Submission on:

BA Policy Memorandum 2008/23. Draft import risk analysis report for fresh Unshu mandarin fruit from Japan (July 2008).

To:

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Essentially, this response raises a number of important general, as well as specific concerns regarding the draft Import risk analysis as listed below followed by a table containing further details:

1. In the citrus canker section starting on page 58, many papers are cited that upon closer inspection do not back up the claims or conclusions stated in the IRA (See specific examples in table below with regards to this from pages 59-62). Quoting papers which do not contain the original data, or do not contain any data directly relevant to Unshiu mandarins, or do not back up statements made in the IRA, while at the same time failing to cite other papers containing relevant data to the issue at hand raises doubts as to the rigour and scientific standards of this document. Numerous cited articles are written in Japanese, with only an abstract in English which prevents checking of facts and conclusions.

   Recommendation: That scientific standards are increased to acceptable levels, literature reviews are conducted in depth, and statements and facts are cited from original sources and checked before a draft is send out for comments. It would be beneficial to the peer-review of the IRA if the English translations of documents written in Japanese used in the production of the IRA by Biosecurity Australia were made available on the Biosecurity Australia website.

2. In the citrus canker section it is stated many times that MAFF informed and advised Biosecurity Australia (BA). Important conclusions are drawn from this advice. However, it is unclear on what basis this advice was provided. Was it done in writing and backed up by relevant scientific data, or was it just a verbal comment? It is not possible to verify the validity of this advice without having seen the data it is based on.

   Recommendation: That advice and information provided by MAFF is substantiated by data and or reports which are made available to be able to verify the claims made.

3. The taxonomy of Satsuma/Unshiu mandarins and their origins is not clear. The range of names used in many of the papers cited in the IRA include “Satsuma”, “Satsuma (*Citrus reticulata* Blanco)”, “Satsuma (*C. unshiu* Marc.)”,“Unshu”, “Unshiu”, “*C. unshu*”, “*C. unshiu*”, “Miyakawa Wase unshiu”, “Sugiyama unshiu”, and others. It is therefore often unclear exactly what germplasm is used in the cited experiments. Many papers contain research on Satsuma’s so it is paramount the exact nature of the relationship between these terms is clarified.

   Recommendation: BA to provide information with regards to the exact nature of the plant material under investigation grown in the production zone and when citing papers clearly indicate the citrus species/varieties this specific claim refers to. Similarly, the taxonomy of *Xanthomonas axonopodis* pv. *citri* (Hasse 1915) Vauterin, Hoste *et al*. 1995 should be brought in line

with the new nomenclature of Schaad et al 2006 (Systematic and Applied Microbiology, Volume 29, Issue 8), and the synonyms updated accordingly.

4. The susceptibility of Unshiu mandarin to citrus canker is not clear. Many papers are cited on page 59 but a close inspection of these reveals that the IRA draws different conclusions from the work than the authors that did the research described in the papers. Many papers contain research on Satsuma's so it is important that point 3 above is addressed to avoid confusion.

   Recommendation: BA when citing papers with regards to susceptibility clearly indicate the citrus species/varieties this specific claim refers to.

5. The terminology of resistance and susceptibility is incorrectly used and is interchanged almost at random. The impression is created that Unshiu mandarins are resistant to citrus canker. A review of the cited literature does not support this, and shows data and pictures of canker lesions on Unshiu mandarins. It appears that Unshiu mandarins are relatively less susceptible to citrus canker than other citrus species but they do not appear to be resistant. Resistance in the discipline of plant pathology is often preserved for the ability to prevent the reproduction of a pathogen.
   NOTE: APHIS in the USA has prohibited the importation of Unshiu fruit from the Jeju region of Korea due to the discovery of citrus canker in 2002 (Global Agriculture Information Network report no. KS6048).

   Recommendation: BA should clearly define what they mean by resistance and susceptibility and use terminology which is commonly accepted within the discipline of Plant Pathology. The terminology used in cited references should also be taken into account in the IRA.

