

# SUBMISSION ON

# Import health standards for importing plant germplasm for propagation

12 May 2023

**To:** The Ministry for Primary Industries

**Name of Submitter:** Horticulture New Zealand

## Contact for Service:

Gabi Hidvegi

Risk Policy Advisor

Horticulture New Zealand

PO Box 10-232 WELLINGTON

Ph: 027 542 7783

Email: [Gabi.Hidvegi@hortnz.co.nz](mailto:Gabi.Hidvegi@hortnz.co.nz)

# OVERVIEW

## Submission structure

- 1 Part 1: HortNZ's Role
- 2 Part 2: Submission

## Our submission

Horticulture New Zealand (HortNZ) thanks the Ministry for Primary Industries (MPI) for the opportunity to submit on the proposed changes to the import health standards for importing plant germplasm for propagation. We welcome any opportunity to continue to work with MPI and to discuss our submission.

The details of HortNZ's submission and decisions we are seeking are set out in our submission below. The following parties support our submission:

- New Zealand Apples & Pears Incorporated
- New Zealand Asparagus Council
- New Zealand Avocado
- Summerfruit New Zealand

# HortNZ's Role

## Background to HortNZ

HortNZ represents the interests of approximately 5,500 commercial fruit and vegetable growers in New Zealand who grow around 100 different fruit, and vegetables. The horticultural sector provides over 40,000 jobs.

There is approximately, 80,000 hectares of land in New Zealand producing fruit and vegetables for domestic consumers and supplying our global trading partners with high quality food.

It is not just the direct economic benefits associated with horticultural production that are important. Horticulture production provides a platform for long term prosperity for communities, supports the growth of knowledge-intensive agri-tech and suppliers along the supply chain; and plays a key role in helping to achieve New Zealand's climate change objectives.

The horticulture sector plays an important role in food security for New Zealanders. Over 80% of vegetables grown are for the domestic market and many varieties of fruits are grown to serve the domestic market.

HortNZ's purpose is to create an enduring environment where growers prosper. This is done through enabling, promoting, and advocating for growers in New Zealand.



# Submission

## 1. Comments on proposed changes

HortNZ understand that MPI are seeking to remove requirements for testing by biological indexing from the below plant germplasm standards to enhance the accuracy of diagnostic screening of pests on imported plants and to increase capacity of level 3B post-entry quarantine (PEQ) facilities.

- *Actinidia Plants for Planting*
- *Citrus Plants for Planting*
- *Importation of Nursery Stock*
- *Prunus Plants for Planting*
- *Seeds for Sowing.*

MPI proposes to use polymerase chain reaction (PCR) and the enzyme-linked immunosorbent assay (ELISA) instead of biological indexing.

MPI also proposes to remove 26 regulated pests from the plants for planting standards as they have determined the scientific literature does not indicate these species pose a significant biosecurity risk to New Zealand.

We support the proposed removal of biological indexing in favour of the updated diagnostic testing methods of PCR and ELISA and do not oppose the removal of the 26 species from the standards.

## 2. Biological indexing, PCR and ELISA

HortNZ recognises that the purpose of biological indexing has been to use a susceptible indicator plant to ensure that any imported or domestically grown host plants are pathogen and disease free (Legrand, 2015).

HortNZ also recognises the New Zealand biosecurity system regularly utilises other tools such as the molecular diagnostic testing methods of PCR and ELISA. These tools are considered sufficient to accurately identify many exotic pests and diseases on imported plants and biological material (MPI, 2023). PCR is an essential diagnostic tool in New Zealand with most validated and accredited diagnostic tests for plant viruses and viroids on germplasm entering New Zealand being PCR assays (Delmiglio et al. 2023). International evidence supports the efficacy and continued use of ELISA for the detection of numerous biosecurity pests e.g. plant pathogens such as tomato brown rugose fruit virus (DAFF, 2019) and tomato ringspot virus (Romaine et al. 1981).

## 3. Limitations of biological indexing

Biological indexing can be an effective method of determining host plant infection but relies upon specific indicator plants that have been selected to assist in identifying disease expression (SCE, 2022). The selected indicator plants are generally susceptible

species or varieties that develop symptoms following pathogenic inoculation (Smith, 1977).

