Ministry for Primary Industries
Manatū Ahu Matua

23 April 2020

Kia ora,

Subject: Consultation on the proposed changes to remove the Apiaceae schedule from Import Health Standard (IHS) 155.02.05: ***Seeds for Sowing***

The Ministry for Primary Industries (MPI) invites feedback on proposed changes to import requirements in the IHS 155.02.05: *Seeds for Sowing*. You are being contacted as a stakeholder with a possible interest in the following matter and are invited to comment on the proposed changes detailed below:

- Removal of the Apiaceae schedule from the IHS 155.02.05: *Seeds for Sowing*.

Please note that the proposed changes will not prevent the importation of Apiaceae seeds for sowing.

This letter includes the following information:

- The MPI assessment for the proposed changes to import requirements (Appendix 1).
- The MPI technical advice on "Review of evidence for seed transmission of liberibacter on carrot seed" (Appendix 2).
- Proposed changes to the IHS 155.02.05: *Seeds for Sowing* (Appendix 3).

MPI seeks comment on the proposed changes by close of business on Thursday, 28 May 2020. Any queries and all submissions should be directed to PlantImports@mpi.govt.nz.

Yours sincerely,

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Appendix 1

Assessment of proposed changes to remove the Apiaceae schedule from the IHS 155.02.05: ***Seeds for Sowing***

Background

1. The Apiaceae schedule currently includes phytosanitary measures to manage the risk of a bacterium '*Candidatus* Liberibacter solanacearum' (Lso) on the import pathway of Apiaceae seeds for sowing (including carrot, celery, chervil, fennel, parsley and parsnip). The reasons for the current phytosanitary measures are outlined below.

2. Lso is a phloem-limited bacterium associated with several vegetative disorders in the Apiaceae, Polygonaceae and Solanaceae families (Munyaneza et al. 2010; Haapalainen et al. 2020). The bacterium has been associated with damage to commercial crops of these families, resulting in significant yield reduction and economic loss (Soukaina et al., 2019).

3. New Zealand produces vegetable seeds for domestic use and export markets, notably radish and carrots. New Zealand supplies nearly 40% of the world production of carrot seed, with the export revenue over $30 million in 2018 (Fresh Facts, 2018). Since New Zealand is one of the major producers of carrot seeds for global trade, Lso could have adverse effects on New Zealand horticulture and vegetable seed industries, if introduced through importation of infected seeds.

4. Lso isolates are currently characterised into nine haplotypes - A, B, C, D, E, F, G, H, and U (Haapalainen et al. 2020). Only haplotype A has been recorded in New Zealand (causing zebra chip in potatoes). Haplotype B (recorded from Solanaceae), and haplotypes C, D, and E (recorded from Apiaceae) are regulated pests in New Zealand, meaning that they are considered to be able to cause unwanted harm in New Zealand.

5. Evidence in one scientific publication and from testing of commercial seed led MPI to conclude in 2017 that imported carrot seed was a risk pathway for the introduction of Lso into NZ.

    a) In 2015, it was reported that Lso was able to be transmitted from carrot seed to carrot seedlings (Bertolini et al., 2015),

    b) In 2016, MPI detected Lso Haplotype D in a consignment of carrot seeds imported into New Zealand for re-exporting to Australia. Following this detection, MPI added a new schedule for Apiaceae seeds in the IHS: *Seeds for Sowing*, as an urgent amendment under the Biosecurity Act s24B(2), in June 2017. This amendment was considered as a provisional measure[1]. Several consignments of carrot seeds contaminated with haplotype D have been intercepted at the border from 2017 to 2020.

6. The Apiaceae schedule requires that all imported seed lots must be accompanied by a phytosanitary certificate with an additional declaration for Lso haplotypes C, D and E. The additional declaration must state that:

    a) the seeds have been sourced from a country free from Lso haplotypes C, D and E; or

[1] A provisional measure is defined in the International Standards for Phytosanitary Measures (ISPM) 5 as '*A phytosanitary regulation or procedure established without full technical justification owing to current lack of adequate information. A provisional measure is subjected to periodic review and full technical justification as soon as possible*.'

b) a representative sample of 10,000 seeds has been sampled and tested for this bacteria (onshore or offshore) and found free; or

c) the seed lot has been treated with hot water at 50°C for at least 30 continuous minutes.

7. This provisional measure was subject to review and full technical assessment within a reasonable period, as per Article 5.7 of the WTO-SPS agreement[2].