6. How infectious is citrus canker ?: The IRA states that bacterial concentrations less than 2×10^3 cfu/ml were unable to cause stomatal infection according to a paper by Gottwald and Graham (1992). However, close inspection of this paper reveals that 2×10^3 cfu/ml were needed to reliably obtain infection every time under artificial conditions. No experiments were done to confirm these numbers under field conditions. At the same time these authors state in their paper that as few as 2 cfu/ml could cause a single lesion and disease. Hence, the figure of 2×10^3 cfu/ml used in the IRA to estimate the "probability of distribution" is underestimating how infectious Xcc can be by a factor of about 1,000.

   Recommendation: Facts and figures should be used only in the context they are intended for. Below a table of specific examples is provided which highlights the need to raise the standard of the document to an acceptable scientific level.

7. At present Australia only accepts fruit from Pest Free Areas with regards to citrus canker. In the case of the Emerald citrus canker outbreak, the movement of asymptomatic fruit from the quarantine area was not permitted to any Australian domestic market by Biosecurity Australia. This IRA proposes to lower Australia's minimum standards for imported fruit, conflicting with the WTO/SPS agreement which clearly states that measures applied domestically to deal with a particular risk need to be applied in the same manner to international sources.

8. The IRA demonstrates a reliance on bacteriophages for Xcc detection, but provides no details of the assay used by MAFF. Furthermore, no details of a diagnostic assay for citrus scab are provided.

   Recommendation: Details of the bacteriophage assay should be provided, along with details of the sensitivity of the assay and justification of this assay over other detection methods (e.g. PCR). Similar details should also be provided for citrus scab detection.

9. The IRA does not provide any efficacy data for mandatory copper sprays for citrus canker or citrus scab.

   Recommendation: Efficacy data should be provided in the IRA.

10. In Appendix A, several pathogens are listed as "present in Australia" but do not appear to have been reported on hosts of the *Citrus* genus, or of the Rutaceae family in Australia. The IRA does not make provision for strains or pathotypes of these pathogens, such as for *Colletotrichum acutatum*, which is present in Australia but does not cause post bloom fruit drop of citrus. Examples of the pathogens "present in Australia", but not known to cause diseases of citrus in Australia include *Rhizobium radiobacter*, *Ascochyta pisi*, *Botryosphaeria dothidea*, *Botryosphaeria ribis*, *Capnodium fuliginodes*, *Diaporthe medusaea*, *Gibberella fujikuroi*, *Mycosphaerella pinodes*, *Phoma pinodella*, *Rosellinia necatrix*, *Schizothyrium pomi*, and *Phytophthora cactorum*. Other pathogens that could be considered for addition to Appendix A are *Colletotrichum gloeosporioides* causing "young fruit apex rot", *Cochliobolus tuberculatus*, and *Leptoxyphium axillatum*, pending relevant literature searches.

    Recommendation: Evidence should be provided in the IRA that the introduction from Japan of pathogens "present in Australia" but not reported on citrus in Australia, does not present a threat to citrus production in Australia i.e. the host specificity, or lack thereof, of these pathogens should be checked in the literature.

