



Australian Government

Department of Agriculture, Fisheries and Forestry

Andrew Metcalfe AO
SECRETARY

Ref: EXEC2013-01801

22 May 2013

Senator the Hon. Bill Heffernan
Chair
Rural and Regional Affairs and Transport References Committee
PO Box 6100
Parliament House
CANBERRA ACT 2600

Dear Senator Heffernan

Thank you for your letter of 25 March 2013 advising me of the committee's work on my department's risk assessment methodologies as it relates to the inquiries into:

- The effect on Australian pineapple growers of importing fresh pineapple from Malaysia
- The effect on Australian ginger growers of importing fresh ginger from Fiji
- The proposed importation of potatoes for processing from New Zealand.

In your letter you asked me that all decisions in relation to the importation of pineapples from Malaysia, ginger from Fiji and potatoes for processing from New Zealand be delayed until after the Senate committee has had the opportunity to further explore the issues raised and table its report on 24 June 2013. As you are aware, the department has published final import risk analysis (IRA) reports for pineapple from Malaysia on 14 December 2012 and ginger from Fiji on 22 January 2013. My predecessor wrote to the Senate committee on 14 December 2012 indicating that the department will commence engagement with the Department of Agriculture in Malaysia to develop operational requirements of the pineapple IRA including an audit of its phytosanitary system. A letter on 24 January 2013 informed the Senate committee that a similar process is required for ginger from Fiji.

The department wrote to Malaysia on 7 February 2013, and to Fiji on 5 April 2013, outlining the next steps required, including the development of a 'work plan' by the exporting country for review by the department before trade can commence. The department has not received a 'work plan' from Malaysia or Fiji for review. As you know, no trade will occur until work plans are established to the department's satisfaction and valid import permits can be issued to an Australian applicant. It is unlikely that all operational requirements will be completed by 24 June 2013 to allow imports of pineapple from Malaysia and ginger from Fiji, in the event that import permits are sought.

The review of import conditions for fresh potatoes for processing from New Zealand was issued as a draft report for a 60 day stakeholder comment period on 3 July 2012. The department received 27 submissions on the draft report and continues to assess the issues raised in the submissions, and the latest scientific information, relevant to developing a final report. As you know, the department sought independent expert advice to assist in the development of this policy. Once a final policy position is reached, and if it recommends that imports can occur, operational requirements will need to be developed so any final import conditions can be implemented to Australia's satisfaction. This work is also unlikely to be completed by 24 June 2013.

In your letter you also asked for a copy of the independent bacteriologist report in relation to the review of import conditions for fresh potatoes for processing from New Zealand. I enclose a copy of the report for your information. The department intends to include the independent bacteriologist review as an attachment to the final report for the review of import conditions for fresh potatoes for processing from New Zealand. Since you mentioned our correspondence may be made public, the contact details of the independent bacteriologist have been redacted.

The committee is aware that, as per the evidence provided by the department during various hearings, at any time we can take into account relevant scientific and technical developments that relate to import conditions necessary to protect Australia from pests and diseases of quarantine concern. Any relevant information that is brought to our attention through the committee's inquiries will be considered in the same way. As such, I await with interest the committee's final reports scheduled for tabling on 24 June 2013.

I note the committee's interest in the department's risk estimation matrix including commissioning a review of the matrix by the New Zealand based risk management consultant, Mr Peace.

The department's risk assessment method, including the estimation matrix, is widely used in biosecurity risk assessment activity in Australia. The risk estimation matrix has been used by the department since 2001 and was endorsed at the Primary Industry Ministerial Council on 2 May 2002. It is used by Plant Health Australia and affiliated industries in assessing risk within their Industry Biosecurity Plans (including the potato and pineapple industries; a ginger biosecurity plan is under development). It has also been used by Australian states in assessing the risk of pests potentially associated with the movement of commodities from domestic sources (e.g. Tasmania's risk assessment for fruit flies completed on 31 March 2012).

Australia's risk assessment methods were considered during the World Trade Organization (WTO) dispute on New Zealand apples. Although the WTO found fault with Australia's overly conservative interpretation of risk in the apple IRA, it did not find fault with the matrix itself. The WTO recognised the function of the risk estimation matrix in defining Australia's appropriate level of protection. It has been the policy of successive Australian Governments that Australia's risk assessments are consistent with Australia's WTO obligations. We provided the committee with advice on Mr Peace's report at the hearing of 12 March and by letter on 8 March 2013.

Australia's risk assessment methods continue to develop under the guidance of the Australian Centre of Excellence for Risk Analysis (ACERA). I note that ACERA was an initiative of the former Government announced in its Quarantine and Border Protection election policy of 2004; established in 2006 to ensure Australia stays at the forefront of world's best practice.

ACERA is guided by a Board that includes as chair Dr Ron Sandland ex deputy head of CSIRO; Emeritus Professor Pauline Ladiges former Head of School of Botany, University of Melbourne; Professor Peter Taylor, Head of Department of Mathematics and Statistics, University of Melbourne; Professor Peter Bardsley, Faculty of Economics and Commerce, University of Melbourne; and Dr Roger Paskin from the Victorian Department of Environment and Primary Industries.

Across Australia there is a network of highly qualified practitioners that are available to and work with the department on risk assessment including ACERA, CSIRO (and the recent Biosecurity Flagship), the Eminent Scientists Group and relevant Universities. I will continue to use the expertise available to me to provide the best possible advice. Given my access to world leading experts in this field, I have asked ACERA to conduct a review of Mr Peace's work. I will provide the Senate committee the ACERA advice.

I should add that the department earlier this week reached a formal agreement with the University of Melbourne to establish a successor body to ACERA, to be known as the Centre of Excellence for Biosecurity Risk Analysis (CEBRA). The objectives of CEBRA are to deliver practical and rigorous research solutions and advice related to the assessment, management, perception, and communication of biosecurity risk. The centre builds upon the world leading work undertaken by the ACERA since 2006. Importantly, it will be used to support current reform initiatives as the department continues to focus on delivering a modern biosecurity system that is responsive and targeted.

As noted in my letter of 30 April 2013, I have been familiarising myself with the role the department plays in assessing the potential biosecurity risks associated with imported commodities. I have received substantial briefing on the legal obligations of the Director of Animal and Plant Quarantine and the department with respect to managing imports and the role that IRAs play within that. I have found this useful and thought the same material may also assist the committee (Attachment A).

As you know the Regulated IRA process is set out in the *Import Risk Analysis Handbook 2011*. It is clear to me that this is only a part of the total picture in the department's role in managing imports into Australia. Given that, and the fact that the Handbook refers to a now defunct institution (Biosecurity Australia) and defunct organisational unit (the Australian Quarantine Inspection Service) I am preparing to withdraw the Handbook and make more up-to-date and comprehensive information available about the department's role in managing imports into Australia, including the IRA process.

I hope that this information is of assistance to your committee.

