

# Stakeholder comments

## Draft review of import conditions for cucurbitaceous crop seeds for sowing into Australia.

### Comments submitted by: Australian Seed Federation, ACT

The Australian Seed Federation does have some comments to make on the references used in the draft Pest Risk Analysis (PRA) to justify the implementation of phytosanitary risk management measures for KGMMV, ZGMMV and *Phomopsis cucurbitae*:

### KGMMV on *Citrillus lanatus*, *Cucumis sativa* and *Cucurbita pepo*

The draft PRA cites Kwon et al 2014 as the reference indicating seed as a pathway for KGMMV in watermelon and squash and Lecoq and Katis 2014 as the reference for cucumber.

The ASF believes that Kwon et al 2014 is scientifically flawed and cannot be relied on as a basis for regulation. The paper describes a PCR method to simultaneously detect a number of viruses. However, no positive and negative plant or seed controls were included in the evaluation of the PCR technique, to show that cross contamination or cross reactivity in a seed background can be excluded. In addition, the positive results of the multiplex PCR were not confirmed using a technique other than PCR, and the primers used by Kwon are 10,000,000 fold more sensitive than conventional primers - this level of detection might be beyond biological relevant levels from a seed transmission perspective. These points severely limit the credibility of the paper and we would suggest that if this is the only evidence that is being used to support the implementation of phytosanitary requirements, as appears to be the case, then this decision should be reconsidered. In addition, we would note there is only one single seed sample of watermelon in this paper, and again the proper controls were not included.

Similarly, the only reference to KGMMV and cucumber seed in Lecoq and Katis 2014 is in Table 5.2. Indeed, this Table is the sum total of the information related to Tobamoviruses contained in this book chapter, and there is no data presented and no references listed to support this information. It is therefore unclear how this conclusion was reached, and would suggest that this cannot be relied on as a basis for regulation.

We do note that Daryono et al. (2005) cite Blancard et al. (1994) as reporting KGMMV is seed transmitted. However, a publication by Muthaiyan (2009) claims KGMMV is not seed transmitted, counteracting this report.

In terms of establishment, the draft PRA states that KGMMV 'has established in areas with a wide range of climatic conditions (Daryono, Somowiyarjo & Natsuaki 2005; ICTVdB Management 2006a), and that virus particles from seed extract were inoculated to *N.bentaminiana* and infectivity was proven. Spread of this virus from the seed pathway depends on human-mediated transport of

infected seed'. However, the ASF contends that this level of virus might not pose a risk in the transmission from seed to seedling - if this is even a pathway. Transmission can even be strain dependent in viruses (Roberts et al., 2003). We would also note that the reference 'ICTVdB Management 2006a' was not possible to find through the included link and could therefore not been discussed.

KGMMV has also been shown to be of limited importance in global cucurbit production and its dispersal is limited to a few Asian countries. This implies that the spread of the pathogen is of a different magnitude than f.e. CGMMV and its economic importance and damage is marginal.

The seed industry therefore does not believe that KGMMV can be considered a quarantine pest to Australia, and indeed the Pest List of the International Seed Federation (ISF) states that no references have been found to date confirming seed as a pathway for KGMMV. To our knowledge there have been no outbreaks that show irrefutable evidence of seed being the source of the disease, and establishment from seed is yet to be proven.

### **ZGMMV on *Citrullus lanatus* and *Cucurbita pepo***

The draft PRA states that seeds provide a pathway for this virus, 'which has been reported naturally occurring as seed-borne in *Citrullus lanatus* (Kwon et al. 2014) and *Cucurbita pepo* (Lecoq & Katis 2014)'.

As per our comments for KGMMV, the ASF believes that Kwon et al 2014 is scientifically flawed and cannot be relied on as a basis for regulation. We would ask the Department of Agriculture and Water Resources to reconsider regulation of this pathogen on watermelon seed if this paper is the only evidence being cited. The ISF Pest List again shows that there is no documented evidence of seed acting as a pathway for ZGMMV. We also believe that there is no data in Lecoq & Katis 2014 to be able to make any sort of evaluation relating to seed as a pathway for this pathogen, for the same reasons as in our comments relating to KGMMV.

In addition, proper scientific evidence of occurrence of ZGMMV only exists for Korea. The presence of ZGMMV on seed from China (Ryu et al.) is based on unpublished (unverifiable) data, and ZGMMV (ZGMMV-SA) in Saudi-Arabia (Al Dosary et al., 2012) has not been characterized by serological or molecular methods. As previously mentioned also, data on host range in this last paper also raises doubts on the nature of the virus studied. This would further suggest that the risk of seed acting as a pathway for entry of this virus is extremely low.

In terms of establishment potential, the draft PRA states that ZGMMV 'has established in areas with a wide range of climatic conditions (Al-Dosary, Marraiki & Aref, 2014; Ryu et al. 2000). Spread of this virus from the seed pathway depends on human-mediated transport of infected seed'. It is not clear to the ASF on which literature reference the statement of seed transmission is based on. The reference made to Al-Dosary's paper published in 2014 is likely mistaken by Al-Dosary 2012. In this paper, Al-Dosary mentions seed, but did not describe how seed transmission did occur or was investigated. Furthermore, in this paper, the data on alternative hosts for ZGMMV described is in contrast with the findings by Yoon et al., 2014. In addition, the symptoms described by Al-Dosay

deviate from those described in other publications. We would therefore ask that the Department review the value of this abstract versus the peer reviewed publication by f.e., Yoon et al, 2014. Similarly, in the publication of Ryu et al.,( 2000) presence of the seed borne nature is claimed, but the evidence is not traceable and cannot be judged, and nor is seed transmission shown.