Specific comments

Page/ Section	Issue
p29, 3.4.1	Trees in the production area are grafted onto *Poncirus trifoliata* rootstock. This rootstock is susceptible to citrus canker (Peltier and Frederich 1920). Production of shoots of the rootstock from below the graft union can occur in citrus production, and with relevance to this IRA potentially provide a *Xanthomonas citri* subsp*. citri* (Xcc) inoculum source. In the case of the Emerald canker outbreak, infection was observed on overgrowth of the canker-susceptible rootstock (see NCCEP report SS-R-003). The possibility exists that the infected rootstock played a role in the establishment and spread of canker in that orchard. The draft IRA does not make provision for inspection for, or management of, rootstock shoots arising from below the graft union that provide an inoculum source within the production areas.
p30, 3.4.3 (Table 3.5)	In Table 3.5 is not clear if products listed above each other, within the same cell, are sprayed simultaneously (i.e. 'tank mixed'), or sprayed separately.
	The standard spray schedule provided in Table 3.5 indicates the need for two applications of copper sulphate and copper carbonate, targeting citrus canker. However, no efficacy data for this spray schedule is provided, or cited, in the IRA even though it is listed as a component of the system approach proposed in the IRA.
	Efficacy data for the standard spray schedule provided in Table 3.5 for citrus scab is not provided, or cited, in the IRA.
	According to the APVMA :"Currently there are no products containing benomyl registered for use in Australia. It became illegal to supply or use products containing benomyl after 6 December 2006." Is the use pattern for Benomyl in the IRA acceptable in Australia? Furthermore, resistance of citrus postharvest mould pathogens (e.g. *Penicillium* spp.) has been reported from various countries. Is data available to demonstrate the efficacy of benomyl in Japan? Reports of resistance of do you need to spell out the genus here?*Elsinoe. fawcettii* to benomyl in Japan may also be of relevance.
p31, 3.4.4	It is not clearly stated if the JA Oigawa packhouse handles fruit from outside the proposed four designated export areas, and if so, what the disease and pest status is of such fruit. It is not clearly stated if the segregation of fruit in the packhouse is temporal or spatial (the flow diagram suggests temporal), or what sanitary precautions are taken to uphold the segregation.
	Has the efficacy of chlorine dips been tested to market access standards? For example, the efficacy of fruit disinfestation treatments is commonly tested on several thousands of fruit.

p58, 4.10	What is the quantitative definition of 'low', as advised by MAFF, in regards to the incidence of scab referred to in paragraph 3 of this section? Is supporting data available?
p59, 4.11.2	"Citrus canker status in the production area": Information from the "Shizuoka Prefectural Plant Protection Office 2007" is not readily available, therefore the information cannot be independently reviewed.
	In the absence of data on the citrus canker status in the production area, the possibility of pathogen inoculum sources located within suggested spread distances (Gottwald *et al.* 1997) cannot be discounted. Assessments of risk should therefore assume canker is present on host plants within the production area, until the absence can be independently confirmed.
	"Susceptibility of unshu mandarin to citrus canker": **Kuhara 1978, Koizumi 1981** – no data and/or not the original source, review article. **Koizumi and Kuhara 1982** – data-based assessments of varieties, but varieties called 'resistant' did still produce symptoms of canker, and support bacterial multiplication. **Leite 2000, Leite 2005** – conference proceedings abstract, no peer review, no data. **Canteros 1992** – Which variety of Satsuma was used in these trials? This reference provides no supporting evidence for the statement "Fruit of unshu mandarin rarely show signs of infection". This paper reports only on artificial inoculation experiments, the results of which indicate the fruit consistently produced fruit symptoms (up to 6.1 lesions per cm^2 in Table 5). **Canteros 2004** – This reference appears to be from a conference proceedings, and is unlikely to have been through the peer review process. The variety used in this study appear to be of the variety 'Okitsu'; according to the IRA the varieties for export from Japan are 'Aoshima' and 'Miyagawa Wase'. No data are provided as to the effect of Unshiu variety on susceptibility to canker. Sufficient weather data (particularly temperature and monthly rainfall in all years) for the trial sites in Argentina are not provided to make comparison to the environmental conditions under which the 'Aoshima' and 'Miyagawa Wase' Unshiu mandarins are grown in Japan. Therefore it is not possible to assess the relevance of the susceptibility of 'Okitsu' Unshiu mandarins grown in Argentina, to 'Aoshima' and 'Miyagawa Wase' Unshiu mandarins grown in the Japanese export plots. This reference would support the comment "Fruit of Okitsu satsuma tangerine (*C. unshiu* Marc.) were found have a disease intensity rating of 0 in trials carried out in Argentina (Canteros 2004)", but does not provide sufficient data to support the statement that "Fruit of unshu mandarin rarely show signs of infection" (this reference is also used further down page 59 to support the blanket susceptibility of "unshu mandarin"