Discussion of new evidence

8. New scientific evidence does not support the finding that Lso is seed transmitted on the Apiaceae family (MPI Technical Advice, 2020, Appendix 2). As a result, MPI no longer considers Apiaceae seed to be a risk pathway for introduction of Lso. This conclusion has been made based on the following:

    a) The finding of Bertolini et al. (2015), indicating transmission of Lso haplotype E from seed to seedling in carrot, was not able to be repeated by further scientific studies:

        i. The seed transmission of Lso haplotypes D and E in carrot was not confirmed using the same seed lots as Bertolini et al. (2015) (Loiseau et al. (2017a). The authors suggested that the discrepancy in results could be due to differing agronomic conditions.

        ii. Loiseau et al., (2017b) confirmed the results obtained in their first experiment despite using the growing conditions similar to those described by Bertolini et al (2015).

        iii. Lso was not detected in the carrot crops grown from seed lots that were known to be infected with Lso haplotype D (Haapalainen et al., 2018). This result is consistent with the results of studies by Loiseau et al (2017a, 2017b) which found no evidence for seed-to-seedling transmission of Lso by carrot seeds.

    b) Research indicates that Lso is vector-transmitted[3] rather than seed-transmitted.

        i. Detection of Lso only from seed coats (Bertolini et al., 2015) provides evidence that Lso is transmitted by its known vector, psyllids. When psyllids feed on mature seeds, they introduce Lso to phloem tissue of seed coat (MPI Pest Risk Assessment, 2105; Monger and Jeffries, 2016). Seed transmitted pathogens should be detected from the embryo of seeds (Singh and Mathur, 2004).

Conclusion

1. MPI proposes to remove the Apiaceae schedule from the IHS, because:

    a) there is insufficient evidence that seed to seedling transmission of '*Candidatus* Liberibacter solanacearum' occurs in species of Apiaceae; and

[2] Article 5.7 of the World Trade Organisation - Sanitary and phytosanitary (WTO-SPS) agreement states '*In cases where relevant scientific evidence is insufficient, a Member may provisionally adopt sanitary or phytosanitary measures on the basis of available pertinent information, including that from the relevant international organizations as well as from sanitary or phytosanitary measures applied by other Members. In such circumstances, Members shall seek to obtain the additional information necessary for a more objective assessment of risk and review the sanitary or phytosanitary measure accordingly within a reasonable period of time*'.

[3] Lso is known to be transmitted by psyllids.

b) the additional measures for the importation of Apiaceae family introduced in June 2017 is no longer technically justified.

2. Following the removal of Apiaceae schedule from the Seeds for Sowing IHS, the import specification of all Apiaceae seeds for sowing species listed in the Plant Biosecurity Index (PBI) will be altered to "Basic".

3. MPI will continue to monitor research findings related to seed transmission of Lso.

References

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Appendix 2

Technical advice on: Review of evidence for seed transmission of liberibacter on carrot seed.

Date: 28 February 2020

Purpose of document

The Plant Germplasm Imports team has requested that the evidence for seed transmission of '*Candidatus* Liberibacter solanacearum' for carrot and other Apiaceae be reviewed.

Background

'Candidatus Liberibacter solanacearum' (Lso) is an unculturable bacterium that lives in the phloem of its plant hosts causing disease on plants in the families Solanaceae and Umbelliferae. It is vectored by psylloids[4] which feed on the phloem contents.

Several haplotypes (A, B, C, D, and E and others) have been recognised within Lso. Haplotype A (Lso A) is present in New Zealand in Solanaceae (Liefting et al, 2009). New Zealand is free of all other known Lso haplotypes, including those that affect Apiaceae (e.g., carrot, *Daucus carota (*Munyaneza et al, 2010)), and these haplotypes are regulated (BORIC[5]). *Bactericera cockerelli*, the vector of Lso haplotypes on solanaceous species, is present in New Zealand. However, the reported vectors for Lso C, D, E and others on Apiaceae, including *Trioza apicalis* and *Bactericera trigonica*, are not known to be present in New Zealand (MPI, 2015; not listed in PPIN, 2020).

In 2015, a single publication (Bertolini et al, 2015) reported seed transmission of Lso in carrot. A subsequent Pest Risk Assessment (MPI, 2015) concluded that if Lso was seed transmitted in Apiaceae then it would be a risk to New Zealand; however, there was insufficient evidence to that date that this pathway (seed to seedling transmission in Apiaceae) occurs. Uncertainty (unquantified) remained because the evidence for seed transmission had not been independently verified, with the issue still under discussion and investigation by international researchers.