Yours sincerely

(Andrew Metcalfe)

Attachment A:

The legal basis for conducting import risk analyses (IRAs) is in Part 6A of the *Quarantine Regulations 2000*. The occasion for an Import Risk Analysis (IRA) usually arises as a result of an import proposal (a request from another country or an importer to import goods into Australia). Under the *Quarantine Act 1908* and the *Quarantine Proclamation 1998*, in deciding whether to grant an import permit, the Director must consider the level of quarantine risk.

One way of managing the process of assessing the risk is by developing an IRA. The IRA process is used when relevant risk management measures have not been established, or relevant risk management measures for a similar good and pest/disease combination do exist, but the likelihood and/or consequences of entry, establishment or spread of pests and diseases could differ significantly from those previously assessed.

Part 6A of the *Quarantine Regulations 2000* (the Regulations) sets out the steps to be followed if a formal IRA process is used. It is worth noting that the Regulations as they currently stand refer to the 'Chief Executive' and Biosecurity Australia as managing the regulated IRA process. Biosecurity Australia was de-prescribed as a statutory agency from 1 July 2009¹, and its functions in conducting risk analyses are now carried out within the department, in the Biosecurity Plant and Biosecurity Animal Divisions. I have nominated a senior person at First Assistant Secretary level to manage the IRA processes for the department.

The current regulations will be updated and revised as part of the modernisation of the Quarantine Act and its subordinate legislation. Regulations covering the Biosecurity Import Risk Analysis process are scheduled for release shortly.

As they currently stand, the Regulations provide for two types of IRA - a standard IRA and an expanded IRA. However, the Regulations do not confer a power or duty on any person to decide whether there should be an IRA, and if so, whether it should be a standard or expanded IRA. In practice, the Chief Executive of Biosecurity Australia, (as it currently stands in the Regulations), makes these decisions as a matter of administration, and not pursuant to any statutory power.

The standard IRA process has a timeframe of 24 months.² It includes a public notice at the commencement of an IRA, release of the draft IRA report for public comment and release of a provisional final report to communicate the results.³ The release of the provisional final report is the last step in the process set out in the Regulations for both a standard and expanded IRA. As an administrative matter, the provisional final IRA report may be reviewed under a non-statutory review process (where stakeholders may appeal to the Import Risk Analysis Appeals Panel within 30 days of publication of the provisional final IRA report). The report becomes final at the end of the 30 day period, or at the end of the appeal, whichever is the later.

An expanded IRA process has a timeframe of 30 months.⁴ In practice, this expanded process is chosen where there are significant differences in scientific opinion, or where significant harm to humans, animals and plants, or the environment may result from an importation. It

¹ *Financial Management and Accountability Amendment Regulations 2009 (No. 5)*, sch 1, item 3.

² *Quarantine Regulations 2000*, sub-reg 69E(1).

³ *Quarantine Regulations 2000*, sub-regs 69C(1).

⁴ *Ibid*, sub-reg 69E(2).

includes, in addition to the steps for a standard IRA, the (optional) development of an issues paper to be released for public comment before preparation of the draft report, and review of the revised draft IRA report by the Eminent Scientists Group (ESG) before preparation of the provisional final IRA report.⁵

The department also seeks independent input and advice outside of the ESG processes. For example, scientific advice was sought on plum pox virus at a workshop with experts in April 2007 in order to complete the US stone fruit IRA. Experts from the South Australian Research and Development Institute also reviewed the pest risk assessment for *Phomopsis viticola* in regards to the Chilean table grape IRA in 2005.

It is important to note that IRA reports remain valid even if the timeframes stipulated in the Regulations are not met.⁶ This prevents the work and effort taken in preparing and commenting on an IRA being set aside because of a failure to meet a timeframe. However, this does not mean that timeframes should not be adhered to (the department has for the most part met the timeframes). The 'Chief Executive' can by public notice 'stop the clock' where it is essential to obtain further information or commission research or expert advice, or where a significant national or international quarantine circumstance prevents the IRA being completed within the time limits.⁷

An IRA report provides advice to the Director of Animal and Plant Quarantine (and his delegates) to consider in determining if an import can be safely imported into the country and, if not, any conditions that could be imposed in order for it to be safely imported. In considering an IRA report, the Director may provide advice to his delegates that the measures contained in the report may be taken into account in the assessment of risk in deciding whether or not to issue an import permit.

It must be noted that the Director's decision to issue a permit is not the sole determinant of an import being allowed into the country, as imports are also subject to the *Customs Act 1901* and other relevant laws depending on the particular class of import, such as the *Imported Food Control Act 1992*.

The Director may delegate the power to grant import permits under the quarantine proclamations⁸ to a quarantine officer or another officer appointed under the *Quarantine Act 1908*.⁹ In considering the level of quarantine risk and whether conditions should be imposed to limit the quarantine risk to an acceptably low level, the Director, or delegate, may take into account any relevant information, including an IRA. However, the decision to issue an import permit is not dependent on an IRA being conducted or completed.¹⁰

A final IRA report is not a statutory decision to allow or prohibit imports into Australia and is not reviewable under the *Administrative Decisions (Judicial Review) Act 1977*. In *Director of Animal and Plant Quarantine v Australian Pork Limited* [2005] FCAFC 206, the Full Court of the Federal Court of Australia held that:

⁵ *Ibid*, sub-regs 69C(2), 69E(2).

⁶ *Ibid*, reg 69F

⁷ *Ibid*, sub-reg 69H.

⁸ *Quarantine Proclamation 1998, Quarantine (Christmas Island) Proclamation 2004 and Quarantine (Cocos Islands) Proclamation 2004*.

⁹ *Quarantine Act 1908*, s 10B.

¹⁰ *Quarantine Regulations 2000*, sub-reg 69C(4). Decisions to issue or not issue import permits are reviewable under the *Administrative Decisions (Judicial Review) Act 1977*.

'The Determination [accepting the final IRA report] did not 'authorise' anything. It did not affect anyone's rights or impose obligations...it did no more than put forward matters to be taken into account by the Director in granting permits. There was no jurisdictional error because no statute conferred jurisdiction to make the Determination; it was a purely internal administrative exercise.'¹¹

A final IRA report is not subject to merits review by the Administrative Appeals Tribunal. An IRA can be reviewed by the department at any time in the light of new scientific information on the pest and/or disease risk assessed in the IRA. This is managed at an administrative level through a non-regulated analysis of existing policy and conditions or measures, consistent with the department's obligation to consider new information to ensure national import conditions continue to meet Australia's appropriate level of protection. For example, Australia has the right to take emergency action and impose sanitary or phytosanitary measures as appropriate in response to a newly reported pest, or changed pest status, in a country that exports to Australia. In such circumstances, Australia is then obliged to conduct an objective assessment of the risk and review the sanitary or phytosanitary measure accordingly within a reasonable period of time. A recent example is the pest risk analysis conducted for *Drosophila suzukii* that was detected in North America.