HortNZ acknowledges that while biological indexing procedures have improved over time due to advancements in growth media, fertigation, lighting systems and greater numbers of host plant cultivars to capture unrecognised graft-transmissible pathogens (Roistacher, 1991), it has several disadvantages compared to molecular techniques e.g., PCR, which are continually improving in sensitivity, accuracy, and reliability (Bustin, 2017; Roche Diagnostics 2023; Zhu et al. 2020).

HortNZ in principle supports MPI's proposal to change diagnostic testing from biological indexing to PCR and ELISA, however we request clarity from MPI on the statement in the risk management proposal "*newer ELISA and PCR tests are now able to sufficiently detect the pests listed*" (MPI, 2023). How confident are MPI that the molecular techniques of ELISA and PCR will accurately and consistently identify regulated pathogens and diseases on imported host plants? Can MPI provide information on test sensitivity e.g., 99 percent or is it highly variable depending on the host and pathogen species?

HortNZ notes the following rationale for changing the plant germplasm standard testing requirements from biological indexing to molecular:

### **Efficacy and environmental influence of results**

It is important to acknowledge that no diagnostic testing method for plant pathogens and diseases is perfect and without the potential risk of environmental factors leading to false-negatives or false-positive results.

Biological indexing can be adversely influenced by a range of factors e.g., analytical specificity, analytical sensitivity, and reliability (repeatability and reproducibility) (Legrand, 2015). Environmental factors such as role of inoculum source, choice of indicator plant, expression of symptoms, number of repetitions, use of positive and healthy controls, differentiation of strains, and means of pathogen transmission may also influence results (Legrand, 2015). Climate can also heavily influence biological indexing. For example, between 2007-2009, grape indicator plants infected with different viruses appeared asymptomatic in a cold climate however, appeared symptomatic in a hot climate (Constable et al. 2012).

For molecular diagnostic tools such as PCR and ELISA, the reagents e.g., assay plate, buffers, enzyme conjugate and substrate can produce erroneous results when contaminated, improperly handled, or stored (MyBioSource, 2023). However, the accuracy of pathogen detection using molecular techniques is considered far greater than biological indexing due to the high specificity of identifying an organism to a molecular level (Moslemkhani et al. 2016).

PCR and ELISA are the primary diagnostic tools used globally as they have several advantages over other testing methods for detecting plant viruses such as enhanced sensitivity, speed, economy, and efficiency (Clark et al. 1986).

### **Physical requirements**

Biological indexing requires an extensive area to physically contain host indicator plants. Currently one third of Level 3B PEQ in New Zealand is designated for biological indexing indicator plants (MPI, 2023). The physical requirements for biological indexing hinders New Zealand's ability to import greater quantities of imported plants including

new cultivars and varieties. The proposal to remove biological indexing will remove the need to have space set aside in Level 3B PEQ for growing indicator plants and therefore, free up space for more imported plants.

### **Scalability**

Biological indexing is generally considered impractical for large-scale testing and is heavily labour intensive (Huttinga, 1996; Singh & Ready, 2003). Molecular diagnostic testing is comparably a more efficient and advantageous option given that small quantities of biological material e.g., segments of DNA from host plants are sufficient to determine pathogenicity (Bhattacharya, 2020) and different parts e.g., roots and leaves of the host plant may be diagnostically analysed (Athman et al. 2014).

### **Timing requirements**

The length of time required to observe and successfully identify a biosecurity pest is highly dependent on the plant pathogen, hosts, and diagnostic technique. Up to 9-12 months is required to grow host indicator citrus plants for biological indexing in a greenhouse using standard protocols (Roistacher, 1991). Lemon and mandarin species inoculated with huanglongbing required 4-7 months before symptoms were visually observable due to disease latency (Razi et al. 2012). Woody plants that require graft transmission assays for the detection of viruses may require continued observation for at least two years before plant hosts can be determined as pathogen free (Martin & Tzanetakis, 2014). The inconsistency associated with the time required for biological indexing to yield positive results is well documented.

