Native Psyllid populations and the distribution of Candidatus phytoplasma australiense

Dr Calum Wilson
Tasmanian Institute of Agriculture

Project Number: PT10001

PT10001

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the potato industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of:
McCain Foods (Aust) Pty Ltd
Simplot Australia Pty Ltd - Tasmania
Snack Brands Australia
Tasmanian Seed Certification Scheme
Smiths Snackfood Company

ISBN 0 7341 3358 8

Published and distributed by:
Horticulture Australia Ltd
Level 7
179 Elizabeth Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

Horticulture Australia

Horticulture Australia

PT10001 (30th May 2014)

Native psyllid populations and the distribution of *Candidatus* phytoplasma australiense

Final Report

Drs Paul Walker & Calum Wilson

Tasmanian Institute of Agriculture
University of Tasmania

HAL Project Number: PT10001

Project Leader's contact name and contact details and a list of other key personnel:

Calum Wilson, Tasmanian Institute of Agriculture (TIA), University of Tasmania, Private Bag 54, Hobart, Tasmania 7001

Tel: (03) 62336841; Email: Calum.Wilson@utas.edu.au

Paul Walker (TIA), Geoff Allen (TIA), Robert Tegg (TIA), Leonie White (TIA), Frank Mulcahy (Simplot Australia Pty Ltd), Brett Pemberton (Smiths Snackfood Company), Allan Smith (Snack Brands Australia) and David Antrobus, (formerly McCains Foods (Aust) Pty Ltd).

Purpose of the Report:

This is the final report for the project "Native psyllid populations and the distribution of *Candidatus* phytoplasma australiense", Project number PT10001. The recent accidental introduction of the tomato potato psyllid (*Bactericera cockerelli*) into New Zealand has raised fears that this insect pest could readily enter Australia and have a similar devastating impact on local solanaceous crop industries. The psyllid is a vector of the bacterium, "*Candidatus* Liberibacter solanacearum", which is associated with psyllid yellows disease in tomatoes, potatoes, capsicums, eggplants and tamarillos, and zebra chip disease in potatoes. Another plant disease "*Candidatus* Phytoplasma australiense" was also recently discovered in New Zealand potatoes and psyllids were implicated in its spread. This report details outcomes from Project PT10001 which aimed to provide potato growers and industry stakeholders with an early warning system to detect incursions of the tomato potato psyllid in the major potato growing areas of eastern Australia, using a network of yellow sticky traps. A literature review on the tomato psyllid, Liberibacter and Phytoplasma complex is provided, and baseline data on the number and types of native psyllids caught in potato fields are given. The likelihood of native psyllids playing a role in the transmission of Liberibacter/Phytoplasma in potatoes is discussed.

Funding Sources:

This project was funded by HAL through voluntary contributions from Simplot Australia Pty, McCain Foods (Aust) Pty, Smiths Snackfood Company, Snack Brands Australia and the Tasmanian Seed Certification Scheme, and matched funds from the Australian Government.

Date of the report: 30 May 2014

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The recent accidental introduction of the tomato potato psyllid (*Bactericera cockerelli*) into New Zealand has raised fears that this insect pest could readily enter Australia and have a similar devastating impact on local solanaceous crop industries. The psyllid is a vector of the bacterium, "*Candidatus* Liberibacter solanacearum", which is associated with psyllid yellows disease in tomatoes, potatoes, capsicums, eggplants and tamarillos, and zebra chip disease in potatoes.

Another plant disease "*Candidatus* Phytoplasma australiense" was also recently discovered in New Zealand potatoes and the tomato potato psyllid was implicated in its spread. This phytoplasma is present in Australia, where it affects several economically important species of plants, but has not been detected in potatoes. The finding of the Phytoplasma in New Zealand potatoes has raised concerns that it could potentially be transmitted into Australian potato crops by native psyllid species.

The tomato potato psyllid could enter Australia either through the accidental importation of infested plant material or by the natural dispersal of psyllids on easterly airflows from New Zealand. Yellow sticky traps are an effective method for detecting the presence of adult tomato potato psyllids.

This project was initiated to set up a network of sticky traps in the major potato growing regions of eastern Australia, to act as an early warning system to detect incursions of the tomato potato psyllid. Information on the number and type of native psyllids occurring in potato fields was collated to examine their potential as vectors of Phytoplasma. Between February 2011 and March 2014, over 2,300 traps were placed in potato growing regions of Tasmania, Victoria, South Australia, New South Wales and Queensland. No tomato potato psyllids were detected but more than 9,600 native psyllids were trapped. The number of native psyllids caught varied considerably between potato growing regions and time of year, but were generally highest in South Australia and lowest in south-eastern Tasmania. In this report we provide details of the common psyllid types caught on traps and their likely plant associations. No evidence was found that native psyllids caught on traps could transmit "*Candidatus* Phytoplasma australiense". Most psyllids caught were incidental captures from nearby host vegetation, such as *Eucalyptus* spp., and are unlikely to feed on potato plants.

A review of the literature on the tomato potato psyllid, Liberibacter and Phytoplasma was completed. Workshops for potato growers and industry stakeholders were held on psyllid identification and recognition of Liberibacter/ Phytoplasma infestations in potato crops to ensure that, if incursions do occur, they are detected as early as possible in order to limit their impact.

Recommendations are made for further research and to extend the trapping program to include other Australian solanaceous crop industries.

TECHNICAL SUMMARY

This project was initiated to set up a network of yellow sticky traps in the major potato growing regions of eastern Australia, to act as an early warning system to detect incursions of the tomato potato psyllid (*Bactericera cockerelli*), the vector of "*Candidatus* Liberibacter solanacearum". The project also aimed to collate baseline data on the number and type of native psyllids caught in potato crops to examine their potential to transmit "*Candidatus* Phytoplasma australiense" which had recently been detected in New Zealand potatoes.

Between February 2011 and March 2014, 2,319 traps were placed in potato growing regions of Tasmania, Victoria, South Australia, New South Wales and Queensland. No tomato potato psyllids were detected but 9,665 native psyllids were trapped. The number of native psyllids caught varied considerably between potato growing regions and time of year, being generally highest in the Penola region of South Australia and lowest in south-eastern Tasmania. Most traps had no (43%) or only one (20%) psyllid present. Only 0.3% of psyllids caught belonged to the same family as *B. cockerelli* (Triozidae). This allowed the rapid differentiation of the majority of trapped psyllids from *B. cockerelli* based on the easily discernable pattern of wing venation. The most prevalent psyllid genera caught were *Ctenarytiana* and *Acizzia*. Other genera less frequently caught included *Creiis*, *Cardiaspina*, *Anoeconeossa*, *Euclyptolyma*, *Phyllolyma*, *Phellopsylla*, *Blastopsylla* and *Cryptoneosea/Ageteopsylla*. No evidence was found that native psyllids caught on traps transmit "*Candidatus* Phytoplasma australiense". Nearly all psyllids caught were associated with nearby vegetation, particularly *Eucalyptus* spp., and would therefore, be unlikely to feed on potato plants and transmit the Liberibacter if was to enter Australia. However, one species of *Acizzia* caught in northern Tasmania, resembled a newly described group which are known to feed on Solanaceous plants. Further collections are needed to confirm its identity and host-plant associations but, due to its rarity and restricted distribution, it is again unlikely to feed on potato plants.