¹¹ *Director of Animal and Plant Quarantine v Australian Pork Limited* [2005] FCAFC 206 (at 85).

13 December 2012

Report to DAFF on the *Draft report for the review of import conditions for fresh potatoes for processing from New Zealand*

and

Recommended quarantine conditions with regard to “*Candidatus Liberibacter solanacearum*”

A. Chris Hayward

(Honorary Reader in Microbiology, School of Chemistry and Molecular Biosciences, the University of Queensland, St. Lucia, 4072, Qld)

Consultant, Bacterial Plant Diseases

[Redacted text block containing several lines of blacked-out text]

Contents

1. Access to the current literature on Zebra Chip disease of potato
2. Entry of the tomato potato psyllid into New Zealand
3. The possible evolutionary origin of “*Candidatus Liberibacter solanacearum*”
4. The tomato potato psyllid: feeding habits and oviposition
5. Chemical and biological methods for control of the insect vector
6. Impact of current literature on zebra chip on quarantine conditions for importation of fresh potatoes for processing from New Zealand
 - i) Tuber transmission
 - ii) Haplotypes of “*Candidatus Liberibacter solanacearum*” and potential vectors
 - iii) Survival of the zebra chip pest external to its insect or plant host
 - iv) Impact of improved diagnostic methods
7. Conclusion

Appendix 1

Current literature on zebra chip of potato and the insect vector
Bactericera cockerelli (December 2012)

Appendix 2

Haplotypes of “*Candidatus Liberibacter solanacearum*”: Hosts, vectors and geographical distribution

1. Access to the current literature on Zebra Chip disease of potato

The literature on the disease is distributed among the scientific periodicals covering plant pathology such as *Phytopathology*, *Plant Disease*, and the *European Journal of Plant Pathology*; those papers primarily concerned with the properties of the insect vector *Bactericera cockerelli* are usually found in the entomological literature in the *Journal of Economic Entomology*, *Environmental Entomology* and *Insect Science*. A few important papers have appeared elsewhere in the *American Journal of Potato Research*, *Crop Protection*, *PLoS ONE*, *Pest Management Science* and *Biological Control*. The journals listed are not the only sources of reference material but most of the research on zebra chip appears within their pages.

An important source of current research information is the proceedings of the Annual Zebra Chip Reporting Session held annually in the United States. The *Proceedings of the 11th Annual Zebra Chip Reporting Session* (Workneh F, Rashed A, Rush C M ed. San Antonio, TX, November 6-9, 2011) have been published and are freely available online. The eight sessions were on the following topics: epidemiology/survey; pathogen/vector management; insect biology/management; resistance/germplasm identification; Zebra chip impact on potato production; host/pathogen interaction; pathogen detection; pathogen/vector management; molecular biology and physiology. The 12th Conference has been held October 30 – November 2, 2012 at the Crowne Plaza, San Antonio, TX. Some conference abstracts are available online. The November issue of *Psyllid News* published by Potatoes New Zealand includes a brief summary of some of the research findings reported at the 12th Conference as well as a valuable account of some of the current New Zealand research and of that being carried out internationally. Two recent conferences provide additional information on zebra chip disease. The World Potato Conference was held in Edinburgh, Scotland, from 27-30 May, 2012 and the proceedings are available online through the following link:

<http://www.potatocongress.org/proceedings-2012/>

The paper presented by Dr Kevin Clayton-Greene gives an assessment of the biosecurity risks to Australia resulting from pest incursion, and that given by Dr. Neil Gudmestad provides an overview of current research on zebra chip and the psyllid vector in North America and elsewhere.

The conference “Psyllid 2012: Tomato Potato Psyllid in New Zealand” was held from 26-27 July, in Auckland, New Zealand, and the proceedings are available online through the following link:

<http://www.potatoesnz.co.nz/Overview/Who-are-we/2012-Potatoes-New-Zealand-Conference.htm>.

All but three of the 30 conference presentations are concerned with current research in New Zealand. Visiting authorities from the USA provided information on the grower experience and research being carried out in North America.

The literature on zebra chip disease is steadily expanding, currently at the rate of about one paper every week. The most recent authoritative review on the subject (Munyaneza 2012a) lists 192 references.

2. Entry of the tomato potato psyllid into New Zealand

The tomato potato psyllid (TPP) *Bactericera cockerelli* was first reported in New Zealand in May, 2006, and is now established throughout the North Island and in most regions of the South Island, including Southland (Thomas et al. 2011). A thorough analysis has been made of the possible entry pathways for this insect pest, with the following conclusions:

“Although a definitive pathway of entry for TPP could not be explicitly identified, this paper has documented the current assessment that (1) New Zealand’s TPP originated from western USA, (2) the original site of establishment in NZ is unclear, (3) the likelihood of introduction on legally imported nursery stock is unlikely, (4) the likelihood of introduction on fresh produce is unlikely (5) the likelihood of introduction by natural dispersal is negligible, and (6) TPP might plausibly have been introduced by the smuggling of primary host material.” (Thomas et al. 2011).

The authors concluded that the most likely pathway of entry of TPP was via smuggling from western USA.

3. The Possible Evolutionary Origin of “*Candidatus Liberibacter solanacearum*”

“*Candidatus Liberibacter solanacearum*” (CLso) associated with zebra chip of potato has emerged as a new pathogen in the past twenty years and its evolutionary origin is the subject of much speculation. A plausible hypothesis is that CLso evolved from being exclusively an endosymbiont in the gut of the tomato potato psyllid to a pathogen adapted to growth in the phloem of a variety of solanaceous host plants including potato. Horizontal (or lateral) gene transfer is well known to occur in prokaryotes. Phage-mediated transduction is one mechanism well known to be a driver of genetic diversity in bacteria and to be a way in which virulence factors can be acquired (Johnson et al. 2011).

Two recent studies using pyrosequencing of the conserved 16S rRNA genes have explored the diversity of bacteria found within the gut of different life stages of the tomato potato psyllid (Nachappa et al. 2011; Hail et al. 2012). Within this diverse community of resident endosymbiotic and transient bacteria, referred to as the microbiome (Hail et al. 2012), horizontal gene transfer may also occur by uptake of naked DNA (transformation) or by conjugation. At this stage there is no evidence to show whether either mechanism has contributed to the evolution of CLso.

4. The Tomato Potato Psyllid: Feeding Habits and Oviposition

Bactericera cockerelli is a species of jumping plant lice, or psyllids (Psylloidea), which comprise a group of around 3000 species of small plant-sap-feeding insects related to the aphids and whiteflies. The common name of jumping lice comes from their well-known habit of jumping when disturbed. A review of life cycle and adaptation in the Psylloidea by Hodkinson (2009) shows they are always associated with above ground plant material for feeding and reproduction oviposition and nymphal development; association with root material is exceedingly rare. Hodkinson (2009) has compiled an exhaustive review of the life history characteristics of Psylloidea world-wide, including the site of overwintering on the plant host and the feeding site (shoot apex, expanded leaf, flower, stem, roots or buds). Feeding on roots is confined to one species, *Craspedolepta subpunctata*; in a further eight species (four confirmed and four suspected) overwintering occurs on root material when seasonal conditions are unfavourable. During favourable seasonal conditions conducive for plant growth, feeding and reproduction occur on shoot material. It is noteworthy that *C. subpunctata* and related species are contained within the family Psyllidae, taxonomically distant from the family Triozidae which includes all of the vectors of CLso and its four haplotypes.

