Stakeholder Comments

Final Pest Risk Analysis for pepino mosaic virus and pospiviroids associated with tomato seed

Comments submitted by: Australian Seed Federation

Thank you very much for the opportunity to comment on this draft Pest Risk Analysis (PRA). In compiling this submission, the Australian Seed Federation has sought and included input from members of the International Seed Federation, the American Seed Trade Association, the New Zealand Grain and Seed Trade Association, the European Seed Association and Plantum.

The international seed industry does have several comments on, and concerns with, the draft PRA. We will go into each of these in more detail below, but the main points are: 1. The evidence provided in the draft PRA does not support the conclusion that the import of tomato seed is a proven pathway for the transmission of pospiviroids into Australia. 2. An internationally harmonized 1,000 seed subsample size should be implemented for pospiviroid testing. 3. There is no clear data presented that supports the statement in the draft PRA that PepMV is not present in Australia, nor its conclusion that the likelihood of the pathogen entering via the imported seed pathway is moderate. 4. Given the low risk of entry of PepMV into Australia via seed, we believe that the current internationally validated 3,000 seed ELISA test should be maintained. 5. Heat treatment of tomato seed imports for PepMV is not a commercially viable option for the seed industry and we ask that other phytosanitary options be provided. 6. We seek clarification that offshore testing of small seed lots will be allowed under the proposed measures. 7. We have concerns relating to the new requirement for approval of offshore testing labs, especially as to how it will apply to testing currently being done in offshore labs and to the capacity of the Department to undertake this work. 8. We would like our 2016 application for a systems approach case study for GSPP-certified seed to be considered and progressed as part of any final phytosanitary measures resulting from this process, and the implementation of alternative phytosanitary options.

We would very much appreciate the opportunity to work with the Department in finalizing the phytosanitary measures to be implemented for these pathogens, with a view to ensuring the safe but also the sustainable access to tomato seed imports – given that the vast majority of Australian growers and consumers depend on this pathway.

1. Determination that seed is a proven pathway for transmission of pospiviroids

The seed industry disputes the conclusion in this draft PRA that the import of tomato seed is a proven pathway for the transmission of pospiviroids into Australia. The International Seed Federation has recently completed a significant exercise of reviewing the scientific literature on all currently regulated pathogens of tomato seed globally, and it is clear that for each of the viroids assessed in the draft PRA to be quarantine pests for which seed is a pathway there is little evidence to support this conclusion. At the very least, the evidence does not warrant or justify implementation of the penalizing, inflexible and non-validated (against other methods currently in use) phytosanitary measures that are being proposed.

As a first point, there is no information provided in the draft PRA of any pospiviroid outbreaks in commercial tomato productions being traced back to infected seeds. Indeed, no completed root cause analysis of any national outbreak has ever been made public. We suggest that the risk from tomato seed needs to be appropriately considered in the light of other data provided in the draft PRA of the presence of pospiviroids within ornamentals. The results of the 2012 survey provided on p.7 reports that the tomato detect resulting from the survey is effectively one tomato crop (we note that no details were given on the number of plants involved here, nor was the crop the same hybrid throughout the glasshouse production) - in a State where PSTVd has been known to occur. Whereas the ornamental detects comprised three independent events of viroids of regulatory status in different hosts and in different States. There were therefore three times the detects on ornamental plants, which comprised only a small portion of the plants sampled. At the very least, these results show that the true risk of viroid introduction and distribution is not tomato seed. This conclusion is further strengthened when viewed in the context of no evidence of any reduced risk since the introduction of the emergency measures - if pospiviroids were such a huge risk in tomato seed imports more outbreaks would have been seen before the emergency measures than afterwards, and this has simply not been the case. Correlation of no evidence linking trade of solanaceous ornamental plants to outbreaks in tomato crops in disparate locations is not causation – this pathway is rarely investigated when there is an outbreak in a commercial tomato production.