Conversely, molecular techniques such as reverse transcription PCR can detect asymptomatic infections of ASBVd in avocado in a few days compared to 8-13 months for bioassays (Schnell et al. 1997). An ELISA test is generally rapid (20-60 minutes) and can detect viruses in asymptomatic host plants (ELISA Technologies, 2023; UNL, 2023).

## **4. A combination of diagnostic tests works best**

Legrand (2015) reviewed a comparison of results between biological indexing with those from serological and molecular testing and highlighted the limitations of detection and identification of many pathogens when using biological indexing. Legrand (2015) recommended that while biological indexing can assist in the detection of pathogens, it should only be used in conjunction with molecular techniques for the identification of plant pathogens.

HortNZ supports the proposal to replace biological indexing with more efficient and accurate molecular techniques of PCR and ELISA where appropriate. We acknowledge that, on a case-by-case basis and particularly when plant disease identification is difficult, a combination of diagnostic techniques may yield the most conclusive results in the identification of pathogens and diseases. In some specific situations, this may include biological indexing.

## **5. Removal of pests from import health standards**

MPI proposes to remove 26 pests from the plants for planting standards as they have determined these pests do not pose a significant biosecurity risk to New Zealand.

## **Experimental pests on *Solanum tuberosum* pest list**

HortNZ note that MPI also proposes to remove 13 experimental pests from the pest list for *Solanum tuberosum* as these have biological indexing listed against them and there is no evidence to support their natural infection of *S. tuberosum* outside of laboratory conditions (MPI, 2023). While we do not oppose the removal of these 13 pests from the *S. tuberosum* pest list, we would appreciate further conversations with MPI to better understand how experimental pests are treated across other Import Health Standards.

## **Pests on plants for planting standards**

HortNZ notes that MPI have reassessed the biosecurity risk of 13 pests in the *Importation of Nursery Stock* and *Prunus for Planting* standards stating that the risk of the pests has changed since the measures were originally added to the standards (MPI, 2023). MPI provides a list of reasons for the removal of these pests from the pest list. In general, the pests that are being proposed for removal are not clearly defined, host-association is extremely limited or, there is a scarcity of evidence in scientific literature to support the pests having a significant biosecurity risk to New Zealand.

While we do not oppose removal of these pests, HortNZ requests clarity from MPI on whether the biosecurity risk of the 13 pests has indeed changed or rather MPI's approach for including or excluding pests has changed. It does not seem like there is significant new information to base the decision on, rather a new way of considering or interpreting the information (or lack of information) to make a decision, compared to when the pest list was originally drafted.

## **6. Conclusion**

We support the proposed removal of biological indexing in favour of the currently used diagnostic testing methods of PCR and ELISA and do not oppose the removal of the 26 species from the standards.

## **7. References**

Athman, A., Tanz, S.K., Conn, V.M., Jordans, C., Mayo, G.M. Ng, W.W., Burton, R.A., Conn, S.J. and Gilliham, M. 2014. Protocol: a fast and simple *in situ* PCR method for localising gene expression in plant tissue. *Plant Methods*. 10: 29.

<https://plantmethods.biomedcentral.com/articles/10.1186/1746-4811-10-29>

Accessed: 21 April 2023.

Bhattacharya, T.K. 2020. Meat Quality Analysis. Chapter 20 - Application of genomics tools in meat quality evaluation. *Advanced Evaluation Methods, Techniques and Technologies*. Pg 369-389.

<https://www.sciencedirect.com/science/article/abs/pii/B9780128192337000203>

Accessed: 21 April 2023.

Bustin, S.A. 2017. How to speed up the polymerase chain reaction. *Biomolecular Detection and Quantification*. June 2017. 12:10-14.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5496742/>

Accessed: 21 April 2023.

Clark, M.F., Lister, R.M. and Bar-Joseph, M. 1986. ELISA Techniques. *Methods in Enzymology*. 118: 742-766.

<https://www.sciencedirect.com/science/article/abs/pii/0076687986181146>

Accessed: 19 April 2023.