The life cycle of the tomato potato psyllid with fresh above ground plant material is consistent with the vast majority of Psylloidea. The tomato potato psyllid was first described in 1909 and has been studied extensively since the 1920's when it was recognised as an important pest of potato and other Solanaceae (Butler and Trumble 2012b). According to the comprehensive review of *B. cockerelli* by Butler and Trumble (2012b) adult psyllids feed primarily on the underside of leaves of host plants, with some individuals also feeding on the upper surface of leaves as well as stems and petioles. For example, in a field study conducted at multiple sites and years, 99% of psyllids are found on leaves with 70% of these on the underside of the leaf (Butler and Trumble 2012a). Histological studies show that this insect, like aphids, are phloem feeders. When leaves are probed by the insect, penetration occurs through the leaf epidermis intercellularly through the spongy mesophyll until the stylets reach the phloem parenchyma cells, which is the region of the leaf where the most extensive feeding occurs.

Despite numerous studies on the tomato potato psyllid and CLso, including repeated sampling of potato tubers, there is no indication in any of the literature read that the tomato potato psyllid feeds on potato tubers below ground or harvested potatoes (Butler and Trumble 2012b; Munyaneza 2012a; Munyaneza and Henne 2013). Further, the jumping behaviour of adult psyllids in response to disturbance is likely to limit any association with potatoes during handling of potatoes during harvest, cleaning and packing.

According to Munyaneza and Henne (2013): "The eggs of *B. cockerelli* are deposited singly, principally on the lower surface of leaves and usually near the leaf edge, but some eggs can

be found throughout suitable host plants. Often, females will lay numerous eggs on a single leaf.”

There is no indication in any of the literature read of oviposition on harvested potato tubers (Butler and Trumble 2012b; Munyaneza 2012a; Munyaneza and Henne 2013). Further, it has been shown that even for preferred host material (actively growing shoots) soil components have a repellency effect on the tomato potato psyllid (see section 5).

5. Chemical and Biological Methods for Control of the Insect Vector

Management of zebra chip disease is targeted against the potato psyllid vector using insecticides (Guenther et al. 2012; Munyaneza 2012a; Butler et al. 2011, 2012). Detection of the insect vector early in the season is essential to minimize psyllid reproduction in the field and spread of the disease throughout fields (Munyaneza 2012a). New treatments are emerging including the finding of insecticides which are deterrents to feeding as well repellents (Butler et al. 2011a). For example, kaolin particle films (a type of clay) on tomato under laboratory and field conditions have been shown to have a repellent effect on the tomato potato psyllid (Peng et al. 2011).

Wuriyangan et al. (2011) have used RNA interference technology to induce mortality in *B. cockerelli* with promising results. The authors conclude that: “RNAi can be a powerful tool for gene function studies in psyllids, and give support for continued efforts for investigating RNAi approaches as possible tools for psyllid and plant disease control.”

Biological control methods are showing promise as tools for the management of zebra chip disease. Two commercially available entomopathogenic fungi, *Metarhizium anisopliae* and *Isaria fumosorosea*, have been shown to give comparable reductions in plant damage and symptoms of zebra chip to the those obtained with an insecticide treatment (Lacey et al. 2011). In New Zealand, O’Connell et al. (2012) have exploited the ‘new species association’ approach based on the ecological principle in which a natural enemy is used that has not coevolved with a pest. In a laboratory based study they were able to show that two New Zealand naturalized coccinellids, *Cryptolaemus montrouzieri* and *Cleobora mellyi* were predatory on and consumed the potato tomato psyllid. Their results suggest that a new species association may potentially exist between the two coccinellids and the psyllid.

6. Impact of Current Literature on Zebra Chip on Quarantine Conditions for Importation of Fresh Potatoes for Processing from New Zealand

Very little of the current literature on zebra chip disease of potato affects the import conditions for importation of potatoes for processing from New Zealand. Four subjects which relate directly to the Draft Report are tuber transmission of zebra chip disease (Pitman et al. 2011), the recognition of the existence of haplotypes in CLso (Nelson et al. 2011; Munyaneza 2012a), consideration of the properties of this bacterial pathogen which might affect its capacity for survival when shed into the environment; and the impact of improved diagnostic methods for diagnosis of zebra chip disease. These aspects are considered in turn.

i) Tuber transmission

Until very recently there has been no interest among North American research workers in risk assessment or in the fate of CLso in potato tubers held in store. This lack of interest is reflected in the session topics covered in the *Proceedings of the Annual Zebra Chip Reporting Session* at least as late as the 11th meeting held in 2011.

Zebra chip disease has recently been reported in the Pacific NorthWest of the United States, in Oregon and Washington (Crosslin et al. 2012). This discovery raises the question of the risk of Zebra Chip developing in stored tubers, in view of the fact that most of the potatoes produced in this part of the United States go into storage following harvest. Because migration of psyllids is late in the season, exposure of potatoes to infection also occurs late in the season. Plants exposed to infected psyllids less than three weeks before harvest usually produce tubers without symptoms of zebra chip disease (Buchman et al. 2012). Munyaneza (2012a) reports unpublished preliminary trials in 2010 and 2011 showing that an average of 10-22% and 46-66.4%, respectively, of symptomless tubers harvested from potato (cv. Atlantic) plants exposed to infected psyllids two to three weeks before harvest developed symptoms of zebra chip after two and three months in storage at 10 °C. Similar results have been obtained by Rashed et al. (2011). They reported symptom development in potato tubers of the chipping cultivar FL1867 after three months in storage at 5 °C. The tubers which were from plants exposed to infected psyllids two weeks prior to harvest were symptomless at harvest. Results from the same laboratory showed that this increase in symptom severity was accompanied by a decrease in titre of CLso (Rush et al. unpublished). If there is a decline in numbers of CLso in stored tubers as symptoms develop this may be a reflection of the profound change in the chemistry of the potato. Symptomatic tubers in comparison with asymptomatic tubers contain higher levels of free amino acids, phenolic compounds and defense-related proteins and enzymes, and many of these compounds also were positively correlated with zebra chip disease severity (Wallis et al. 2012; Yang et al. 2011). Similar observations have been made using tomato as the host plant (Casteel et al. 2012).