For the same reason, we question the statement on p.36 that reports of outbreaks in disparate locations present a picture of global distribution that is best explained by international transport of the pathogens with seed, and their introduction to crops through seed, along with instances of local transmission. This statement is assumptive given the above survey information. More work needs to be done to understand potential sources in the context of all materials and potential entry pathways of viroids in tomato productions.

This conclusion is further weakened when viewed in the context of what is known about each pospiviroid in particular. CLVd, for example, is found on several herbaceous plants, and not just tomato. There is conflicting scientific information on whether imported seed is a pathway for this pathogen in tomato. One reference indicates that CLVd could be detected on seed, however another indicates that transmission did not occur. A recent reference also indicates seed transmission occurred in 3 out of 4 varieties of tomato inoculated and grown under experimental conditions, but this was not tested in situ. What is more, CLVd infection in tomato is considered selflimiting in that extremely small numbers of seed are produced in infected tomato plants – meaning that any infection is likely to be picked up during the production and processing stages.

Little information also exists in relation to PCFVd. One abstract indicates the presence of PCFVd sequences on tomato seed through the use of RT-PCR. But a second references found seed transmission of PCFVd in only one out of four tomato varieties when seed was grown, again, from inoculated plants. No information has been given on whether the seed was extracted or sanitized in a commercial manner and, therefore, whether this is representative of commercial seed production in practice.

Several references make statements or suggest that seed is a pathway for TASVd. However, in reality no real data has been presented to support this. One reference indicates that seed could be a pathway under experimental conditions, while another reference indicates that seed is NOT a

pathway under experimental conditions. The international seed industry cannot find any other references indicating that seed as a pathway, with supporting data.

Again, there is also conflicting data as to whether seed is a pathway for TCDVd in tomato. One reference found low levels of seed transmission to seedlings in one commercial seed lot when tested by PCR. Another reference indicates the presence of TCDVd in seeds and seedlings when one isolate of TCDVd was inoculated to one variety of tomato under experimental conditions and tested by PCR. In this latter study, the seeds were not harvested and treated in a commercial manner. Yet other references indicate no transmission of TCDVd to seedlings.

In the case of TPMVd, one reference states that seed as a pathway could not be demonstrated. Another reference records seed transmission of TPMVd in one out of four tomato varieties when seed was grown from inoculated plants. However, no information was given on whether the seed was processed in a commercial manner. This lack of consideration of information relating to the reality of seed production practices in coming to the conclusions in this draft PRA is a significant concern to industry.

The 10.3% positive hit number presented on p.42 in the draft PRA also has to be viewed carefully. It only represents the results of the lots that were tested upon import. To better understand the rate of infected seed that is being sent, one needs to consider all the seed lots that were imported to Australia. Any seed lot that was not tested at the border met the import requirement of being tested and found free of pospiviroids. Therefore, the incursion rate is much lower than 10.3%. The origin of the seed and where it was sent from should also be differentiated, as it is highly unlikely that contamination will occur during processing (treating, packing and shipping).

While we appreciate that Australia adopts a very high Appropriate Level of Protection in its consideration of quarantine matters, the evidence provided in the draft PRA does not support the introduction of the current proposed costly and restrictive phytosanitary measures.

2. Testing requirements for pospiviroids

The seed industry notes that there is no proposal in this draft PRA to reduce the sample sizes for pospiviroid testing from the 20,000 seed and 400 seed maximum subsample sizes implemented as part of the emergency measures.

The international seed industry has previously provided arguments that a testing protocol using 3,000 seeds as a sample size with 3 subsamples of 1,000 seeds is sufficient to meet the Australian government's appropriate level of protection - should a decision be made to formally regulate these pathogens. Industry has been using such a testing protocol for several years, in cooperation with governments and public seed health labs, with no evidence of any increased risk associated with the pathogens.

We would also make the point that if, as stated in the draft PRA, the transmission rate for these pathogens is so high (even 1% is very high), then there should not be a need to test at 20,000 seeds as the pathogens will definitely also be detected just as well in a 3,000 seed sample. The argument that a 20,000 seed test is more sensitive does not hold up when examining the biology of the pests involved.