Since 2015 other studies on seed to seedling transmission of Lso on carrot have been published. The evidence for seed to seedling transmission of Lso in Apiaceae is re-evaluated here taking these publications into account.

Summary of advice

There is insufficient evidence that seed to seedling transmission of '***Candidatus*** Liberibacter solanacearum' occurs in carrot or other species of Apiaceae.

A single publication has reported seed transmission of Lso in carrot (Bertolini et al, 2015).

However, this publication was insufficient on its own to clearly demonstrate that seed transmission occurs on this pathway for several reasons: it had not been independently verified; the PCR primers used to detect Lso in seed and leaf midrib tissue are capable of detecting a wide range of other bacteria; DNA sequences from the study are unavailable (e.g. through GenBank) for analysis by other researchers; and transmission electron

[4] Psylloids are members of the superfamily Psylloidea. The superfamily contains several families including Psyllidae and Triozidae. Members of the Psyllidae (e.g., Diaphorina citri) are known as psyllids and members of the Triozidae (e.g., Trioza erytreae) are known as triozids.

[5] https://www.mpi.govt.nz/news-and-resources/resources/registers-and-lists/biosecurity-organisms-register-for-imported-commodities

microscopy cannot be used alone as a diagnostic tool for liberibacter without diagnostic DNA sequence information.

Other more recently published studies have investigated seed transmission of Lso in Apiaceae. However, neither of these was able to demonstrate seed transmission of Lso in carrot. Loiseau et al (2017b) in an independent laboratory used the same three lots of seed as Bertolini et al (2015). The authors suggested the discrepancy could be due to differing agronomic conditions. An additional study was conducted by Loiseau et al (2017a) which confirmed the results obtained in their first experiment despite using the growing conditions described by Bertolini et al (2015). Other hypotheses may explain the results obtained by Bertolini et al (2015).

Supporting information

Original report of seed transmission

The study by Bertolini et al (2015) suggested that Lso is transmitted by seed in carrot. The authors developed a real-time PCR protocol for the specific detection and quantification of Lso in carrot seeds. This was used to detect Lso in carrot seed lots including those collected from diseased mother plants. Lso was also detected post-germination in seedlings grown from PCR positive seed lots in an insect-proof PC2 glasshouse. It was detected as early as 30 days post-germination, but more consistently after 90 days. Some of the positive samples were sequenced by the authors and determined to be Lso haplotype E based on the 16S and 50S rRNA genes. Electron microscopy was used to show the presence of BLOs (bacteria-like organisms) in the phloem sieve tubes of the seed coat the phloem of the carrot mid-rib seedlings grown from positive seed.

The MPI pest risk analysis (MPI 2015) considered the Bertolini et al (2015) publication when reaching the conclusion that there was insufficient evidence that seed to seedling transmission occurs in Apiaceae. The following is taken from the pest risk analysis (MPI, 2015):

> A recent study by Bertolini et al. (2014[6]) suggests that Lso is transmitted by seed in carrot. However, this remains to be confirmed by an independent laboratory or further studies. The real-time PCR assay used in this study can potentially amplify a wide range of other bacteria that may be present as environmental contamination. Positive samples were sequenced by the authors and determined to be Lso haplotype E based on the 16S and 50S rRNA genes. However, the sequences were not deposited in GenBank (or a similar sequence database) which is a standard requirement of publishing, and as a result are not readily available for analysis. Although electron microscopy showed the presence of BLOs (bacteria-like organisms) in the phloem sieve tubes of the seed coat and in the phloem of the carrot mid-rib seedlings grown from positive seed, this result is inconclusive as liberibacters cannot be diagnosed from morphology only. Seed transmission has not yet been clearly demonstrated for other liberibacter species (personal communication, Lia Liefting, October 2014; Hilf et al. 2013, Hilf 2011, van Vuuren et al. 2011).

Additional studies of seed transmission

Other more recently published studies have investigated seed transmission of Lso in Apiaceae (Loiseau et al, 2017a, b). However, neither of these was able to demonstrate seed transmission of Lso in carrot.