The fate of the bacterial pathogen in tubers has implications for the transmission to daughter tubers. This will have most significance for potato tubers used for propagation where the growth of tubers is the primary purpose of the imported material. The pathway considered in the draft report is only for potatoes for processing. Processing will stop potatoes from growing and therefore prevent tuber transmission.

ii) Haplotypes of '*Candidatus Liberibacter solanacearum*' and potential vectors

There are four haplotypes of CLso; haplotypes A and B associated with zebra chip of potato and both with the same insect vector; haplotypes C and D with a disease of carrot and with different psyllid species that are suspected to be the vector in Scandinavia and parts of Spain because of their consistent association with infected hosts (Appendix 2). The recognition of different haplotypes of CLso occurring on different host plants and insect vectors, and in widely separate geographical locations, is an important finding (Nelson et al. 2011; Nelson et al. 2012). To quote Nelson et al. (2011): "These apparently stable haplotypes suggest separate bacterial populations of long standing."

Phylogenetic analysis of the 16S rRNA gene has shown that two of the haplotypes, CLsoA in the Americas and CLsoC in northern Europe are closer to each other in spite of a large geographic separation and differences in plant host and insect vector (Nelson et al. 2012).

The genetic diversity of CLso populations in different geographical locations has also been explored using multilocus sequence typing (MLST) (Glynn et al. 2012) and by typing using a panel of eight simple sequence repeat (SSR) markers (Lin et al 2012). Both MLST and SSR typing systems have provided information that confirms and extends the haplotyping scheme.

The number of psyllid vectors for CLso that are associated with particular haplotypes may suggest a very highly specific pathogen/vector relationship. Recent work in the USA has shown that there are three biotypes of the tomato potato psyllid though only two have been shown to acquire CLso (Swisher et al. 2012), evidence suggesting a very high level of specificity between pathogen and vector. However, the potential for native psyllid vectors to acquire the pathogen from sprouting shoot material is not ruled out. The pathway considered in the draft report is only for potatoes for processing. Processing will stop tubers from growing and therefore prevent shoot growth that could allow any potential native psyllid vector(s) to feed and acquire the bacterium.

No published information has been found indicating that haplotypes C and D are transmissible to potato.

iii) Survival of the Zebra Chip Pathogen external to its insect or plant host.

CLso is an obligate intracellular parasite that resides in the phloem cells of the plant which it infects and which is transmitted by insect vectors. CLso is a fastidious bacterium which has so far defied all efforts to obtain pure cultures on an artificial medium. There is no indication in the literature that resting cells are produced by the bacterium which would enable survival when exposed to environmental stresses of desiccation, ultraviolet radiation and high temperature. If imported potatoes carrying CLso were accidentally released into the environment and then washed into water courses or crushed, it is likely that the pest population would lose viability and numbers decline to zero, when subjected to environmental stress or in competition with native microbiota, antibiotic-producing bacteria

or predatory protozoa, for example. Like "*Candidatus Liberibacter africanus*", CLso is known to be heat sensitive to temperatures above 32 °C (Munyaneza et al 2012b). Genetic analysis also shows that CLso has reduced metabolic capabilities reflecting its fastidious and obligate parasitic nature (Doddapaneni et al. 2010), including a limited capacity to utilise complex carbohydrates (Lin et al. 2011). The reduced metabolic capacity of CLso would limit any ability to compete with specialist saprophytes.

iv) Impact of improved diagnostic method

Diagnostic methods of the required sensitivity and specificity are an essential prerequisite for an understanding of the transmission pathway of zebra chip, the epidemiology of the disease, as well as for screening of potato germplasm and in seed certification programs to ensure the availability of clean potato seed (Li et al. 2009). Diagnostic methods based on cultural procedures, including the use of selective media are not available because CLso has not been obtained in culture. Accordingly there has been the need for development of DNA-based diagnostic procedures.

There have been several attempts to develop early detection of CLso in plant tissue and the tomato potato psyllid using polymerase chain reaction (PCR) assays and primer sets located in the conserved 16S rRNA gene, with unreliable and somewhat variable results depending on the primer sets used (Li et al. 2009; Wen et al. 2009; Ravindran et al. 2011). New primer sets have been developed targeting a conserved intergenic region between the 16S and 23S rRNA genes and a conserved bacterial housekeeping gene, adenylate kinase. These primer sets have proved to be more sensitive and reliable in detecting CLso (Ravindran et al. 2011) in plant tissue and insects. Quantitative real-time PCR (qPCR) enables the quantification of CLso populations in environmental samples, in terms of genome equivalents /g plant tissue (Li et al. 2009). The Loop-mediated isothermal amplification procedure or LAMP is a further improvement in methodology with the advantage that a thermocycler is not needed for amplification or agarose gel electrophoresis for resolution (Ravindran et al. 2012). LAMP shows promise as a reliable, rapid and cost-effective method of detecting CLso and other *Liberibacter* pathogens in psyllids and field-grown potato plants and tubers.

In summary DNA-based diagnostic procedures enable, for example, early detection and monitoring of infective populations of the tomato potato psyllid throughout the growing season. Control of psyllid populations through timely application of insecticides is made possible. The same methodologies have made possible fundamental studies on the acquisition of CLso by its insect vector and its transmission to host plants (Rashed et al. 2012).

7. Conclusion

It is required that New Zealand potatoes are imported in insect proof containers and opened only within quarantine approved premises in a metropolitan area, as specified in the *Draft report for the review of import conditions for fresh potatoes for processing from New*

Zealand, 3 July 2012. When these conditions are applied the risks of importing an exotic pest are minimised, as previously concluded (Hayward 2011).

There are numerous studies on the biology of the zebra complex that provide a very good understanding of the behaviour of the tomato potato psyllid in relation to the potato plant. These studies also assist to answer key questions raised by authorities carrying out pest risk analysis for importation of potatoes for processing from a country such as New Zealand in which zebra chip disease is endemic. In addition expert opinion can also be sought to provide additional guidance. In this context Munyaneza (2012b) writes as follows:

“ZC-infected tubers potentially being a source of the disease spread is a major concern, especially for national and international trade of fresh and seed potatoes. Recent research conducted in the United States by Dr. Munyaneza and collaborators showed that potato seed quality of ZC-infected tubers is significantly diminished as the tubers generally do not sprout and if they do, produce hair sprouts and weak plants.

However, the study concluded that the risk of spreading ZC through disease-infected tubers is extremely low and not significant because the number of ZC-infected tubers giving rise to infected plants is generally negligible and these potatoes are short-lived. Most importantly, potato psyllids must be present to spread the disease.

The main pathway for introducing the disease into potato and other solanaceous crops in regions where ZC is absent would be the introduction of infective potato psyllids, rather than infected seed material or fresh tubers. All life stages of the psyllid can easily be transported on live plant material that serves as hosts to potato psyllid, including produce for sale as well as plants meant for propagation.

Because potato tubers are not a suitable host of the psyllid, exported potato tubers are much less likely to contribute to psyllid movement. Therefore more emphasis should be on developing strategies and phytosanitary measures to effectively exclude the potato/tomato psyllid instead of focusing on preventing export of fresh and seed potatoes.”