	based on the assessment of only one variety of Unshiu mandarin, only under Argentinean conditions). **Goto 2005** – This reference shows a photograph of a "*Citrus unshiu*" fruit with typical canker symptoms, which would indicate a certain level of susceptibility. Important details such as country of origin, variety, means of infection (i.e. natural or inoculated) etc do not seem to be available. This information would be useful for assessing the relevance of the image. Is more robust evidence available in the literature?
	Canteros 2004 (cont) - This is the only reference cited in the 2nd last dot point on page 59. This reference is cited as showing that the Unshiu mandarin ["Okitsu satsuma tangerine (*C. unshiu* Marc.)"] was the least susceptible of all cultivars tested. This statement is statistically supported [by ANOVA and Duncan RMT ($p<0.01$ or 0.05)] in 2 of 12 assessments in Table 5. In the majority of the assessments in Tables 4 & 5 (respectively: 9 of 14, and 4 of 6 assessments, where cultivar had a significant effect within the statistical parameters detailed in the reference) the "Satsuma tangerine" had significantly [by ANOVA and Duncan RMT ($p<0.01$ or 0.05)] lower disease intensity than Grapefruit, but was not significantly different from the other varieties. NOTE: Goto 1992 lists *Citrus unshu* fruit to be "susceptible" (page 181-182), and foliage to be "resistant", however the definition of "resistant" is "no infection of twigs, but scattered small lesions on leaves; epidermis of leaf spots remains intact and does not rupture to form canker", i.e. infection does occur. The original work cited in this review should be investigated further.
	Leaf and fruit damage of the Unshiu mandarins due to wind/weather is 'not known to occur in the designated export areas', however Table 3.5 in section 3.4.3 outlines that 11-31 sprays (depending on the interpretation of the table) are applied. Some spray application methods use high velocity air (can be as high as 200km/h), or high pressure (>300psi) liquid to delivery the chemical to the target. The use of such equipment could lead to leaf and fruit damage (unpublished work in Australia has shown this), and possibly facilitate spread of Xcc and water congestion of tissues. How are chemicals applied in the production area? Spray application method also affects coverage of citrus trees, with implications for the efficacy of protectant chemicals such as copper.
p60	"Timing for infection": 1st dot point **Koizumi 1981** – no data and/or not the original source, review article. Koizumi (1981) cites Ohta (1967) – but this could not easily be sourced for comment. **Gottwald and Graham 1992** – the data in this reference was produced only from Duncan grapefruit. Additional literature should be sought with regards to Unshiu mandarins. It should be easy to add references for the comment: "Disease symptoms become visible first on leaves"

	2nd dot point: **Schubert et al 2001** – Exact quote from reference: “Appearance of fruit and twig lesions also assumes that in most cases a certain prior inoculum level must have been reached on leaves to further advance the disease.” The statement in the IRA is based on an assumption by the reference’s author, but has this hypothesis ever been tested? **Kuhara 1978** – no data and/or not the original source, review article. **Canteros 1992** – not the original source, should be Stall et al 1981. **Canteros 2004** - no data and/or not the original source, review article. This reference refers to “selective localized pruning” as “a method used for years in Japan” etc but doesn’t present or cite any supporting data that this reduces disease. 3rd dot point: **Koizumi 1972** – According to the CABI Cab Direct database this article is written in Japanese, with only an abstract in English. 4th dot point: “The time interval for fruit to become infected is limited…” **Koizumi 1972** – According to the CABI Cab Direct database this article is written in Japanese, with only an abstract in English. **Canteros 1992** – It is not clear which variety of Unshiu mandarin was used in the experiments. Table 5 shows that fruit could be infected from Nov 17 to Mar 7 (~4 months), with declining susceptibility as fruit expand. The reported time interval for fruit infection should be stated more quantitatively, as supported by the experimental evidence. **Graham et al 1992b** – The reference provides supporting data for the statement, though is not specific to Unshiu/satsuma, and also cites several other sources that should be considered in the IRA. “Natural (stomatal) citrus canker infection…” **Goto 1962 and Koizumi 1972**- According to the CABI Cab Direct database this article is written in Japanese, with only an abstract in English. An English translation is required to fully appraise the publication. It is not clear from the Goto abstract what variety of satsuma mandarin was studied. “Unshu mandarin fruits become increasingly resistant…” **Koizumi 1972** – According to the CABI Cab Direct database this article is written in Japanese, with only an abstract in English. **Canteros 1992** – It is not clear which variety of Unshiu mandarin was used in the experiments. Is data available to demonstrate that the variety of Unshiu does not affect the susceptibility period? It would be beneficial to provide a quantitative definition of “resistant” as used in this reference, as fruit termed “resistant” may have several lesions per cm^2.