Loiseau et al (2017b) in an independent laboratory used the same three lots of seed as Bertolini et al (2015). Their study in France was carried out in parallel with the Spanish laboratory (Bertolini et al, 2015) to show that seed transmission is reproducible. However, they were unable to demonstrate the transmission of Lso by carrot seeds and their results were not consistent with those of Bertolini et al (2015). The authors suggested

[6] Bertolini et al (2015) first became available in 2014 as an electronic publication.

that one of the most probable hypotheses for the discrepancy could be due to differing agronomic conditions. They had subjected their seedlings to an overwintering regime to produce seeds and this could have inhibited the development of the bacteria. The authors noted there are other possible hypotheses such as cross-contamination.

An additional study was conducted by Loiseau et al (2017a) which confirmed the results obtained in their first experiment despite using the growing conditions equivalent to those described by Bertolini et al (2015). These authors concluded: 'If the transmission of 'Ca. L. solanacearum' by carrot seed is at all possible, it is probably rare and difficult to reproduce.' In this study the plants were grown for 6 months in an insect-proof greenhouse. Sets of plants from the contaminated lots and from the healthy lots were individually analysed each month using real-time PCR to detect Lso. Tests on seeds and plants from healthy lots were always negative. During the 6 months of the trial, no plants from the contaminated seed lots tested positive for Lso or showed any typical infection symptoms.

During the course of the study, a small number of inconclusive results from the PCR assays were obtained from the third month samples to the sixth month (Loiseau et al, 2017a). In these cases, the detection of the bacterium was repeated on those carrots that gave inconclusive results. No positive results were obtained on the stored crude extracts or on new samples of the same plants. In addition, the plants that gave inconclusive results during any one sampling period were not found to give inconclusive results in any other sampling period. As a result, the nine inconclusive results obtained during the study were considered by the authors to be micro-contaminations during DNA extraction or amplification even though positive and negative controls in both steps were clear.

This and the previous study (Loiseau et al 2017b) used the real-time PCR primers developed by Li et al (2009) rather than those used in Bertolini et al (2015). Real-time PCR primers used by Li et al (2009) were found to be the most sensitive and robust of those tested in an evaluation by Ilardi et al (2019) of a diagnostic protocol for Lso in carrot seeds. The primers used by Teresani et al (2014), which are the same as those used by Bertolini et al (2015), were included in their evaluation.

In Japan, a comparison of protocols to detect Lso in carrot seeds was undertaken by Oishi et al (2017). Their study used real-time PCR to evaluate 1,438 seedlings grown from five Lso positive seed lots and seed transmission of Lso was not observed. This finding supports those of Loiseau et al (2017a, b) but this paper has not been assessed thoroughly here as the full text was not in English.

The positive results obtained by Bertolini et al (2015) were most likely due to either cross-contamination during DNA extraction or PCR amplification and/or due to non-specific amplification of organisms closely related to liberibacters.

Additional observations

Haapalainen et al (2018) investigated the occurrence of Lso haplotypes in various psyllids and plant hosts in Finland. Samples included carrot seeds used for carrot production by commercial carrot growers. The commercial growers use imported carrot seeds since there is no domestic carrot seed production in Finland. Plants (carrot, parsnip, and wild plants growing as weeds in and next to carrot fields) and psyllids feeding on these plants were also sampled. At the time of the study, only Lso haplotype C was known to occur in Finland. Lso haplotype D was confirmed in 2 of the 34 imported carrot seed lots tested. Plants grown in the field from the infected imported seed lots were tested at the end of the season. Haplotype D was not detected in these plants nor had it been detected in any other plants or psyllids in Finland. Haapalainen et al (2018) stated that their results agreed with the Loiseau et al (2017a) study which found no evidence for vertical transmission of Lso by carrot seeds. The authors also noted the possibility that haplotype D or its vector might not suit the cool conditions found in Finland. However, if it was able to survive and spread then it would be expected to be found in carrots in Finland because field-scale cultivation of carrots had occurred there since the late nineteenth century.

Monger and Jeffries (2018) carried out a survey of seed of carrot and related Apiaceae species held in three seed collections in the United Kingdom. Accessions went back as far as the 1970s and the seed had been sourced worldwide. They found that a high proportion of commercial carrot seed lots from European sources were infected with Lso dating back to 1973; 74% of European carrot from the 1970s and 63% from the 1980s and 90s were positive for Lso indicating that it has been present in European crops for many years and at high levels. Given the international trade in Apiaceae seed, it is expected that Lso positive seed would have been exported worldwide. However, the incidence of Lso in the seeds in the study showed a clear distinction between the European-Mediterranean region and the rest of the world with few findings in seed from countries in the latter category. The authors conclude that Lso is not an emerging pathogen in European Apiaceae crops but rather has gone unnoticed and undetermined for a long time and reaches economic levels when vector populations increase under favourable environmental conditions to cause epidemics.