Workshops for potato growers and industry stakeholders were held on psyllid identification and recognition of Liberibacter/ Phytoplasma infestations in potato crops to ensure that, if incursions do occur, they are detected as early as possible in order to limit their impact.

Recommendations are made for further research to model the potential distribution of *B. cockerelli* and "*Candidatus* Liberibacter solanacearum" in Australia and to extend the trapping program to include other Australian solanaceous crop industries.

INTRODUCTION

A comprehensive literature review covering the tomato potato psyllid and associated plant diseases was conducted at the beginning of the project and is provided in the Appendices (Appendix 2). The following introduction is a summary of the main points, including some additional references that were published after the completion of the review. For more details on the biology and identification of *B. cockerelli* and disease diagnostics of the psyllid/Liberibacter complex, please refer to this review.

Emergence of *Bactericera cockerelli* as a pest of Solanaceous crops

The tomato potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), has recently undergone a considerable expansion in its range and become a major pest of solanaceous crops in several countries (Butler & Trumble 2012). The psyllid is a vector of "*Candidatus* Liberibacter solanacearum" (syn. "*Ca*. L. psyllaurous"), which is associated with 'psyllid yellows' disease in several solanaceous crops and 'zebra chip' disease in potatoes (*Solanum tuberosum* L.) (Munyaneza *et al*. 2010). Both the vector and pathogen are currently absent in Australia. However, it is feared that they may become established in this country after their accidental introduction into New Zealand in the mid-2000's (Teulon *et al*. 2009).

Bactericera cockerelli was described in 1909 by Šulc who collected large numbers of nymphs from capsicums (*Capsicum annum* L.) in Colorado, USA (Butler & Trumble 2012). The first recorded outbreak of *B. cockerelli* in potato crops was in 1927, starting in Utah and then spreading to other Rocky Mountain states (Colorado, Idaho, Montana and Wyoming). However, Wallis (1946) suggested that previous reported losses in tomatoes (*Lycopersicon esculentum* Mill.) and potatoes in Colorado, dating back to the late 1800's and early 1900's, may have been due to *B. cockerelli* but at the time psyllid injury to these crops was not recognised and were attributed to other causes. Early studies established that the source of *B. cockerelli* in agricultural areas of western USA was through annual migrations from parts of Texas and Mexico, where the psyllid infested potato and tomato crops and overwintered on various solanaceous hosts (Butler & Trumble 2012). While *B. cockerelli* has long been known to occur in Lower Rio Grande Valley in Texas (Wallis 1946), it has only recently become a major economic pest of potatoes, tomatoes, capsicums and eggplant (*Solanum melongena* L.) in this region (Yang & Liu 2009). Infestations of *B. cockerelli* in western USA were relatively rare during the 1930's and 1940's and only sporadic populations were reported in relatively small geographic areas within California which seldom persisted for more than a year or two before disappearing (Liu & Trumble 2007). However, since 1999-2000, *B. cockerelli* has undergone a western expansion in distribution into southern and central California from Baja, Mexico (Liu *et al*. 2006). These recent invasions in western areas have been unusual both for the duration of the infestation and for the extent of the geographic range invaded (Liu & Trumble 2007). They have been associated with an 'invasive' biotype of *B. cockerelli* which is genetically distinct from a 'native' biotype associated with central and eastern parts of North America (Liu *et al*. 2006).

In March 2006, *B. cockerelli* was reported infesting glasshouse tomatoes near Auckland, New Zealand, but several authors suggest that it may have entered the country in 2005 (Teulon *et al*. 2009). Subsequent surveys found that *B. cockerelli* populations were well established in numerous glasshouse facilities and outdoors on volunteer potato plants around Auckland and eradication was

considered unattainable (Teulon *et al*. 2009). Between 2006 and 2009, *B. cockerelli* spread to other glasshouse crops, outdoor crops, nurseries and gardens throughout the North and South Islands, infesting mainly solanaceous crops and weeds. This rapid spread was likely to have been through a combination of natural and human mediated dispersal, particularly through the distribution of infested host-plant material (Teulon *et al*. 2009).

Identification of "*Candidatus* Liberibacter solanacearum"

Psyllid yellows were first observed in potatoes during the 1927 outbreak of *B. cockerelli* in the USA (Munyaneza *et al*. 2007). For many decades it was thought that psyllid yellows was caused by toxins in the saliva of *B. cockerelli* but this has since been discounted by grafting experiments (Munyaneza *et al*. 2007). In 2007-2008, symptoms resembling psyllid yellows were observed in tomato plants in California and Arizona, and in greenhouse tomatoes and capsicums in New Zealand (Crosslin *et al*. 2010). Zebra chip was first documented affecting potatoes in 1994 near Saltillo, Coahuila, Mexico (Munyaneza *et al*. 2007). Infected tubers displayed a distinct internal brown discolouration which darkened on frying , hence the name 'zebra chip'. Similar internal discolouration of potato tubers was reported in the USA in the 1940's and 1950's, suggesting zebra chip may have been present for a long time but in a latent state (Rubio-Covarrubias *et al*. 2011; Munyaneza *et al*. 2007). In 2000, zebra chip was detected in potato crops in the Lower Rio Grande Valley, Texas, but remained of sporadic importance until the 2004-2006 growing seasons when economic losses due to the disease rose dramatically in several locations in the USA and Mexico, (Munyaneza *et al*. 2007). Zebra chip rapidly spread to other potato growing regions and it has now been recorded in solanaceous plants in: Arizona, California, Colorado, Idaho, Kansas, Montana, Nebraska, Nevada, New Mexico, North Dakota, Oregon, Texas, and Washington in the USA; Alberta in Canada; Coahuila and Nuevo León in Mexico; Guatemala and Honduras (Butler & Trumble 2012; Rodon & Murphy 2012). Symptoms resembling zebra chip were first observed in New Zealand in potato tubers harvested from a breeding trial in South Auckland in May 2008 (Liefting *et al*. 2008a).