Appendix 1

Current Literature on Zebra Chip of Potato and the

Insect Vector *Bactericera cockerelli* (December, 2012)

- Alfaro-Fernandez A, Cebrian M C, Villaescusa F J, Hermoso de Mendoza A, Ferrandiz J C, Sanjuan S, Font M I (2012a) First report of “*Candidatus Liberibacter solanacearum*” in carrot in mainland Spain. *Plant Disease* 96: 582.
- Alfaro-Fernandez A, Siverio F, Cebrian M C, Villaescusa F J, Font M I (2012b) “*Candidatus Liberibacter solanacearum*” associated with *Bactericera trigonica*-affected carrots in the Canary Islands. *Plant Disease* 96: 581.
- Alvarado V Y, Odokonyero D, Duncan O, Mirkov T E, Scholthof H B (2012) Molecular and physiological properties associated with zebra complex disease in potatoes and its relation with *Candidatus Liberibacter* contents in psyllid vectors. *PLoS ONE* 7: e37345.
- Buchman J L, Fisher T W, Sengoda V G, Munyaneza J E, (2012) Zebra chip progression from inoculation of potato plants with *Liberibacter* to development of disease symptoms in tubers. *American Journal of Potato Research* 80: 159-168.
- Buchman J L, Heilman B E, Munyaneza J.E. (2011) Effects of *Liberibacter*-infective *Bactericera cockerelli* (Hemiptera: Triozidae) density on zebra chip potato disease incidence, potato yield and tuber processing quality. *Journal of Economic Entomology* 104: 1783-1792.
- Buchman J L, Sengoda V G, Munyaneza J E. (2011) Vector transmission efficiency of *Liberibacter* by *Bactericera cockerelli* (Hemiptera: Triozidae) in zebra chip potato disease: effects of psyllid life stage and inoculation access period. *Journal of Economic Entomology* 104: 1486-1495.
- Butler C D, Byrne F J, Keremane M L, Lee R F, Trumble J T (2011) Effects of insecticides on behaviour of adult *Bactericera cockerelli* (Hemiptera: Triozidae) and transmission of *Candidatus Liberibacter psyllae*. *Journal of Economic Entomology* 104: 586-594.
- Butler C D, Trumble J T (2012a) Spatial dispersion and binomial sequential sampling for the potato psyllid (Hemiptera: Triozidae) on potato. *Pest Management Science*. 68: 865-869.
- Butler C D, Trumble J T (2012b) The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae): life history, relationship to plant diseases, and management strategies. *Tropical Arthropod Reviews* 5: 87-111.

- Butler C D, Trumble J T (2012c) Identification and impact of natural enemies of *Bactericera cockerelli* (Hemiptera: Triozidae) in Southern California. *Journal of Economic Entomology* 105:1509-1519.
- Butler C D, Walker G P, Trumble J T (2012) Feeding disruption of potato psyllid, *Bactericera cockerelli*, by imidacloprid as measured by electrical penetration graphs *Entomologia Experimentalis et Applicata*. 142: 247-257.
- Butler C.D, Gonzalez B, Keremanc M L, Lee R F, Novy R G, Miller J C, Trumble J T (2011) Behavioural responses of adult potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), to potato germplasm and transmission of *Candidatus Liberibacter psyllauros*. *Crop Protection* 30: 1233-1238.
- Casteel C L, Hansen A K, Walling L L, Paine T D (2012) Manipulation of plant defense responses by the tomato psyllid (*Bactericera cockerelli*) and its associated endosymbiont (*Candidatus Liberibacter psyllauros*). *PLoS ONE* 7(4): e35191. doi: 10.1371/journal.pone.0035191.
- Chapman R (2012) Population Genetics of the Potato Psyllid, *Bactericera cockerelli*. A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Biology, University of Texas at Tyler, May 2012, 77pp.
- Crosslin J M, Hamm P B, Eggers J E, Rondon S I, Sengoda, V G, Munyaneza J E (2012) First report of zebra chip disease and “*Candidatus Liberibacter solanacearum*” on potatoes in Oregon and Washington State. *Plant Disease* 96: 452.
- Crosslin J M, Olsen N, Nolte, P (2012) First report of zebra chip disease and “*Candidatus Liberibacter solanacearum*” on potatoes in Idaho. *Plant Disease* 96: 453.
- Davis T S, Horton D R, Munyaneza J E, Landolt P J (2012) Experimental infection of plants with an herbivore-associated bacterial endosymbiont influences herbivore host selection behaviour. *PLoS ONE* 7(11): e49330. doi: 10.1371/journal.pone.0049330.
- Doddapaneni H, Lin H, Duan Y, Lou V, Chen C, Vahling C, Lijuan Z, Civerolo, E.L. (2010) Comparative analyses of the ‘*Candidatus Liberibacter*’ species reductive genome features. *Phytopathology* 100: S30.
- Fisher T W, Cicero J M, Brown J K (2012) Cellular interactions and transcript profiling of “*Candidatus Liberibacter solanacearum*” during psyllid infection and vector-mediated transmission. *Phytopathology* 102: S4.39.
- Glynn J M, Islam M S, Bai Y, Lan S, Wen A, Gudmestad N C, Civerolo E L, Lin H. (2012) Multilocus sequence typing of “*Candidatus Liberibacter solanacearum*” isolates from North America and New Zealand. *Journal of Plant Pathology* 94: 223-228.
- Goolsby J A, Adamezyk J L Jr, Crosslin J M (and 16 other authors) (2012) Seasonal population dynamics of the potato psyllid (Hemiptera: Triozidae) and its associated

- pathogen “*Candidatus Liberibacter solanacearum*” in potatoes in the Southern Great Plains of North America. *Journal of Economic Entomology* 105: 1268-1276.
- Guedot C, Horton D R, Landolt P J (2012) Age at reproductive maturity and effect of age and time of day on sex attraction in the potato psyllid *Bactericera cockerelli*. *Insect Science* 19: 585-594.
- Guenther J, Goolsby J, Greenway G (2012) Use and cost of insecticides to control potato psyllids and zebra chip on potatoes. *Southwestern Entomologist* 37: 263-270.
- Hail D, Dowd S E, Bextine B (2012) Identification and location of symbionts associated with potato psyllid (*Bactericera cockerelli*) lifestages. *Environmental Entomology* 41: 98-107.
- Hayward A C (2011) Report to Biosecurity Australia on the draft revised import conditions for entry of potatoes from New Zealand for processing and the position paper for the review of import conditions for fresh potatoes from New Zealand.
- Henne D C, Workneh F, Rush C M (2012) Spatial patterns and spread of potato zebra chip disease in the Texas Panhandle. *Plant Disease* 96: 948-956.
- Hodkinson I D (2009) Life cycle variation and adaptation in jumping plant lice (Insecta: Hemiptera: Psylloidea): a global synthesis. *Journal of Natural History* 43: 65-179.
- Hunter W B, Glick E, Paldi N, Bextine B R (2012) Advances in RNA interference: dsRNA treatment in trees and grapevines for insect pest suppression. *Southwestern Entomologist* 37: 85-87.
- Johnson C P, Duan Y P, Wen A, Gudmestad N C (2011) A comparison of phage-like regions in “*Candidatus Liberibacter solanacearum*” and “*Candidatus Liberibacter asiaticus*”. pp.163-167. In: *Proceedings of the 11th Annual Zebra Chip Reporting Session*. (eds. Workneh F, Rashed A, Rush C M). San Antonio, TX (November 6-9, 2011).
- Kent D, Taylor G (2010) Two new species of *Acizzia* Crawford (Hemiptera: Psyllidae) from the Solanaceae with a potential new economic pest of eggplant, *Solanum melongena*. *Australian Journal of Entomology* 49: 73-81.
- Lacey L A, Liu, T-X, Buchman J L, Munyaneza J E, Goolsby J A, Horton D R (2011) Entomopathogenic fungi (Hymenozoa) for control of potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) in an area endemic for zebra chip disease of potato. *Biological Control* 56: 271-278.
- Li W, Abad J A, French-Monar R D, Rascoe J, Wen A, Gudmestad N C, Secor G A, Lee I-M, Duan Y, 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.