	5th dot point: **Koizumi 1972** – According to the CABI Cab Direct database this article is written in Japanese, with only an abstract in English. **Gottwald and Graham 1992** – Only the results of 'experiment 2' are reported in the IRA from this reference. In the context in which these results are considered later in the IRA (4.11.2. Probability of Distribution, Sufficient inoculum would need to be present to cause infection, paragraph 4, page 62), more of the findings of this reference should be reported in the IRA. In particular the conclusion of the authors, based on all the experiments conducted, that "as few as 2 cfu were required to cause a single lesion". "Conditions promoting infection": 3rd dot point: The first sentence makes mention of leaves and fruit only, but makes no mention of shoots. **Goto 1962** - According to the CABI Cab Direct database this article is written in Japanese, with only an abstract in English. It is not clear from the abstract what variety of satsuma mandarin was studied. **Serizawa et al. 1969** - According to the CABI Cab Direct database this article is written in Japanese, with only an abstract in English. **Koizumi 1981** – no data and/or not the original source, review article. The original paper cited by Koizumi (1981) cites Ohta (1967) and should be appraised and cited if appropriate. 4th dot point: In the production area surrounding the designated export areas, what is the frequency of "severe bacterial infection of the tree canopy" that is stated as leading to Unshiu fruit infection?
p61	"Existing commercial control program": The IRA cites/provides no efficacy data for the MAFF Unshiu mandarin spray calendar. "Conditions for transport": **Koizumi 1976** - According to the CABI Cab Direct database this article is written in Japanese, with only an abstract in English. **Koizumi 1985** – Not readily available for appraisal "The unknown status of citrus canker and its hosts in the production area outside the designated export areas": In the absence of data on the citrus canker status in the production area, the possibility of pathogen inoculum sources located within possible spread distances cannot be discounted. Assessments of risk should therefore assume canker is present on host plants within the production area unless otherwise demonstrated by rigorous surveillance data.

	"Probability of distribution": "**2. Sufficient inoculum would need to be present to cause infection**" **Graham et al. 2000** – This reference is an abstract in a conference proceedings, and doesn't provide sufficient information or the data to fully appraise the work undertaken. It is surprising this information has not been subject to peer review and publication in a reputable journal. **Koizumi 1972, Goto et al 1975b, Goto 1962** – According to the CABI Cab Direct database this article is written in Japanese, with only an abstract in English. It would be beneficial to the peer-review of the IRA if the English translations used in the production of the IRA were made available on the Biosecurity Australia website. **Gottwald et al. 1992** – This reference undertakes experiments with X. *campestris* pv. *citrumelo* (*X. alfalfae* subsp. *citrumelonis*), the cause of citrus bacterial spot, and not with *X. axonopodis* pv. *citri* (*Xanthomonas citri* subsp*. citri*) causing true citrus canker. Furthermore, the effect of direct sunlight on bacterial desiccation does not appear to be assessed in the experiments. **Graham et al. 2000** – This reference is an abstract in a conference proceedings, and doesn't provide sufficient information to fully appraise the work undertaken. It is surprising this information has not been subject to peer review and publication in a reputable journal.
p62	"Probability of distribution": "**2. Sufficient inoculum would need to be present to cause infection**" (cont.) "*Epiphytic bacterial proliferation is epidemiologically insignificant*" **Goto 1992** – This reference provides no data to support the above statement, and cites only unpublished work. Furthermore the author states "the epiphytic form of survival should have only minor importance from the epidemiological viewpoint of citrus canker". Quantitative assessment of the importance of the epiphytic form is required. **Brunings and Gabriel 2003** – This reference provides no data to support the above statement, nor does it cite any data to support the statement. The authors state "there is no evident epiphytic growth stage". The paper makes no attempt to experimentally evaluate the importance of epiphytic growth to the epidemiology of citrus canker, as this was not the theme of the paper. Quantitative assessment of the importance of the epiphytic form is required. "*Sufficient inoculum to cause infection was estimated to be more than $2x10^3$ cfu/mL for stomatal infection…*" Refer to the comments made under point 6 regarding **Gottwald and Graham 1992** and **Koizumi 1972**. "*In contrast, populations of X. axonopodis pv. citri declined rapidly in lesions in picked leaves or fruit…*" **Graham et al. 1989** – This reference does not provide evidence to support the statement. Effect of time after "picking" on bacterial populations does not appear to have been investigated in this reference. No experiments were conducted on fruit.