Munyaneza *et al*. (2007) confirmed that *B. cockerelli* transmitted zebra chip to potatoes but the causal agent remained unknown until Liefting *et al*. (2008a) (in New Zealand) and Hansen *et al*. (2008) (in the USA) independently identified a new Huanglongbing bacterium species in infected tomato and potato plants. The bacterium belonged to the genus "*Candidatus* Liberibacter" which are phloem-restricted, non-culturable, alpha-proteobacteria usually associated with citrus (Liefting *et al*. 2009; Hansen *et al*. 2008). Liefting *et al*. (2009) named the Liberibacter "*Ca*. L. solanacearum" while Hansen *et al*. (2008) named it "*Ca.* L. psyllaurous". Comparisons of the DNA sequence of the Liberibacter from New Zealand and the USA suggested that they were the same organism (Wen *et al*. 2009). Both names are currently used in the literature and which names presides awaits further research (Butler & Trumble 2012). The Liberibacter is now known to infect several other solanaceous crops and plants including: tamarillos (*Solanum betaceum* Cav.), cape gooseberry (*Physalis peruviana* L.), silverleaf nightshade (*Solanum elaegnifolium* Cav.), *S. nigrum* and matrimony vine (*Lycium halimifolium* Mill.) (Butler & Trumble 2010; Liefting *et al*. 2008b).

Economic impact of *Bactericera cockerelli* and "*Candidatus* Liberibacter solanacearum"

The economic impact of *B. cockerelli* and "*Ca*. L. solanacearum" to solanaceous crop industries around the world has been severe. Psyllid management has now become critical for maximising crop

yields and the quality of produce for export (Crosslin *et al*. 2010; Teulon *et al*. 2009). This has resulted in a major increase in the number of insecticide applications for the control of *B. cockerelli* in greenhouse tomato and capsicums and outdoor tomatoes and potatoes, which has jeopardised the ongoing development of Integrated Pest Management (IPM) and biological control programmes in these crops (Ogden 2011; Walker *et al*. 2011; Teulon *et al*. 2009). Consequently, if *B. cockerelli* was to become established in Australia, the potential impact on current IPM strategies in indoor and outdoor crops is of major concern (Horne & Page 2009).

In New Zealand, the greatest impact of *B. cockerelli* and "*Ca*. L. solanacearum" has been in the potato and tamarillo industries (Ogden 2011). Although *B. cockerelli* occurs in tamarillos in very low numbers, transmission of "*Ca*. L. solanacearum" and its persistence in this perennial crop can cause tree losses of over 50% and has led many growers to exit the industry (Anon. 2011; Ogden 2011; Watson 2009). New Zealand tomato and capsicum growers have sustained lesser impacts but at the expense of increased insecticide applications and a range of new requirements to meet certification of export produce (Teulon *et al*. 2009). In 2011, the estimated cost of *B. cockerelli* and "*Ca*. L. solanacearum" to tomato and capsicum growers was NZ$5 million, mainly through increased psyllid monitoring and control costs, and the rigorous removal of Liberibacter infected plants to limit disease spread (Ogden 2011).

The cost of *B. cockerelli* and "*Ca*. L. solanacearum" to the New Zealand potato industry has exceeded NZ$120 million (Ogden 2011). In 2010 alone, extra costs induced by the psyllid and pathogen was over NZ$28 million, of which *ca*: NZ$21.7 m was incurred by growers through reduced yields and increased management costs; NZ$5.3 million was incurred by processors and seed industry; and NZ$1 million was spent on research and other associated costs (Kale 2011). The main impact of the psyllid and pathogen on processing potatoes in New Zealand is the lower specific gravity and this appears to be a much bigger problem than the discolouration of tubers, which is relatively uncommon compared to the USA (Ogden 2011). North Island process growers incur greater costs per hectare perhaps due to the longer growing period of these crops (around 150 days) compared to table (100-120 days) or seed crops (90 days), and the much lower psyllid numbers in the South Island (Ogden 2011; Kale 2011). When zebra chip first became a major problem in the USA and Mexico during the 2004-2006 growing seasons, entire fields were often abandoned (Munyaneza *et al*. 2007). A high incidence of zebra chip also destroyed the seed potato production industry in the central plateau of Mexico (Rubio-Covarrubias *et al*. 2011). However, the adoption of aggressive insecticide programmes by USA growers has greatly reduced the damage caused by zebra chip disease (Butler & Trumble 2012; Crosslin *et al*. 2010) but has significantly increased production costs.

"*Candidatus* Phytoplasma australiense" in New Zealand potatoes

Not long after the discovery of the psyllid/Liberibacter complex in New Zealand, considerable confusion arose when another plant disease, "*Candidatus* Phytoplasma australiense", was also found infecting potato plants (Liefting *et al.* 2009; 2011). This phytoplasma was known to occur in several other plants, including economically important species, in both New Zealand and Australia but had never previously been found in a solanaceous plant. The highly polyphagous *B. cockerelli* was implicated, but not proven, to be involved in the spread of "*Candidatus* Phytoplasma australiense"

from non-solanaceous hosts plants into potato crops. This raised concerns that the phytoplasma may also spread to Australian potato crops by native species of psyllids occurring in crops.

Pathways of entry of *B. cockerelli*/Liberibacter into Australia

Bactericera cockerelli and "*Ca*. L. solanacearum" could enter Australia together or independently through the accidental or intentional importation on fresh fruit, potato tubers or nursery stock, or through hitchhiker transportation of psyllids on plant or non-plant material (Biosecurity Australia 2009; Australian Government Department of Agriculture Fisheries and Forestry 2012a, b; Plant Health Australia 2011). In Hawaii, the most highly rejected commodity by airport quarantine authorities were capsicums, originating from California, infested with live *B. cockerelli* nymphs (Hara 2012). *Bactericera cockerelli* most likely entered New Zealand as a result of smuggling psyllid-infected primary host material (possibly chilli peppers) from the Americas, rather than through the accidental transportation on consignments of fresh produce through regulated pathways (Thomas *et al*. 2011). Given the wide host-plant range of *B. cockerelli*, the risk of importation of plant material contaminated with psyllids and/or infected with "*Ca*. L. solanacearum" is not limited to the Solanaceae.

In potatoes "*Ca*. L. solanacearum" can be transmitted from infected mother tubers both to the foliage of growing plants and to progeny tubers, in the absence of *B. cockerelli* (Pitman *et al*. 2011). Therefore, tuber transmission could play a role in the movement of the pathogen to other regions, including Australia, via the transport of infected tubers for the fresh market or for seed. However, the likelihood of spread and establishment of the pathogen in other plants or areas beyond the point of entry in the absence of *B. cockerelli*, is considered to be low or moderate as plant material displaying symptoms of "*Ca*. L. solanacearum" are likely to be discarded or destroyed (Biosecurity Australia 2009).