- Lin H, Islam M S, Bai, Y, Wen A, Lan S, Gudmestad N C, Civerolo E L (2012) Genetic diversity of “*Candidatus Liberibacter solanacearum*” strains in the United States and Mexico revealed by simple sequence repeat markers. *European Journal of Plant Pathology* 132: 297-308.
- Lin H, Islam M S, Wen A, Gudmestad N (2012) Genotyping and population genetic analysis of “*Candidatus Liberibacter solanacearum*”, bacterium associated with potato zebra chip disease. *Phytopathology* 102: S4.71.
- Lin H, Lou B, Glynn J M, Doddapaneni H, Civerolo E L, Chen C, Duan Y, Zhou L, Vahling C M (2011) The complete genome sequence of ‘*Candidatus Liberibacter solanacearum*’ the bacterium associated with potato zebra chip disease. *PLoS ONE* 6: e19135. DOI:10.1371/journal.pone.0019135.
- Lin H, Nwugo C C, Duan Y (2012) Physiological and proteomic characterizations of “*Candidatus Liberibacter*” associated diseases to citrus and potato plants *Phytopathology* 102: S4.71.
- Munyaneza J E, Fisher T W, Sengoda, V G, Garczynski S F, Nissinen A, Lemmetty A (2010a) First report of “*Candidatus Liberibacter solanacearum*” associated with psyllid-affected carrots in Europe. *Plant Disease* 94: 639.
- Munyaneza J E, Fisher T W, Sengoda V G, Garczynski S F, Nissinen A, Lemmetty A (2010b) Association of “*Candidatus Liberibacter solanacearum*” with the psyllid, *Trioza apicalis* (Hemiptera: Triozidae) in Europe. *Journal of Economic Entomology* 103: 1060-1070.
- Munyaneza J E, Fisher T W, Sengodagounder V, Garczynski S F, Nissinen A, Lemmetty A (2010c) Association of “*Candidatus Liberibacter solanacearum*” with psyllid-affected carrots in Europe. *Phytopathology* 100: S88.
- Munyaneza J E, Lemmetty A, Nissinen A I, Sengoda V G, Fisher, T W (2011) Molecular detection of Aster Yellows Phytoplasma and “*Candidatus Liberibacter solanacearum*” in carrots affected by the psyllid *Trioza apicalis* (Hemiptera: Triozidae) in Finland. *Journal of Plant Pathology* 93: 697-700.
- Munyaneza J E (2012a) Zebra chip disease of potato: biology, epidemiology and management *American Journal of Potato Research*. Published online: 30 August 2012.
- Munyaneza J E (2012b) Assessing the risks of spreading zebra chip disease. *The Global Herald* October 26, 2012.
- Munyaneza J E, Buchman J L, Sengoda V G, Goolsby J A, Ochoa A P, Trevino J, Schuster. G, (2012a) Impact of potato planting time on incidence of potato zebra chip disease in the lower Rio Grande valley of Texas. *Southwestern Entomologist* 37: 253-262.

- Munyaneza J E, Sengoda V G, Buchman J L, Fisher T W (2012b) Effects of temperature on “*Candidatus Liberibacter solanacearum*” and zebra chip potato disease symptom development. *Plant Disease* 96: 18-23.
- Munyaneza J E, Sengoda V G, Stegmark R, Arvidsson A K, Anderbrant O, Yuvaraj J K, Ramert B, Nissinen A, (2012c) First report of “*Candidatus Liberibacter solanacearum*” associated with psyllid-affected carrots in Sweden. *Plant Disease* 96: 453.
- Munyaneza J E, Sengoda V G, Sundheim L, Meadow R (2012d) First report of “*Candidatus Liberibacter solanacearum*” associated with psyllid-affected carrots in Norway. *Plant Disease* 96: 454.
- Munyaneza J E, Henne D C. (2013) Leafhopper and psyllid pests of potato. pp. 79-89. In: *Insect Pests of Potato* (eds. Giordanengo P, Vincent C and Alyokhin A.). Elsevier: Amsterdam.
- Nachappa P, Levy J, Pierson E, Tamborindoguy C (2011) Diversity of endosymbionts in the potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), vector of zebra chip disease of potato. *Current Microbiology* 62: 1510-1520.
- Nachappa P, Levy J, Tamborindoguy C (2012) Transcriptome analyses of *Bactericera cockerelli* adults in response to “*Candidatus Liberibacter solanacearum*” infection *Molecular Genetics and Genomics* 287: 803-817.
- Nachappa P, Shapiro A A, Tamborindoguy C (2012) Effects of “*Candidatus Liberibacter solanacearum*” on fitness of its insect vector, *Bactericera cockerelli* (Hemiptera: Triozidae), on tomato. *Phytopathology* 102: 41-46.
- Nelson W R, Fisher T W, Munyaneza J E (2011) Haplotypes of “*Candidatus Liberibacter solanacearum*” suggest long-standing separation. *European Journal of Plant Pathology* 130: 5-12.
- Nelson W R, Sengoda V G, Alfaro-Fernandez A O, Font M I, Crosslin J M, Munyaneza J E (2012) A new haplotype of ‘*Candidatus Liberibacter solanacearum*’ identified in the mediterranean region. *European Journal of Plant Pathology* DOI 10.1007/s10658-012-0121-3
- Nissinen A I, Lemmetty A, Pihlava J M, Jauhiainen L, Munyaneza J E, Vanhala P (2012) Effects of carrot psyllid (*Triozia apicalis*) feeding on carrot yield and content of sugars and phenolic compounds. *Annals of Applied Biology* 161: 68-80.
- O’Connell D M, Wratten S D, Pugh, A.R, Barnes A-M (2012) ‘New species association’ biological control ? Two coccinellid species and an invasive psyllid pest in New Zealand *Biological Control* 62: 86-92.
- Peng L, Trumble J T, Munyaneza J E, Liu T-X. (2011) Repellency of a kaolin particle film to potato psyllid, *Bactericera cockerelli* (Hemiptera: Psyllidae), on tomato under laboratory and field conditions. *Pest Management Science* 67: 815-824.