The ASF therefore considers the statement that ZGMMV can occur in a wide range of climates insufficiently supported with the provided data. As it is also not proven that contaminated seed is indeed causing infected seedlings, we would suggest that the risk of establishment of ZGMMV is at best very low.

### **Phomopsis cucurbitae on Cucumis melo**

This fungal pathogen is very widespread and known to the seed industry, and the ASF would like to see evidence in a final Pest Risk Analysis that indeed it is not present in Australia and can be considered a quarantine pest. It is unclear if this should be a local industry concern.

In addition, the only citation given in the draft PRA for seed as a pathway for this pathogen is Garibaldi et al 2011. The ASF notes that this reference is only a “first report” abstract and the only mention of seed as a pathway is the final sentence: “However, attention must be paid considering that the pathogen can be transmitted through seeds.” No evidence is actually provided to support this statement. We have also reviewed the three (3) other references cited in this abstract and there is no information regarding seed as a pathway in any of these references.

Further review of the literature has only yielded one other mention of seed as a potential pathway for this pathogen. It is in the APS compendium entry for Phomopsis Black Rot and Purple Stem. Again, the only mention of seed as a pathway in this compendium entry is one statement: “The pathogen can be seedborne in melon.” The ASF would also contend that as the primary source of infection for this pathogen is still unknown, the phytosanitary risks and quarantine issues associated with P.cucurbitae are at best very low.

Given all of these reasons, the ASF would therefore question the basis for regulating this pathogen at the border. There would not appear to be any scientific justification for doing so, and the decision would not be science-based as per Australia’s obligations under World Trade Organisation rules.

### **CGMMV and MNSV**

The ASF understands that the Department’s consideration of the risk posed by seed for these two pathogens has been completed. We do note, however, that these pathogens are listed as being ‘under official control’ in Australia in this present draft PRA. The ASF would like to request that the seed industry be included and consulted in the ongoing monitoring, biosecurity and international reporting plans for these viruses to ensure that there is sufficient surveillance of crops and wild hosts to maintain this status and to determine whether the risk posed by seed is significant enough relative to the risk posed by endemic sources in order to justify and maintain this statement.

It is unclear to the ASF what the basis is for imposing a single proposed measure for KGMMV and ZGMMV from all exporting countries. The reported geographical distribution of KGMMV is limited to three countries (Korea, Japan and Indonesia) and that of ZGMMV to only one. Only these countries would possibly warrant the requirement of a seed test. Due to its limited distribution, the ASF would like to ask that Additional Declarations such as 'Pest free area', 'Pest free area of production' or 'absent / not known to occur in the country' also be considered as suitable alternative measures for adequately addressing any risk that may exist. These should provide enough confidence that Australia's ALOP is being met, given the limited distribution and limited importance of these viruses in global cucurbit production.

Another point to consider when imposing a single phytosanitary measure is the availability of test kits to meet demand. The ASF is already aware of shortages of KGMMV and ZGMMV test kits as a result of the emergency measures being imposed by the Department for these pathogens – as demand for these kits has been negligible prior to these measures. In addition, many laboratories have not validated these protocols or used them on the specific crop species. We would also note that the requirement for an ISTA test method to be used makes the ELISA testing option not feasible, as there are currently no ISTA methods available for KGMMV or ZGMMV.

In addition, the ASF would also like to point out that additional tests will add more cost to the seed, even if the sample is the same (an additional ELISA test for MNSV is currently approximately USD250 offshore for example, but is likely to be higher for ZGMMV and KGMMV as kits are in short supply). This will add significant burden to the Australian industry and it is likely that elite lines will not make their way to Australia as a result. The ASF is already aware that the new costs associated with importing cucumber seed following the introduction of the new emergency measures has forced some of its members to remove these cucumbers from their portfolio. Requiring four separate tests for watermelon seed imports, in particular, has the potential to make this market non-viable – initial quotes from Australian labs, for example, suggest that a 9,400 sample test for 4 viruses will cost almost double that currently required for 2 viruses (\$2077.40 compared to \$1175), and 250% more than the cost of a single virus test (\$817.80).

Again, we would therefore strongly encourage consideration of alternative phytosanitary measures to address any perceived risk. This same request would apply to the application of a fungicide treatment to address the risk of *P.cucurbitae* on melon seed. Some melon seed is imported into Australia untreated in order to meet organic market demands. R&D seed is also often imported without any treatments, as this seed is not always obtained from commercial seed lots and is handled differently by breeders and trial coordinators in order not to mask any original characteristics. The ASF would therefore request the Department to also allow alternative phytosanitary measures to address this pathogen.

The ASF very much appreciates the opportunity to comment on the draft Pest Risk Assessment and would be very happy to discuss any of these points in further detail if desired. We hope that a workable system can be implemented to allow the continued growth of Australia's cucurbit industry.