Currently, *B. cockerelli* is the only known vector of "*Ca*. L. solanacearum" in solanaceous plants. However, recent outbreaks of "*Ca*. L. solanacearum" in carrots in southern Finland and in the Canary Islands (Tenerife, Spain) were associated with two other psyllid species, *Trioza apicalis* Förster and *Bactericera trigonica* (Hodkinson), respectively (Alfaro-Fernández *et al*. 2011; Munyaneza *et al*. 2010). This suggests that "*Ca*. L. solanacearum" is not solely restricted to solanaceous hosts and that Liberibacters may be more widespread in different psyllid species and their host plants than previously thought (Munyaneza 2010).

Another possible pathway of entry of *B. cockerelli* into Australia is through natural long-distance dispersal by wind or flight from New Zealand. In its native range, *B. cockerelli* is highly migratory (Liu & Trumble 2007; Liu *et al*. 2006) and may be capable of reaching Australia by travelling on strong easterly airflows, similar to how the currant lettuce aphid (*Nasonovia ribisnigri* Mosley) is thought to have invaded Tasmania in 2004 (Hill 2012). Such weather systems are relatively rare but can occur when several high pressure weather systems (anticyclones) are situated at uncommonly southern latitudes (45-50° S) and form a blocking pattern over southeast Australia and the Tasman Sea extending to New Zealand (Hill 2012).

If *B. cockerelli* was to enter Australia it is vital that any incursions are detected as early as possible in order to undertake appropriate actions to limit the spread and damage caused by the insect and

Liberibacter. Yellow sticky traps are highly attractive to adult *B. cockerelli* and they are used regularly to detect and monitor psyllid populations in New Zealand and North America (Taylor *et al*. 2014; Crosslin *et al*. 2012; Walker *et al*. 2011). In trapping studies conducted in New Zealand, yellow sticky traps outperformed all other methods tested for the detection of *B. cockerelli* in potato crops (sweep netting, vacuum sampling and direct searching) except water traps (Yen *et al*. (2012). Yellow sticky traps are also more effective at detecting low, early season populations of *B. cockerelli* than direct visual searches (Vereijssen *et al*. 2012; Cameron *et al*. 2009). Yen *et al*. (2012) stated that in the event of a suspected incursion by *B. cockerelli* in Australia, a programme of surveillance using yellow sticky or water traps would be easy and time-effective to implement and maintain, and would provide a high probability of detection if the psyllid was in fact present.

Project aims

1. Conduct a literature review on the tomato potato psyllid and associated vectored diseases.
2. Establish a network of yellow sticky traps in the major potato growing areas of eastern Australia to provide an early warning system to detect incursions of the tomato potato psyllid.
3. Provide baseline information on the numbers and types of native psyllids caught in potato crops.
4. In liaison with DPI Victoria, test native psyllids for the presence of "*Candidatus* Phytoplasma australiense".
5. Conduct workshops for potato growers and industry personnel on recognizing the tomato potato psyllid and infestations on "*Candidatus* Liberibacter solanacearum" and "*Candidatus* Phytoplasma australiense" in potato plants.

MATERIAL AND METHODS

Trap placement

Double sided, yellow sticky traps (each side = 15 x 10 cm) (Bugs for Bugs Ltd, Mundubbera, Queensland) (Fig. 1) were distributed to industry partners for placement in the field. Trapping was mainly conducted in the potato growing regions of northern Tasmania (Devonport-Ulverstone, Scottsdale-Herrick), south-eastern Tasmania (Swansea-Orford-Copping-Richmond), western Victoria (Ballarat) and South Australia (Penola) (Fig. 2). Traps were also occasionally placed in the potato growing regions of New South Wales (Riverina), eastern Victoria (Koo-wee-rup, Thorpedale), southern Queensland (Lockyer Valley), central Queensland (Bundaberg) and northern Queensland (Atherton Tableland) (Fig. 2). Trapping commenced in February 2011 and finished in March 2014, covering part of the 2010-11 potato growing season and the entire 2011-2012, 2012-13 and 2013-2014 growing seasons.

Fig. 1. A yellow sticky trap placed in a potato field to monitor for incursions of the tomato potato psyllid.

A lower number of traps (2-5 per region) were also operated outside the potato growing season and placed in or close to fallow potato fields. The number of fields that traps were placed in during the potato growing season varied from 5-25 per region. Traps were changed at approximately weekly or fortnightly intervals during the potato growing season and usually every 3-4 weeks at other times. Traps were suspended from wooden or metal stakes at crop canopy level. Between 15 February and 10 May 2011, two traps were placed in each potato field in north-west Tasmania (*n* = 36 trapping events) and the central Victoria (*n* = 45 trapping events) to compare the number of Psylloidea caught on traps placed at 1-2 m from the crop edge (within the crop) with those placed at least 100 m from the edge of the crop. In other districts and for other trapping dates, only one trap was placed in each field, at 1-2 m from the crop edge. After exposure in the field, plastic sheeting was placed over the sticky sides before folding together and returning the traps to TIA for screening for *B. cockerelli*.