We are disappointed that our efforts to collaborate with the Australian government on developing a more globally harmonized testing protocol, using 1,000 seed subsamples, have also not been considered or referenced in the draft PRA. We provide the following examples: • In 2013, the Department requested that seed companies provide it with seed samples that had tested positive to PSTVd so that it could conduct trials to ascertain whether there was any difference in sensitivity between using 1,000 seed and 400 seed subsamples. This was provided at extensive cost to companies, as this seed could still be sold in other markets. There have never been any results presented from these trials, and it is unclear whether experimental work was ever undertaken. • In 2014, the Department presented industry was an experimental design for a trial to compare the sensitivity of the two different subsample sizes in detecting pospiviroids in tomato seed on arrival onshore in Australia. This involved testing 10,000 seeds at a 400 seed subsample frequency and 10,000 seeds at a 1,000 seed subsample frequency. The industry was grateful for this opportunity and provided technical feedback on the design, with the assistance of ISHI-Veg - with a view to enhancing its statistical strength. However, a response was never received to these comments and we must assume that this trial was never proceeded with. • ISHI-Veg engaged the Victorian testing lab in a ring test with several other key seed pathology testing labs to compare the sensitivity of subsample sizes in detecting pospiviroids. The results of this testing were presented at a Symposium organized by the Victorian lab in September 2017, at which the Department was present. A conclusion from this test, and reaffirmed during the Symposium, was that there would appear to be no clear difference in sensitivity between the use of 1,000 seed or 400 seed subsamples.

We would therefore ask that strong consideration be given to implementing a globally-harmonised testing protocol that adopts a 1,000 seed subsample size.

On another point, we note that Section 7.3.3 states that “samples must be drawn for testing prior to fungicide treatment or coating of the seeds”. Such a requirement is currently not being enforced as an emergency measure for tomato seed imports (excluding pelleted seed) under Condition 2.2e because it is not always the case that companies have a raw seed sample available. We would ask that such a requirement not be made mandatory as it could affect the ability to meet on-time demand of seed in Australia since many commercial lots are processed for many countries and not specifically for Australia.

3. Presence of PepMV in Australia and seed as the pathway

There has been no clear data presented in the draft PRA to support its conclusion that PepMV is not already present in Australia. Given the arguments presented in the document relating to the highly contagious biology and epidemiology of PepMV, in conjunction with the global production and trade of tomato seed, such a statement is speculative. Similarly, the argument presented in the document that countries do not know what diseases are present due to asymptomatic or mild infections also holds true for Australia. To come to such a conclusion, clear supporting data should be provided, along with information demonstrating how this pest freedom status is being maintained.

In addition, the difficulty with the references to PepMV outbreaks and infections provided in the draft PRA is that there is no ability to understand what is due specifically to seed movement and what is due to transplant movement or to fruit movement. Plant products may move easily over borders depending on the various agreements in place among trading partners or within unions

(e.g., EU). As demonstrated, this virus can produce high titers in all plant parts and can move with any plant products that are traded or moved for production reasons.

For growers in the Netherlands it has become common practice for growers to also cross protect plants during transplant productions. Cross protecting means that the plants are purposefully inoculated with an attenuated strain of PepMV as it is believed that this minimizes the impact of late season PepMV infections (Spence et al., 2006). It therefore cannot be ruled out that this practice has become more widespread as growers attempt to maximize plant outputs (fruit yields). Nor can it be ruled out that some detects could be the result of purposeful infections (tomato fruits from cross protected plants). Seed can play a role in PepMV spread, but it is highly unlikely (in fact, almost impossible) that infected seed has played a role in reported outbreaks, considering that PepMV has not been detected in any seed lot since testing was implemented.

