 | 471 | ~~A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat (ippc@fao.org), which will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).~~ | **Viet Nam**<br>move to Appendix 1<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This is in line with the IPPC format for diagnostic protocols. |
| 120 | 472 | ~~**7. Acknowledgements**~~ | **Viet Nam**<br>move to Appendix 2<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This is in line with the IPPC format for diagnostic protocols. |
| 121 | 473 | ~~The first draft of this protocol was written by J. Edwards (Agriculture Victoria, Department of Economic Development, Jobs, Transport and Resources, Australia (see preceding section)), M. Glen (School of Land and Food, University of Tasmania, Australia (see preceding section)), J. Hubert (Mycology Unit, ANSES, France) (see preceding section)), J. Hernandez (USDA-APHIS, United States of America (see preceding section)) and K. Shakiru Adewale (General Research and Biotechnology Unit, Nigeria Agricultural Quarantine Service, Nigeria (see preceding section)). In addition, the following experts were significantly involved in the development of this protocol: M. Piepenbring (Department of Mycology, Goethe University Frankfurt am Main, Germany), C. Rodriguez-Delgado (Department of Agriculture and Water Resources, Australia), F. Sorgoni (Ministero delle Politiche~~ | **Viet Nam**<br>move to Appendix 2<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This is in line with the IPPC format for diagnostic protocols. |

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| | | ~~Agricole Alimentari e Forestali and Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Italy) and H. Shirato (Ministry of Agriculture, Forestry and Fisheries, Yokohama Plant Protection Station, Japan).~~ | | |
| 122 | 474 | ~~8~~6. **References** | **Viet Nam**<br><br>*Category : EDITORIAL* | **Considered but not incorporated.** |
| 123 | 502 | ~~9~~7. **Figures** | **Viet Nam**<br><br>*Category : EDITORIAL* | **Considered but not incorporated.** |
| 124 | 502 | **9. Figures** | **EPPO**<br>This figure does not accommodate the real time PCR and nested conventional PCR methods.<br><br>- 98% should be replaced by >98% or actually even better by ≥99% based on my comment above<br><br>- Is the situation in the bottom left needed (diamond with "Identification of different rust species?")? If the species is not P. psidii but belongs to Pucciniales, then you know it is a rust species other P. psidii. Is it relevant that the exact rust species cannot be identified?<br><br>*Category : TECHNICAL* | **Modified.** It is really designed for the first initial identification. The figure heading indicates that. Not sure how to incorporate the real time and nested PCR tests.<br><br>The diagram was changed to ≥99%. Also, P. psidii was changed to A. psidii. |
| 125 | 503 | Modify the figure 1 about paraphyeses. | **China**<br>It is difficult to distinguish Austropuccinia psidii from other related rust pathogen of Myrtaceae, such as Phakopsora rossmaniae、Physopella jueli, Physopella xanthostemonis, Puccinia cygnorum and so on, by the character of paraphyeses.<br>See Simpson et al. (2006). Uredinales species pathogenic on species of Myrtaceae. Australasian Plant Pathology. 35 (5) 549–562. Cummins GB, Hiratsuka Y. (2003). Illustrated genera of rust fungi. 3rd edn. (APS Press: St Paul, MN). LUDWIG BEENKEN (2017). Austropuccinia: a new genus name for the myrtle rust Puccinia psidii placed within the redefined family Sphaerophragmiaceae (Pucciniales)<br>*Category : TECHNICAL* | **Considered but not incorporated.** The Simpson *et al.* (2006) paper is no longer considered relevant.<br>The flow chart says that if paraphyses are observed, then the rust is not *A. psidii*. That is a valid statement. If you see paraphyses, it is not *A. psidii*.<br><br>If you don't see paraphyses, then keep going down the flow chart. If the host is Eucalyptus, then go straight to a molecular test. There are only 2 species known on Eucalyptus and neither have paraphyses. If it is not, then check for morphological characters consistent with P. psidii eg spore size, fragile pedicel on teliospores. |

| # | Para | Text | Comment | SC's response |
|---|---|---|---|---|
| 126 | 517 | **Figure 5.** *Puccinia psidii*: (a), (b) urediniospores; (c)–(d), teliospores and urediniospores; (e) teliospore, and (f), germinated teliospores and basidiospores. Scale bars: 20 µm. | **European Union**<br>The scale bar in figure f) appears to be slightly out as the description says basidiospores are 8-11 µm in diameter but two of them appear to be the same length in diameter as the scale bar which is 20 µm.<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This figure was taken as published from Machado *et al*. |
| 127 | 517 | **Figure 5.** *Puccinia psidii*: (a), (b) urediniospores; (c)–(d), teliospores and urediniospores; (e) teliospore, and (f), germinated teliospores and basidiospores. Scale bars: 20 µm. | **EPPO**<br>The scale bar in figure f) appears to be slightly out as the description says basidiospores are 8-11 µm in diameter but two of them appear to be the same length in diameter as the scale bar which is 20 µm.<br>*Category : EDITORIAL* | **Considered but not incorporated.**<br>This figure was taken as published from Machado *et al*. |