Constable, F.E., Connellan, J., Nicholas, P. and Rodoni, B.C. 2012. The reliability of woody indexing for detection of grapevine virus-associated diseases in three different climatic conditions in Australia. *Australian Journal of Grape and Wine Research*. Short Research Paper.

<https://onlinelibrary.wiley.com/doi/full/10.1111/j.1755-0238.2012.00204.x>

Accessed: 21 April 2023.

Department of Agriculture, Fisheries and Forestry (DAFF). 2019. Emergency measures for tomato and capsicum seed commencing 5 March 2019.

<https://bicon.agriculture.gov.au/BiconWeb4.0/ViewElement/Element/Alert?elementPk=990138>

Accessed: 19 April 2023.

Delmiglio, C., Waite, D.W., Lilly, S.T., Yan, J., Elliott, C.E., Pattemore, J., Guy, P.L. and Thompson, J.R. 2023. New virus diagnostic approaches to ensuring the ongoing plant biosecurity of Aotearoa New Zealand. *Viruses*. 15: 418.

[https://www.researchgate.net/publication/368243425\\_New\\_Virus\\_Diagnostic\\_Approaches\\_to\\_Ensuring\\_the\\_Ongoing\\_Plant\\_Biosecurity\\_of\\_Aotearoa\\_New\\_Zealand](https://www.researchgate.net/publication/368243425_New_Virus_Diagnostic_Approaches_to_Ensuring_the_Ongoing_Plant_Biosecurity_of_Aotearoa_New_Zealand)

Accessed: 18 April 2023.

ELISA Technologies. 2023. FAQs: In-House Analytical Testing.

[https://www.elisa-tek.com/faqs/#:~:text=Product%20%23510821\).-,How%20long%20does%20testing%20take%20and%20how%20many%20samples%20can,two%20and%20a%20half%20hours.](https://www.elisa-tek.com/faqs/#:~:text=Product%20%23510821).-,How%20long%20does%20testing%20take%20and%20how%20many%20samples%20can,two%20and%20a%20half%20hours.)

Accessed: 21 April 2023.



Huttinga, H. 1996. Sensitivity of indexing procedures for viruses and viroids. *Advances in Botanical Research*. 23: 59-71.

[https://books.google.co.nz/books?hl=en&lr=&id=3we8eM3govAC&oi=fnd&pg=PA59&dq=Sensitivity+of+indexing+procedures+for+viruses+and+viroids&ots=6jvJNyobmk&sig=KKezVrwZ5nhDpy6nOTgBHsoi3oU&redir\\_esc=y#v=onepage&q=Sensitivity%20of%20indexing%20procedures%20for%20viruses%20and%20viroids&f=false](https://books.google.co.nz/books?hl=en&lr=&id=3we8eM3govAC&oi=fnd&pg=PA59&dq=Sensitivity+of+indexing+procedures+for+viruses+and+viroids&ots=6jvJNyobmk&sig=KKezVrwZ5nhDpy6nOTgBHsoi3oU&redir_esc=y#v=onepage&q=Sensitivity%20of%20indexing%20procedures%20for%20viruses%20and%20viroids&f=false)

Accessed: 19 April 2023.

Legrand, P. 2015. Biological assays for plant viruses and other graft-transmissible pathogens diagnoses: a review. *Bulletin OEPP. EPPO Bulletin*. 45(2): 240-251.

<https://onlinelibrary.wiley.com/doi/10.1111/epp.12222>

Accessed: 18 April 2023.

Martin, R.R. & Tzanetakis, I.E. 2014. Pathogen-Tested Planting Material. *Encyclopedia of Agriculture and Food Systems*. 304-312.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7099661/>

Accessed: 19 April 2023.

Ministry for Primary Industries (MPI)roche. 2023. Risk Management Proposal: Amendments to the import health standards *Actinidia Plants for Planting, Citrus Plants for Planting, Importation of Nursery Stock, Prunus Plants for Planting and Seeds for Sowing*.

<https://www.mpi.govt.nz/dmsdocument/56419-Risk-Management-Proposal>

Accessed: 18 April 2023.