It should also be noted that there are tens of thousands of tomato seed shipments annually, many of which are through the EU, and that there are many different companies (seed producers to seed distributors, wholesalers, etc.) who partake in this business. Given that context, it is not surprising that there are detects; what is more surprising is that these are not more numerable. This is the reflection that for many seed companies, PepMV is an important quality target and that there are efforts to ensure parental seed and productions remain free of this virus. Additionally, quality testing is applied on final seed lots to ensure this goal has been achieved. These practices are reflected in the testing results that have come from the Australian import records, in which no detections have occurred since testing was implemented.

We would therefore propose that the likelihood of entry of PepMV through imported tomato seed is very low, and certainly not moderate as concluded in the draft PRA. The appropriate context needs to be considered n light of the amount of shipments that occur annually versus the number of detects. This is supported by the data in the draft PRA and by import experience.

4. Increase of PepMV testing sample to 20,000 seeds and 400 maximum seed subsamples

The international seed industry strongly believes that the draft PRA provides, at best, extremely theoretical arguments for increasing the current sample size required for testing PepMV. This is certainly not based on the evaluation of any new and real identified risk, as is recognised in the draft PRA itself where it states that 'since the emergency measures were installed, no PepMV infected lots have been picked up in imported seed lots by Australian laboratories'.

The 3,000 seed sample size has been the standard within the seed industry for 15 years. During that time, there has also not been a single outbreak. It is the experience of the industry that this seed sample size provides full confidence in the detection of PepMV and that the current sample size is adequate to detect and protect Australia. The highly contagious nature of the virus also adds to the conclusion that it is extremely unlikely that there would be a limited contamination of plants within a seed production population. In grow-outs with freshly harvested, partly cleaned seeds (the situation with the highest risk of transmission of PepMV via seed), less than 1 in 1,000 seedlings was found infected. All seeds used in the grow-outs were tested and found to be ELISA-positive. This very low transmission rate was confirmed in a European research project 'PEPEIRA'. To be able to detect 1 infected seed in 1,000 seeds with a 95% confidence level, a sample size of 3,000 seeds is

needed. When one infected seed in a sample of 1,000 can be detected, a transmission rate of at least 1 in 1,000,000 (1,000 x 1,000) is covered.

There is therefore a robust data set that supports the validation and routine use of this method. The method using 3,000 seed as the sample size is also widely accepted outside of the seed industry (e.g. by EPPO, NSHS). In contrast, to date, there is no validated PepMV RT-PCR method. In addition, current industry practices involve using the same 3,000 seed sample to test for both PepMV and for tobamoviruses – since these are both seed health quality targets. As such, combining this test with the pospiviroid sample will not result in an overall saving of seed for the industry.

We would therefore very like to request that this proposed amendment to the current emergency measures be removed. There are no outbreaks, no detection of positive seed lots, nor is there any new epidemiological data presented in the draft PRA that justify a larger sample size being implemented - not in Australia, and not in other parts of the world. Introducing an non-validated method is likely to lead to more detection problems, not less, and could potentially put Australia more at risk because of an increase in false negatives. On this point, the ASF understands that ISHIVeg is currently undertaking a project in which PCR primers are being developed for PepMV, for use as a confirmatory test. ISHI-Veg would like to work with the Australian authorities on this effort, with a view to sharing knowledge and the PCR-protocol, and to collaborating with the Australian government on any seed health test development that is ongoing. A letter of approach from ISHIVeg is at Annex 1 to this submission.

On another point, the industry would also like to point out that in subsection 7.3.2 of the draft PRA, there is reference to testing for PepMV requiring an Additional Declaration, unlike current conditions for pospiviroids where the testing certificate alone is sufficient. This is because a number of NPPOs will not endorse use of a non-validated test on their phytosanitary certificates. Should the Australian government insist on implementing a new testing protocol for PepMV, the latter option will need to be made available.