In addition, there is no evidence that Lso is seed transmitted through true seed from infected solanaceous plants (potato, tomato, pepper, tamarillo) (Munyaneza 2012; Liefting, personal communication).

Nor is there sufficient evidence that other liberibacter species are seed transmitted. In particular. '*Candidatus* Liberibacter asiaticus', associated with huanglongbing disease of citrus, has been the subject of much research effort in this area. Older studies suggesting seed transmission have not been supported by more recent more robust studies (e.g., Hartung et al, 2010; Hilf et al. 2013, Hilf 2011). Alternative explanations such as PCR contamination or reliance on non-specific symptoms can explain results of earlier studies.

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Appendix 3

It is proposed to remove Apiaceae schedule from the IHS 155.02.05: *Seeds for Sowing*

~~*2.7 Apiaceae*~~

~~The following requirements only apply to species in the Plant Biosecurity Index listed under Import Specifications for Seed as "see 155.02.05 under *Apiaceae*".~~

~~Approved countries: All~~

~~Quarantine pests: '*Candidatus* Liberibacter solanacearum' haplotypes C, D and E~~

~~Import permit: Not required~~

~~PEQ: Not required~~

~~Phytosanitary certificate: Required~~

~~Approved treatment~~

~~If the treatment option is selected:~~

~~(1) Each seed lot must be treated using a hot water dip, for the treatment of bacterial organisms ('*Candidatus* Liberibacter solanacearum' haplotypes C, D and E) as per MPI Standard MPI-STD-ABTRT Approved Biosecurity Treatments.~~

~~(2) The hot water treatment is required to be completed offshore prior to export, or on arrival in New Zealand.~~

~~(3) Pre-export treatment for each seed lot must be endorsed by the NPPO in the treatment section on the phytosanitary certificate, where the temperature and time must be clearly stated.~~

~~Phytosanitary certificate – Additional declarations~~

~~(4) The required additional declarations must be endorsed in full on the phytosanitary certificate, no variations in the wording will be accepted by MPI, with exception of translation artifacts.~~

~~(5) The exporting country NPPO must confirm any treatment(s) as required by the IHS in the disinfestation and/or disinfection treatment section.~~

~~(6) If satisfied that the pre-shipment activities have been undertaken, the exporting country NPPO must confirm this by providing the certifying statement as per Part 1.5.2 of this import health standard and also the following additional declaration (s) to the phytosanitary certificate:~~

~~a) '*Candidatus* Liberibacter solanacearum' haplotypes C, D, and E are absent/not known to occur in _____ (name of country).~~

~~OR~~

~~b) the seeds for sowing have been sourced from a seed lot officially sampled according to ISTA or AOSA methodology, and tested using a NPPO approved PCR method and found free from *Candidatus* Liberibacter solanacearum haplotypes C, D and E.~~

~~OR~~

~~c) the seeds for sowing have been treated with hot water at a minimum temperature of 50ºC for at least 20 continuous minutes.~~

Testing Requirements

If the testing option is selected:

(7) Testing is required to be completed offshore prior to export, or on arrival in New Zealand.

(8) Pre-export testing for each seed lot must be endorsed by the NPPO on the phytosanitary certificate, or if tested on arrival in New Zealand, must be completed by an MPI-approved testing laboratory.

(9) A representative sample of a minimum of 10,000 seeds, is required from each seed lot and tested using the real-time PCR assay described by Li *et al*., 2009 to show the consignment is free of '*Candidatus* Liberibacter solanacearum' haplotypes C, D and E.

Guidance:

Refer section 1.11 Seeds of MPI Standard MPI-STD-ATBRT Approved Biosecurity Treatments

References:

Li, W., Abad, J. A., French-Monar, R. D., Rascoe, J., Wen, A., Gudmestad, N. C., Secor, G. A., Lee, I. M., Duan, Y., and Levy, L. 2009. Multiplex real-time PCR for detection, identification and quantification of '*Candidatus* Liberibacter solanacearum' in potato plants with zebra chip. Journal of Microbiological Methods 78:59-65.