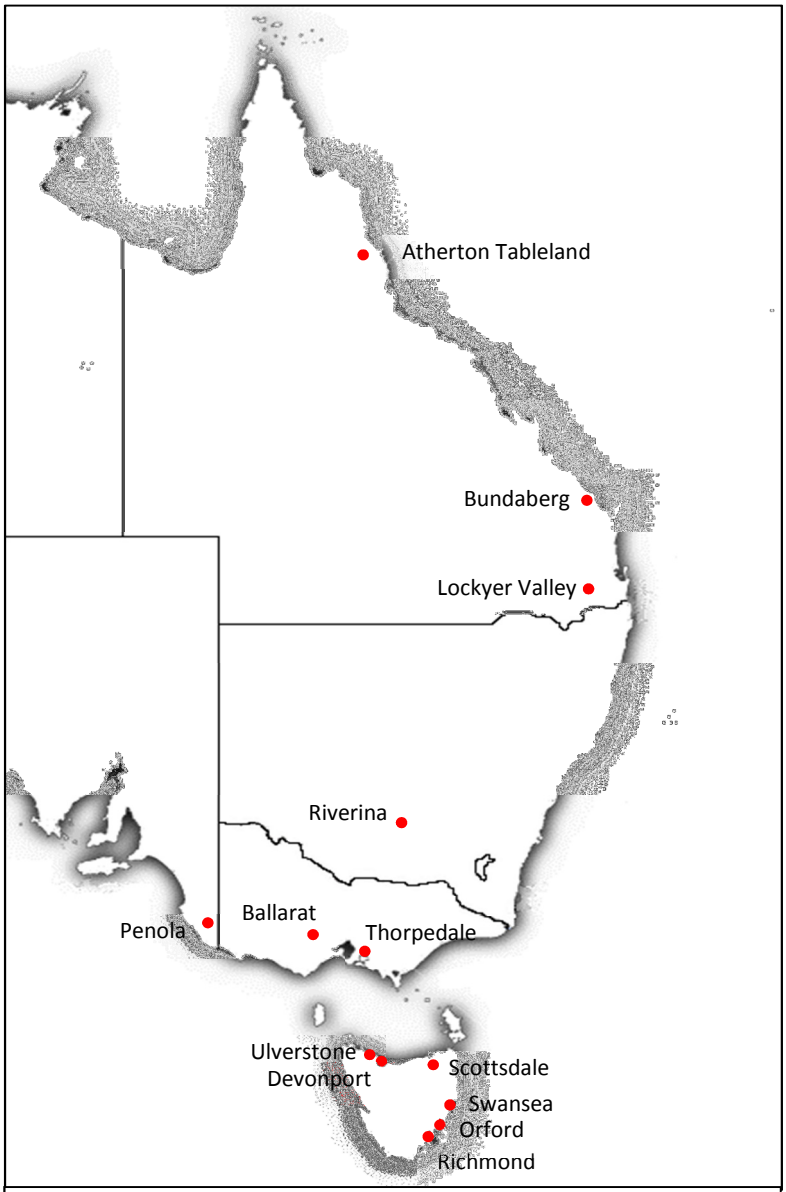

Fig. 2. Potato growing regions in eastern Australia where yellow sticky traps were placed to monitor for incursions of *Bactericera cockerelli* between February 2011 and March 2014.

Identification of psyllids

The number of Psylloidea caught on traps was counted under a binocular microscope. Trapped Psylloidea were distinguished from the genus *Bactericera*, which is absent in Australia, using the diagnostic characters and keys provided by the Australian Government Department of Agriculture Fisheries and Forestry (2012a) identification protocol. Briefly these are: trifurcate branching of the forewing basal vein; short and compact cubital cell; lack of well-developed genal cones; two inner apical spurs on hind leg tibia; distinctive white markings and absence of long setae on the vertex and dorsal thoracic surfaces; presence of a disk-shaped rhinarium on the fourth antennal segment and the shape of the genitalia.

Identification was aided by comparing trapped psyllids with validated voucher specimens of *B. cockerelli* obtained from New Zealand by Dr Alan Yen (Department of Primary Industries, Victoria). Representative specimens of the most common psyllid species caught were pinned and identified to the genus level using the keys of Hollis (2004) and verified by Dr Gary Taylor (University of Adelaide). Specimens were photographed using a digital microscope (Dino-Lite Basic microscope with DinoCapture 2.0 software, AnMo Electronics Corporation). Psyllid specimens that were very similar in appearance to *B. cockerelli* were sent to Dr Alan Yen (Department of Primary Industries, Victoria) for verification. Samples of psyllids were also sent to Dr Fiona Constable (Department of Primary Industries, Victoria) for molecular screening the presence of "*Candidatus* Phytoplasma australiense".

Data analysis

The number of Psylloidea caught/trap/day was averaged for each trapping period and trapping region. The frequency of captures of Psylloidea on traps was compared for each potato cropping region. Paired t-tests were used to compare the number of Psylloidea caught on traps placed near the crop edge with those placed towards the centre.

RESULTS

No *B. cockerelli* were caught on 2,319 yellow sticky traps placed in eastern Australian potato fields between February 2011 and March 2014. However, 9,665 other Psylloidea were caught on traps (Table 1) ranging in densities from 0 up to 22.2 psyllids/trap/day.

The number of psyllids caught on traps was generally low compared to other insects (Fig. 2). Trap catches usually predominantly consisted of various species of flies (Diptera), thrips (Thysanoptera), wasps (Hymenoptera), leafhoppers and aphids (Hemiptera), depending on the time of year. Traps that had been left in the field for more than *ca*. two weeks during spring and summer were more difficult to screen for psyllids due to the large number of other insects trapped. Putrefaction of trapped insects was also a problem in traps left in the field for more than two weeks, making identification of any psyllids caught difficult. Also, traps placed in or near fallow fields were often covered in soil particles which decreased their stickiness thus reducing their capacity to retain attracted insects.

Fig. 2. Typical yellow sticky trap catch with high numbers of flies (Diptera) and other insects.

The number of native Psylloidea caught on traps varied greatly within and between regions, and between trapping months and years (Table 1). Most traps had no (43%) or only one (20%) psyllid present (Fig. 3). Comparing regions, a higher proportion of traps in South Australia (81%) caught one or more Psylloidea than in other regions (46-59%) (Table 2). Occasionally, high numbers of psyllids were caught and this was more likely to occur in South Australia than in other regions (Table 2). The highest number of psyllids found on a single trap was 444 at Mingbool, near Penola, South Australia. Consequently, traps placed in potato fields in South Australia had the highest overall mean number of Psylloidea at 12.0 psyllids/trap, while traps in northern Tasmania and Victoria had similar numbers at 3.8 and 3.6 psyllids/trap, respectively, and south-eastern Tasmania the lowest at 2.1 psyllids/trap. In Queensland, the average number of Psylloidea caught was 0.3, 1.6 and 3.4 psyllids/trap in southern, central and northern potato growing areas, respectively. In the Riverina area of New South Wales, only two traps were operated in January 2012 and February 2014 and a total of two psyllids were caught.

Table 1. Number of traps and Psylloidea catches in potato growing regions of eastern Australia between February 2011 and March 2014.

	Potato growing region						
	Northern Tasmania	**South-eastern Tasmania**	**Victoria**	**South Australia**	**New South Wales**	**Queensland**	**All regions**
No. traps							
Year 1 2010/11	243	-	273	57	-	-	**573**
Year 2 2011/12	399	179	207	61	-	9	**855**
Year 3 2012/13	222	147	55	65	2	57	**548**
Year 4 2013/14	112	150	28	51	2	-	**342**
Total	**976**	**476**	**563**	**234**	**4**	**66**	**2319**
No. psyllids							
Year 1 2010/11	182	-	139	229	-	-	**550**
Year 2 2011/12	1864	364	551	204	-	97	**3080**
Year 3 2012/13	1506	251	910	1245	0	63	**3975**
Year 4 2013/14	160	379	397	1122	2	-	**2060**
Total	**3712**	**994**	**1997**	**2800**	**2**	**160**	**9665**
No. psyllids/trap							
Year 1 2010/11	0.8	-	0.5	4.0	-	-	1.0
Year 2 2011/12	4.7	2.0	2.7	3.3	-	10.8	3.6
Year 3 2012/13	6.8	1.7	16.6	19.2	0	0.8	7.3
Year 4 2013/14	1.4	2.5	14.2	22.0	1.0	-	6.0
All	**3.8**	**2.1**	**3.6**	**12.0**	**0.5**	**3.4**	**4.2**