5. Proposal to introduce heat treatment as an option for PepMV

The draft PRA proposes dry heat as an alternative option to testing for PepMV. The proposed 80C/72h heat treatment negatively impacts germination of treated seed lots (Ling 2010), which results in seed lots that do not meet growers' expectations. The impacts of the heat treatment result in on average a 5% drop in germination. It also negatively impacts the shelf life of seed lots. Some tomato hybrids may be even more sensitive, resulting in more than a 10% drop in germ. This impact on germ and shelf life is unacceptable to the protected culture commercial tomato growers paying high prices for this seed. In short, the proposed seed treatment is not a feasible commercial option for routine application on seed lots.

Other phytosanitary options could be considered. The seed industry believes that it is premature to assume that chemical treatments are ineffective and to rule this out as an alternative phytosanitary measure. The work reflected in the referenced Ling (2010) publication was not a robust data set and based on the method performance, the results on the chemical treatments can and should be questioned. To begin with, the equipment used to perform the grinding of seed samples was not properly disinfected. Ethanol is not an appropriate disinfectant - the author noted this in his work and proceeded to process samples in the way that would keep the untreated control (the Pepino

infected seed) from contaminating other samples (these samples were ground last). But there is no proof that one of the treatments that failed to work, HCl for example, did not impact the results of other chemical treatments. Additionally, only 1-2000 seed samples treated per chemical treatment were evaluated; the samples were then split and analysed as 250 seed subsamples. Based on this work it is premature to assume that chemical treatments are ineffective. In fact, there were promising results from the 1.0% commercial bleach (effectively 0.05% NaOCl concentration) that should have been further investigated by the researcher.

We would therefore ask the Australian government to further consider chemical treatment as an option for addressing the risk of PepMV in tomato seed imports. High value tomato seed is typically extracted using pectinase and hydrochloric acid to facilitate the separation of the seed and the surrounding gel. Existing regulations for the import of tomato seed to France require that tomato seeds be treated with 1.85% NaOCl for 15 minutes on dry seed, or 0.38% NaOCl for 30 minutes on wet seed. Additionally, seeds are typically treated with a 10% trisodium phosphate solution to remove surface contaminants. Cordoba-Selles et al. (2007) evaluated some of these chemical treatment options. Such a multistep chemical seed sanitation option could be presented as an equivalent to the heat treatment, or at the very least allow for the current 3,000 seed ELISA test to be used.

We would also note that in the case of certification, the draft PRA seems to propose in Chapter 7.4 that this dry heat treatment also has to be followed by PCR-testing on 20,000 seeds with subsamples of maximum 400 seeds. However, in 7.3 this requirement is not mentioned. We would like to get some clarity around this, as there is no use in testing dry heat-treated seed with PCR because PCR detects living and dead 'genetic material' and this will not be useful in determining risk.

6. Small seed lots

As a general point, we would like to request confirmation that small seed lots will be allowed to be tested offshore under any final phytosanitary arrangements. The Australian government has previously acknowledged that there is no difference in sensitivity in small seed lot testing conducted onshore and offshore in the case of cucurbit and carrot seed imports, and we would ask that the same considerations be made in the case of tomato seed imports. Section 7.3.2 does seem to suggest that tomato seed lots that weigh 300g or less and that are tested offshore for the regulated pathogens must be accompanied by an official government Phytosanitary Certificate endorsed an additional declaration, but it is not clearly stated that this is a change from the current emergency conditions.

We would make the point here too (as we did for PepMV) that an AD may not be possible from exporting governments when using this testing protocol and we would therefore ask that consideration be given to allowing the test certificate to accompany the consignment (as is the case now for commercial lots tested offshore).

Section 7.3.1 also states that "laboratories will not be permitted to pool (batch) samples for testing from different seed lots or seed batches". We would like to know the rationale behind this request and whether this applies to small seed lots in addition to commercial lots. Currently for imports of small seed lots into Australia, it is permissible to pool 20% from each of the samples. We would like the current practice to continue. Not allowing pooling of small sample lots for testing may adversely

affect breeder access to highly valued material to use in specific breeding programs for the Australia market. As there have not been any increased hits for PepMV on samples tested onshore, we do not believe that there is any increased risk associated with pooling of small lots.