Moslemkhani, C., Ravari, S.B. and Khodaygan, P. 2016. Problems encountered with nested PCR to diagnosis of phytoplasmas. *Iranian Plant Protection Research*. 29:4:4. 624-917.

[https://jpp.um.ac.ir/article\\_35990.html?lang=en](https://jpp.um.ac.ir/article_35990.html?lang=en)

Accessed: 20 April 2023.

MyBioSource. 2023. Factors affecting the accuracy and reliability of ELISA results.

<https://www.mybiosource.com/learn/factors-affecting-the-accuracy-and-reliability-of-elisa-results/>

Accessed: 21 April 2023.

Razi, M.F., Khan, I.A., Jaskani, M.J. and Basra, S.M.A. 2012. Graft transmission and biological indexing of huanglongbing on local citrus germplasm. *Science International (Lahore)*. 24(2): 153-157.

<https://swfrec.ifas.ufl.edu/hlb/database/pdf/00002971.pdf>

Accessed: 19 April 2023.

Roche Diagnostics. 2023. Innovation in PCR.

<https://diagnostics.roche.com/global/en/article-listing/pcr-innovation-is-in-our-dna.html>

Accessed: 21 April 2023.

Roistacher, C.N. 1991. Graft transmissible diseases of citrus. *Handbook for Detection and Diagnosis*. F.A.O. Rome (1991).

[https://books.google.co.nz/books?hl=en&lr=&id=9zY1uFGchAgC&oi=fnd&pg=PR3&ots=GouxMbd26s&sig=hl25KCiSHlu9KpabvG7FEfGhkrU&redir\\_esc=y#v=onepage&q&f=false](https://books.google.co.nz/books?hl=en&lr=&id=9zY1uFGchAgC&oi=fnd&pg=PR3&ots=GouxMbd26s&sig=hl25KCiSHlu9KpabvG7FEfGhkrU&redir_esc=y#v=onepage&q&f=false)

Accessed: 19 April 2023.

Romaine, C.P., Newhart, S.R. and Anzola, D. 1981. Enzyme-linked Immunosorbent Assay for Plant Viruses in Intact Leaf Tissue Disks. *The American Phytopathological Society. Plant. Phytopathology*. 308-312.

[https://www.apsnet.org/publications/phytopathology/backissues/Documents/1981Articles/Phyto71n03\\_308.pdf](https://www.apsnet.org/publications/phytopathology/backissues/Documents/1981Articles/Phyto71n03_308.pdf)

Accessed: 19 April 2023.

Scholarly Community Encyclopedia (SCE). 2022. Plant Viral Disease Detection.

<https://encyclopedia.pub/entry/21490>

Accessed: 20 April 2023.

Schnell, R.J., Kuhn, D.N., Ronning, C.M., and Harkins, D. 1997. Application of RT-PCR for indexing avocado sunblotch viroid. *The American Phytopathological Society. Plant Disease*. 1023-1026.

<https://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS.1997.81.9.1023>

Accessed: 19 April 2023.

Singh, R.P. & Ready, K.F.M. 2003. Biological Indexing. *Viroids*: 89-94. ISBN: 0643067892.

<https://www.cabdirect.org/cabdirect/abstract/20033106053#:~:text=Biological%20indexing%20or%20bioassay%20was,reliably%20distinguished%20from%20other%20pathogens.>

Accessed: 19 April 2023.

Smith, K.M. 1977. Testing for viruses: Indicator plants. In Plant Viruses; Smith, K.M. Ed: Springer: Dordrecht, The Netherlands. Pg 175-180.

University of Nebraska-Lincoln (UNL). 2023. ELISA testing.

[https://cropwatch.unl.edu/potato/elisa\\_testing](https://cropwatch.unl.edu/potato/elisa_testing)

Accessed: 19 April 2023.

Zhu, H., Zhang, H., Xu, Y., Lassakova, S., Korabecna, M. and Neuzil, P. 2020. PCR past, present and future. Biotechniques. doi: 10.2144/btn-2020-0057.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7439763/>

Accessed: 19 April 2023.