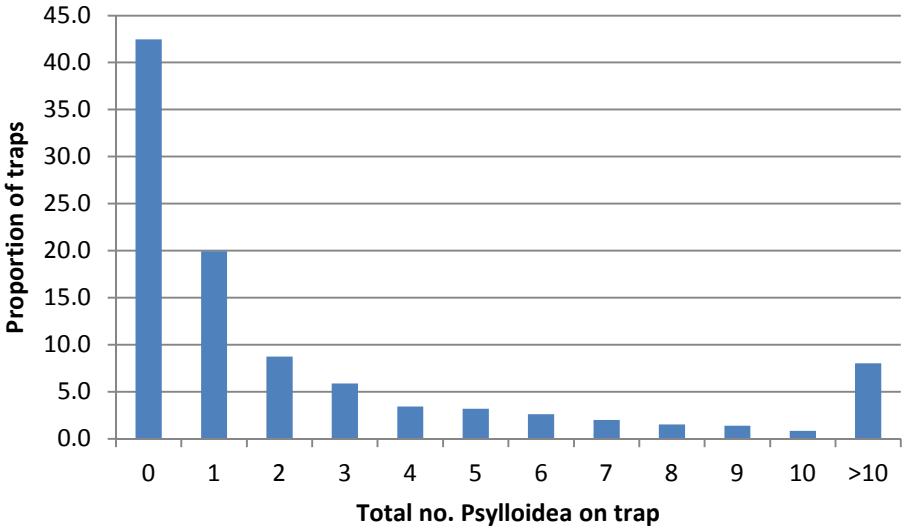

Fig. 3. Frequency of catches of native Psylloidea on yellow sticky traps for all regions combined.

Table 2. Frequency of catches of native Psylloidea on yellow sticky traps for each potato growing region sampled.

Total no. Psylloidea on traps	% traps with n Psylloidea				
	Northern Tasmania	South-eastern Tasmania	Victoria	South Australia	Queensland
0	40.7	46.5	50.7	19.0	54.5
1	21.3	17.8	20.6	16.9	19.7
2	8.4	10.0	7.1	11.7	9.1
3	5.3	8.0	4.1	9.1	3.0
4	4.2	3.0	2.1	5.2	0.0
5	3.1	3.3	2.3	6.5	0.0
6	2.4	3.5	2.1	3.9	0.0
7	2.1	2.0	1.6	2.2	3.0
8	1.2	2.0	1.1	3.0	1.5
9	1.7	1.1	0.5	2.6	1.5
10	0.9	0.0	0.5	3.0	0.0
> 10	8.6	2.8	7.1	16.9	7.6

In northern Tasmania, captures of Psylloidea peaked in November-December during 2011 and 2013, but peaked in February during 2012 (Fig. 4a), largely due to high captures at one location near Devonport. In south-eastern Tasmania, very low numbers of Psylloidea were caught over the potato growing season except for a single peak in captures in January 2012 (Fig. 4b). In Victoria, captures of Psylloidea peaked in mid-late October in 2011 and 2012, gradually declining through summer and

into early autumn (Fig. 4c). In 2013, cool, wet conditions in spring in the Ballarat region delayed the planting of potatoes and placement of traps until December, and peak captures of Psylloidea occurred in early January 2014 (Fig. 4c). In the Penola region, captures of Psylloidea were generally highest in September-October (Fig. 4d). In all regions, no or very low numbers of Psylloidea were caught during winter months. There was no significant difference in the number of Psylloidea caught on traps placed near the crop edge (mean = 0.456 psyllids/trap; ± 0.096 SE) with those placed 100 m from the crop edge (mean = 0.469 psyllids/trap; ± 0.108 SE) (Paired t-test, t = -0.088, p = 0.93, df = 80).