- Pitman A R, Drayton G M, Kraberger S J, Genet R A, Scott I A W (2011) Tuber transmission of “*Candidatus Liberibacter solanacearum*” and its association with zebra chip on potato in New Zealand. *European Journal of Plant Pathology* 129: 389-398.
- Ramirez-Davila J F, Porcayo-Camargo E, Sanchez-Pale J R, Vazquez-Garcia L M (2012) Spatial behaviour comparison of *Bactericera cockerelli* Sulc. (Hemiptera: Triozidae) in Mexico. *Neotropical Entomology* 41: 9-16.
- Rashed A, Nash T D, Paetzold L, Workneh F, Rush C M (2012) Transmission efficiency of “*Candidatus Liberibacter solanacearum*” and potato zebra chip disease progress in relation to pathogen titre, vector numbers, and feeding sites. *Phytopathology* 102: 1079-1085.
- Rashed A, Workneh F, Paetzold L, Gray J, Wallis C, Rush C M (2011) Zebra chip disease severity and “*Candidatus Liberibacter solanacearum*” titer load of the potato hosts inoculated throughout the field season. pp 27-31. In *Proceedings of the 11th Annual Zebra Chip Reporting Session*, ed. Workneh F, Rashed A, Rush C M. San Antonio, TX (November 6-9, 2011).
- Ravindran A, Levy J, Pierson E, Gross D C (2011) Development of primers for improved PCR detection of the potato zebra chip pathogen “*Candidatus Liberibacter solanacearum*”. *Plant Disease* 95: 1542-1546.
- Swisher K D, Munyaneza J E, Crosslin J M (2012) High resolution melting analysis of the cytochrome oxidase 1 gene identifies three haplotypes of the potato psyllid in the United States. *Environmental Entomology* 41: 1019-1028.
- Thomas K L, Jones D C, Kumarasinghe L B, Richmond J E, Gill G S C, Bullians M S (2011) Investigation into the entry pathway for tomato potato psyllid *Bactericera cockerelli*. *New Zealand Plant Protection* 64: 259-268.
- Tran L T, Worner S P, Hale R J, Teulon D A J (2012) Estimating development rate and thermal requirements of *Bactericera cockerelli* (Hemiptera: Triozidae) reared on potato and tomato by using linear and nonlinear models. *Environmental Entomology* 41: 1190-1198.
- Valterova I, Nehlin G, Borg-Karlson A-K (1997) Host plant chemistry and preferences in egg-laying *Trioza apicalis* (Homoptera, Psylloidea). *Biochemical Systematics and Ecology* 25: 477-491.
- Walker G P, MacDonald F H, Puketapu A J, Fergusson II A, Connolly P G, Wright P J, Anderson J A D (2012) A field trial to assess damage by *Bactericera cockerelli* to early potatoes at Pukekohe. *New Zealand Plant Protection* 65: 148-154.
- Wallis C M, Chen J C, Civerolo E L (2012) Zebra chip-diseased potato tubers are characterized by increased levels of host phenolics, amino acids, and defense-related proteins. *PMPP Physiological and Molecular Plant Pathology* 78: 66-72.

- Wallis C, Rashed A, Rush C.M. (2012) Large-scale shifts in potato (*Solanum tuberosum*) tuber physiology occur following infection by “*Candidatus Liberibacter solanacearum*” *Phytopathology* 102: S4.129.
- Wen A, Johnson C, Gudmestad N C (2012) Genetic characterization of “*Candidatus Liberibacter solanacearum*” associated with zebra chip disease of potato in Washington and Idaho. *Phytopathology* 102: S4.134.
- Wen A, Mallik I, Alvarado V Y, Pasche J S, Wang X, Li W, Levy L, Lin H, Scholthof H B, Mirkov T E, Rush C M, Gudmestad N C (2009) Detection, distribution, and genetic variability of ‘*Candidatus Liberibacter*’ species associated with zebra complex disease of potato in North America. *Plant Disease* 93: 1102-1115.
- Workneh F, Henne D C, Childers A C, Pactzold L, Rush C M (2012) Assessments of the edge effect in intensity of potato zebra chip disease. *Plant Disease* 96: 943-947.
- Workneh F, Mirik M, Rashed A, Hamm P B, Ansley J, Rush C M (2012) Potato zebra chip in the Pacific Northwest: impact and probable psyllid source assessments. *Phytopathology* 102: S4.137.
- Wuriyangan H, Rosa C, Falk B W. (2011) Oral delivery of double-stranded RNAs and siRNAs induces RNAi effects in the potato/tomato psyllid, *Bactericera cockerelli*. *PLoS ONE* 6: e27736.
- Yang X-B, Malik N S A, Perez J L, Liu T-X (2011) Impact of potato psyllid (Hemiptera: Triozidae) feeding on free amino acid composition in potato. *Insect Science* 18: 663-670.
- Yen A L, Madge D G, Berry N A, Yen J D L (2012) Evaluating the effectiveness of five sampling methods for the detection of the tomato potato psyllid *Bactericera cockerelli* (Sulc) (Hemiptera: Psylloidea: Triozidae) *Australian Journal of Entomology* doi: 10.1111/aen.12006.

Appendix 2

Haplotypes of “*Candidatus Liberibacter solanacearum*”: Hosts, Vectors and Geographical Distribution.

Haplotype	Host Plant	Insect vector	Geographical distribution	Reference
A	Potato	<i>Bactericera cockerelli</i>	Honduras, Guatemala, western Mexico, Arizona, California, Idaho, Oregon, Washington, New Zealand	Nelson et al. 2012; Wen et al. 2012
B	Potato	<i>Bactericera cockerelli</i>	eastern Mexico, Texas, south, central Washington	Wen et al. 2012
C	Carrot	<i>Trioza apicalis</i>	Finland, Sweden, Norway	Nissinen et al. 2012; Munyaneza et al. 2010a, b, c; 2011; 2012c, d
D	Carrot	<i>Bactericera trigonica</i>	Canary Islands, mainland Spain	Alfaro-Fernandez et al 2012a, b

1) CLso has been reported on carrots in association with *Trioza apicalis* in France but the haplotype involved has not been determined (<http://archives.eppo.int/EPPORreporting/2012/Rse-1210.pdf>).

2) CLso has been reported on celery (Apiaceae) in Spain in association with *Bactericera trigonica* ([www.http://archives.eppo.int/EPPORreporting/2012/Rse-1206.pdf](http://archives.eppo.int/EPPORreporting/2012/Rse-1206.pdf)).