	"*Inoculum loads are higher in symptomatic lesions...*" **Gottwald et al. 1992** – This reference is a study of *X. campestris* pv. *citrumelo* (*X. alfalfae* subsp. *citrumelonis*), and does not provide data on the inoculum loads of *X. axonopodis* pv. *citri* (*Xanthomonas citri* subsp*. citri*) in symptomatic or symptomless infection sites or contaminated peel. Some cited references in Gottwald et al. 1992 may provide this information, and could be cited if so. **Canteros 2004** – This reference states "Numbers detected ranged from undetected level on lesionless leaves and fruits of orange and lemon from sprayed low disease plots and 0 to 10^6 cells of Xac per leaf or fruit (mean: <10) from highly infected unsprayed plots of grapefruit, lemon and orange (Rybak and Canteros, 2001)." If the original work is presented in Rybak and Canteros (2001), only Rybak and Canteros should be cited. Furthermore, the above quote from Canteros 2004 does not provide sufficient evidence for the comparison of inoculum loads on host tissues. Other literature relevant to this issue should be found and cited. "*The rate and number of bacterial cells exuding…*" **Timmer et al 1991** – The comparison of bacterial exudation was made between "young" and "old" "lesions". The use of the word "infections" could be mistaken for symptomatic or symptomless "infections". The term "lesions" would be better to use. **Koizumi 1972** – According to the CABI Cab Direct database this article is written in Japanese, with only an abstract in English. "**3. Free water is necessary to enable the release of viable bacteria from infected tissue**" **Goto 1962** - According to the CABI Cab Direct database this article is written in Japanese, with only an abstract in English. An English translation is required to fully appraise the publication. The findings of Timmer et al 1991, that immediately after wetting of young leaf lesions, 10^4-10^5 bacteria/mL were exuded, could be cited here. "Bacterial proliferation depends on…" **Koizumi 1976** - According to the CABI Cab Direct database this article is written in Japanese, with only an abstract in English. An English translation is required to fully appraise the publication. The English summary states "The maximum and minimum temperatures for disease development after inoculation were 36-38 deg and 13 deg C, respectively" however bacterial proliferation is not explicitly referred to. **Kuhara 1978** – This review article does not provide details of the environmental conditions required for bacterial proliferation, supported by experimental data. The most relevant quote would appear to be "In early spring as the average temperature reaches 13^{o}C, the pathogen after overwintering begins to multiply in infected tissues." No details of how this was determined are provided. **Koizumi 1972** - According to the CABI Cab Direct database this article

	is written in Japanese, with only an abstract in English. **Goto 1992** – This review article is frequently cited, but is not the original source of most of the data, or no data is provided or cited. "There is no evidence that fruit without visible symptoms at harvest…" **Graham et al. 1992b** – The experiments described in this reference do not investigate the likelihood of fruit without visible symptoms at harvest developing lesions after harvest. This reference shouldn't be cited.

Additional References:

Gottwald TR, Graham JH, Schubert TS (1997) An epidemiological analysis of the spread of citrus canker in urban Miami, Florida, and synergistic interaction with the Asian citrus leafminer. *Fruits (Paris)* **52**, 383-390.

Peltier GL, Frederich WJ (1920) Relative susceptibility to citrus-canker of different species and hybrids of the genus citrus, including the wild relatives. *Journal of Agricultural Research* **14**, 339-362.