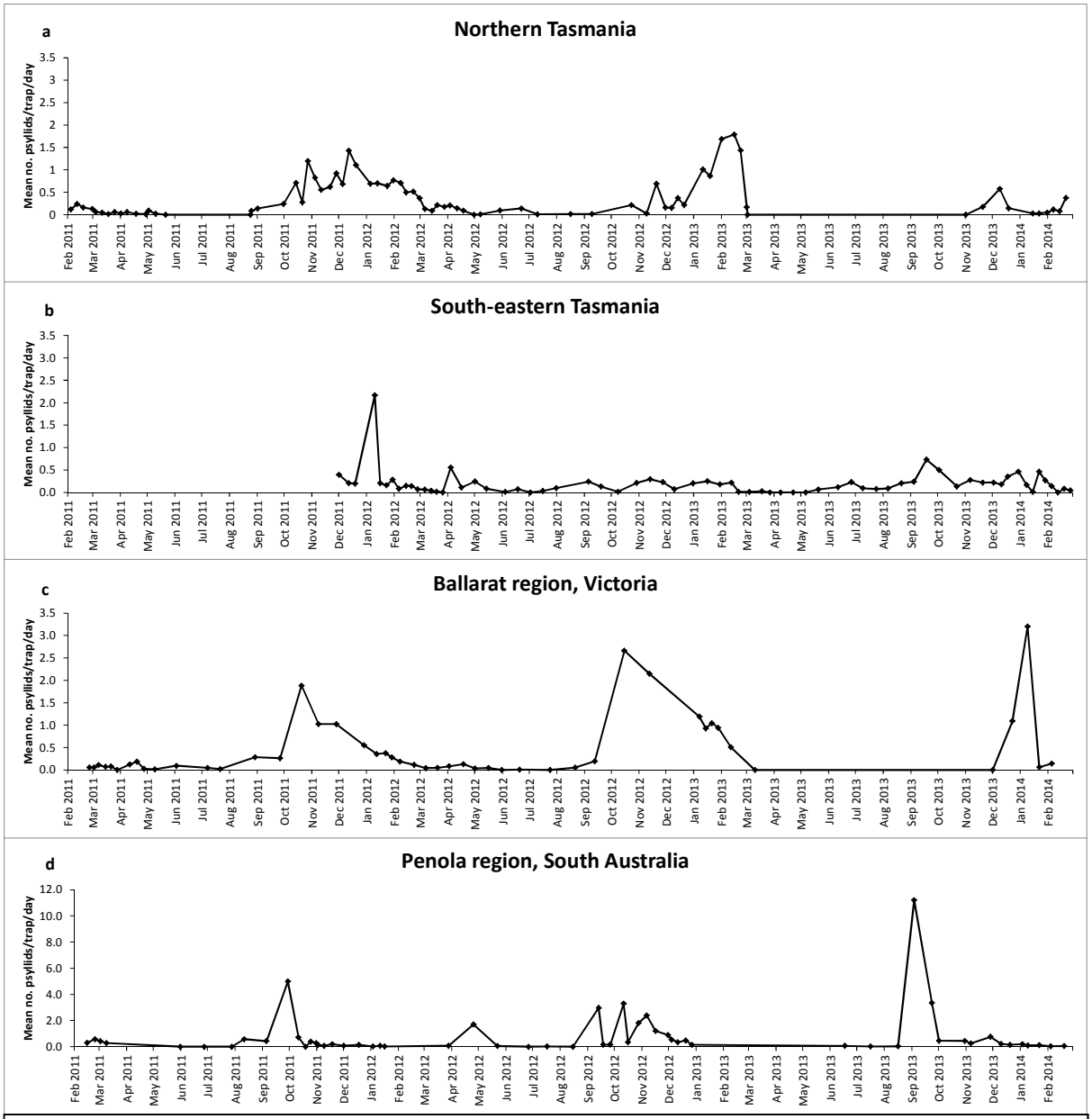

Fig. 4. Mean number of Psylloidea caught on yellow sticky traps/day placed in potato fields in eastern Australia between February 2011 and March 2014; a) northern Tasmania; b) south-eastern Tasmania; c) Ballarat region, Victoria; d) Penola region, South Australia. Note: the different scale used on the vertical axis in graph (d).

Over 99% of all Psylloidea caught belonged to the family Psyllidae. The dominant genera caught were *Ctenarytiana* and *Acizzia*. Other genera less frequently caught included: *Creiis*, *Cardiaspina*, *Anoeconeossa*, ?*Eucalyptolmya* (tentative generic identification), *Phyllolyma*, *Phellopsylla*, *Blastopsylla* and *Cryptoneosea/Ageteopsylla*. Photographs of representative specimens of psyllids from each genera, with notes on the regions they were collected from and their likely plant associations, are given in Appendix 1.

Only very low numbers (*n* = 25) of trapped psyllids belonged to the same family as *B. cockerelli*, the Triozidae. In the main trapping regions, the Triozidae represented from 0.14% (western Victoria) to 0.4% (northern and south-eastern Tasmania) of all Psylloidea caught. One Triozidae was caught in both northern and central Queensland but none were caught in eastern Victoria, southern Queensland and New South Wales where lower numbers of traps were operated for shorter periods of time. The genera of Triozidae caught (and likely plant associations in brackets) included *Trioza* spp. (host unknown), *Aacanthocnema dobsoni* (*Allocasuarina*), *Acanthocasuarina tasmanica* (*Allocasuarina*), ?*Cerotrioza* sp. (tentative generic identification, host unknown) and an undescribed genus (*Allocasuarina*). One *Trioza* spp. caught was very similar to *B. cockerelli* and was sent to Dr Alan Yen (DPI Victoria) who confirmed that it was an undescribed native species. Equal numbers of Triozidae were trapped in spring (44%) and summer (44%) with fewer being caught in autumn (8%) and winter (4%).

DISCUSSION

As predicted by Yen *et al*. (2012), yellow sticky traps used in this study to monitor for *B. cockerelli* incursions were also attractive to a large number of native species, thus requiring careful identification. However, as only 0.3% of all Psylloidea caught belonged to the family Triozidae, the vast majority of specimens could rapidly be discounted as *B. cockerelli* based on the branching of the forewing basal vein, which is trifurcate in the Triozidae and bifurcate in all other Psylloidea families (Hollis 2004). This character was easy to discern even on specimens in poor condition. Nevertheless, due to a lack of a comprehensive key to Australian Triozidae, species belonging to the genus *Trioza* could be very difficult to differentiate from *B. cockerelli*, requiring careful examination of the diagnostic characters as described in the identification protocol produced by the Australian Government Department of Agriculture Fisheries and Forestry (2012a). By world standards, the Australian Triozidae fauna is relatively poor, comprising 37 described species in seven genera (Taylor *et al*. 2013, Taylor *et al*. 2010). Currently, nine species of *Trioza* have been described in Australia with many more species awaiting description (Taylor *et al*. 2013).

We recommend that the trapping programme established during this project is continued and extended to include other solanaceous crops (see Recommendations). If an incursion of *B. cockerelli* originates from other host-plants, such as glasshouse crops, then placement of sticky traps in potato crops may not detect until psyllid populations are well established in an area. Therefore, it is essential that additional monitoring programmes are implemented to increase the chance of early detection. There is a need to extend the trapping programme to include all other indoor and outdoor commercial solanaceous crops grown in Australia (tomatoes, capsicums, eggplants, chillies and tamarillos) and other non-solanaceous crop host-plants of *B. cockerelli* such as sweet potato.

There is a need to be continually vigilant for the possible dispersal of *B. cockerelli* into Australia via easterly weather systems originating from New Zealand and to increase surveillance when such systems occur. At least two weather events occurred during this project (in 2011 and 2013) which were conducive for psyllid migration. During these events, we alerted industry partners to be extra alert for possible appearance of *B. cockerelli* in potato crops and to ensure traps were in place.

No Psylloidea in Australia are currently known to be associated with potato plants, therefore those caught on traps were likely to have originated from nearby vegetation, such as trees or weeds bordering crops (Appendix 1). Some of the *Acizzia* spp. trapped in northern Tasmania did resemble a newly described species group that are associated with solanaceous plants (G. Taylor, pers. comm. May 2014). Until recently, Australian species of *Acizzia* were mainly associated with host-plants belonging to the family Mimosaceae, particularly *Acacia* spp. (Hollis 2004). However, recently, three new species of *Acizzia* were found feeding on several solanaceous plants in Australia (Taylor & Kent 2013; Kent & Taylor 2010). *Acizzia solanicola* Kent & Taylor was recorded damaging eggplant in commercial crops and gardens and also occurring on wild tobacco bush (*Solanum mauritianum* Scop.), rock nightshade (*S. petrophilium*), cape gooseberry, *Brugmansia* sp. and *Datura* sp. in eastern Australia (Taylor & Kent 2013; Kent & Taylor 2010). *Acizzia alternata* Kent & Taylor feeds on *S. mauritianum* in eastern Australia while *Acizzia credoensis* Taylor & Kent was described from a single population feeding on *Solanum lasiophyllum* from semi-arid Western Australia (Taylor & Kent 2013; Kent & Taylor 2013). It is not known whether the recently described solanaceous-associated species

are native and recently switched hosts or were accidentally introduced from elsewhere (Kent & Taylor 2010). However, the occurrence of *A. credoensis* and *A. solanicola* on native *Solanum* spp. supports an Australian origin (Taylor & Kent 2013). While *A. solanicola* is a potential new pest of eggplant, preliminary caging trials suggest that they do not feed on tomatoes or capsicums (Kent & Taylor 2010) but further experiments are needed to test the host range of this species on other solanaceous plants, including potatoes. *Acizzia solanicola* was also recently detected in New Zealand in 2012 where the only known host-plants are *S. mauritianum* and eggplant (Taylor & Kent 2013; Anon. 2012). Establishment of *A. solanicola* in New Zealand has the potential to provide an additional vector of "*Ca*. L. solanacearum", although its ability to transmit the bacterium has yet to be assessed (Taylor & Kent 2013). Currently, psyllids belonging to the family Triozidae are the only known vectors of "*Ca*. L. solanacearum" (in potato and carrot plants). Further collections are of *Acizzia* spp. from northern Tasmania are in progress to confirm their identity, distribution and host-plant associations.