7. Approval of offshore testing labs

The draft PRA proposes that testing protocols used in off-shore testing (for both PepMV and pospiviroids) will require Departmental approval under any final phytosanitary measures. It is unclear what this will entail, and whether current labs already being allowed to conduct testing offshore will be automatically approved under any such arrangement. There is no more detail provided on how this requirement will be implemented. This could have a significant impact on seed supply should seed that has already been tested in these labs not be allowed import until the protocols have been formally re-approved by the Department. It will be important to put in place proper transitional arrangements if the small number of labs currently being used for offshore testing of tomato seed need to undergo a new approval process. The seed industry would ask that, if it has not already done so, the Department approach these laboratories and exporting NPPOs so as to minimise disruptions.

Given that outbreaks of PepMV have not occurred in Australia, and that no data has been provided to support the Australian government's expertise in detecting this virus (and pospiviroids for the that matter) over that of other exporting governments, we would also question the local ability to approve such methods. This is especially so given that there are no validated PCR methods for these pathogens, and so there is likely to be more variability when compared with any existing ELISA method that has been validated and with which competency has been shown many times over. Industry preference is also for a qRT-PCR (real time) protocol for any PCR testing, without a mandatory requirement for manual RNA extraction (using a hammer). There are lab accreditations in place in the United States and the Netherlands that leverage data review processes to establish routine seed testing methods for export and it is suggested that DAWR work with the USDA and with NVWA to identify a process to mutually recognize methods should it be decided to go down this path.

8. Alternative measures

The draft PRA makes mentions of the fact that alternative phytosanitary measures are possible. The seed industry is certainly supportive of such an approach. However, it has been our experience that such alternatives do not seem to be fully considered or implemented upon request by the Department, and in any event testing is nevertheless still required. For example, p.91 of the draft PRA states that area freedom is difficult to establish without testing seed imports, and p.92 states that pest free places of production need to implement testing of seeds or parent plants.

We have often called for field inspection regimes, and area freedom declarations from exporting governments, to be considered and offered as alternatives. Such schemes are in place in a number of developed countries for these pathogens, including in the EU and New Zealand, and are very well established as mechanisms for mitigating risk. A t the very least, such programs should result in lighter testing regimes being required (that would be more in line with global and industry best practice).

It is very positive to see reference in the draft PRA to the fact that Australia embraces the principle of a Systems Approach to seed imports (although again testing of seed is mentioned as being required). The seed industry would like to congratulate the Australian government for the role they have played to date in seeking global alignment in this space. As such, we expected consideration of the ASF's application to allow a systems approach option for GSPP-certified tomato seed imports (submitted in December 2016 in discussion with the Department, and submitted again with this submission) to have been addressed in this draft PRA and, potentially, for such a system to be proposed as an alternative measure.

We are therefore disappointed to see on p.22 of the draft PRA that, in discussing GSPP, only one quote from the IIGB report into the import of tomato and carrot seed is referenced, relating to apparent quality differences between production systems in Thailand and the Netherlands. Please note that irrespective of the production practices applied, the goal is the same: plants and seed free of Cmm. Net houses in Thailand are reliable seed suppliers and compliant with GSPP (and audited by Dutch testing laboratory officials). The seed production practices are determined based on a comprehensive risk assessment process, per site, which permits companies to look holistically across the seed production process for areas of disease risk and to implement those practices that mitigate given the risks and based on the resources available. In fact, the IIGB recommended in his Report that GSPP be approved as a systems approach for importing tomato seed into Australia, and this recommendation was accepted by the Department – this conclusion should have been mentioned in the draft PRA as well. We would still like the Australian government to consider the ASF's GSPP application, and to implement a lighter testing regime for seed that has been produced through this extensive and audited seed health quality system. At the very least, the seed industry would like to work with you in implementing this as a case study.

We thank you again for the opportunity to comment on this draft PRA and look forward to seeing our comments incorporated into a final version. Please do not hesitate to contact me if you have any questions or would like any further information.