Initial speculation that *B. cockerelli* was responsible for the spread of "*Candidatus* Phytoplasma australiense" to potato plants in New Zealand, now appears to be unlikely (L. Liefting pers. comm. March 2014). The most probable vector is the highly polyphagous planthopper, *Zeoliarus oppositus,* which was previously known as the vector of the phytoplasma in other native and exotic plants in New Zealand (Liefting et al. 2011). This planthopper is absent from Australia. While the vector of "*Candidatus* P. australiense" in Australia remains unknown, no positive detections of the phytoplasma were evident from the psyllid samples commonly caught on sticky traps in this study sent to DPI Victoria for phytoplasmas testing.

In summary, this project provided assurance to potato industry stakeholders that incursions of the *B. cockerelli* in the major potato growing areas of eastern Australia were being continuously monitored using static insect traps. Incursion preparedness was increased through the training of personnel in identifying tomato potato psyllids and recognising symptoms of "*Ca*. L. solanacearum" infection in potato plants, as well as through the extension of the projects aims and results. The network of sticky traps established during the project found no evidence for the presence of populations *B. cockerelli* in potato fields. Baseline data on the type of native psyllids caught on traps was collected. All native psyllids caught were likely to have been incidental captures from nearby host plants and therefore, highly unlikely to transmit "*Ca*. L. solanacearum" (if the disease was to become established in Australia) or spread "*Candidatus* P. australiense" from other hosts to potato plants.

TECHNOLOGY TRANSFER

This project has successfully provided the potato industry with an early warning system to monitor for incursions of the tomato potato psyllid in the major potato growing areas of eastern Australia.

This project has also aided incursion preparedness by:

1. Creating scientific expertise within TIA in tomato potato psyllid identification and disease diagnostics.
2. Augmenting the awareness of tomato potato psyllid/Liberibacter threat through the extension of project aims and results to the potato industry, general public and scientific community at several meetings and through several publications (Table 3 and 4).
3. Providing training of potato industry field officers in the identification of psyllids and recognition of symptoms of tomato potato psyllid infestations in the field.
4. Providing training of potato industry field officers in recognizing symptoms of "*Candidatus* Liberibacter solanacearum" and "*Candidatus* Phytoplasma australiense" in the field.
5. Providing baseline data on native psyllids commonly caught in potato fields in eastern Australia and assessment of their potential for disease transmission.

Communication, training and extension activities over the life of the project are summarised in Table 3 and publications are listed in Table 4.

Table 3. PT10001 communication, training and extension activities.

Dates and personnel	Activity	Outcomes
14-18th February 2011 Calum Wilson, Paul Walker, Leonie White, Michael Hicks (Snack Brands Australia), Rod Lay (McCain Foods, Victoria), Andrew Vadenburg (Tasmania)	Discussions in New Zealand with main personnel involved in research on *B. cockerelli*/Liberibacter complex and field tour to examine affected potato fields	Links formed with key NZ researchers, training on psyllid/Liberibacter identification
7th March 2012 Paul Walker	Attended psyllid workshop at DPI Victoria, Knoxfield run by Dr A. Yen and Dr D. Burckhardt	Training on identification of exotic psyllid species
14th March 2012 Calum Wilson, Robert Tegg	Talk at the Potato Industry Extension Program workshop, Devonport, Tasmania	Dissemination of project aims and update on results to industry stakeholders
3rd September 2012 Calum Wilson, Paul Walker, Fiona Constable (DPI Victoria)	Workshop on psyllid identification and Liberibacter/Phytoplasma held at Ulverstone, Tasmania	Training workshop for Tasmanian potato industry field officers in identifying psyllids and recognising disease symptoms in plants
4th September 2012 Calum Wilson, Paul Walker, Fiona Constable (DPI Victoria)	Workshop on psyllid identification and Liberibacter/Phytoplasma held at McCain Foods, Ballarat, Victoria	Training workshop for Victorian/SA potato industry field officers in identifying psyllids and recognising disease symptoms in plants
15th November 2012 Calum Wilson	Talk and industry discussion at Potato Industry Extension Program field day, Atherton, Queensland	Dissemination of project aims and update on results to industry stakeholders
23rd November 2012 Paul Walker	Talk presented at TIA field day, Forthside Research Farm Poster presentation and leaflet handed out.	Dissemination of project aims and update on results to growers and other industry stakeholders
25-28th November 2012 Paul Walker	Poster presentation at Australian Entomological Society and Australian Arachnological Society Conference, Hobart	Dissemination of project aims and results to scientific community
26th February 2013 Paul Walker	Talk presented Potato Industry Extension Program workshop held in Ballarat, Victoria	Dissemination of project aims and update on results to industry stakeholders
12th March 2013 Paul Walker	Talk presented at Potato Industry Extension Program workshop held in Sassafras, Tasmania	Dissemination of project aims and update on results to growers and other industry stakeholders
19th June 2013 Calum Wilson	Talk presented at Potato R & D workshop held in Devonport, Tasmania	Dissemination of project aims and update on results to growers and other industry stakeholders
27th May 2014 Calum Wilson	Talk presented at the Potato Industry Extension Program workshop, Lockyer Valley, Queensland	Update of project findings to growers and other industry stakeholders

Table 4. PT10001 publications.

Authors	Title	Publication type
Calum Wilson	Knowing our psyllids	Article in Potatoes Australia, August/September 2011 edition, pp. 13-14.
Paul W Walker, Geoff R Allen, Robert S Tegg, Leonie R White and Calum R Wilson	Monitoring for incursions of the tomato potato psyllid (*Bactericera cockerelli*) in Australian potato fields	Poster presented at the Australian Entomological Society and Australian Arachnological Society Conference, Hobart, 25-28 November, 2012.
Calum Wilson and Paul Walker	Monitoring for tomato-potato psyllid	TIA Research Extension handout produced for extension meetings and workshops in April 2012.
Calum Wilson, Paul Walker, Geoff Allen, Robert Tegg and Leonie White	Monitoring for invasions of the tomato/potato psyllid, native psyllid populations and phytoplasmas in Australian potato crops	Factsheet posted on TIA website in 2012 and available from: http://www.tia.tas.edu.au/__data/assets/pdf_file/0008/354572/Monitoring-the-tomatopotato-psyllid.pdf
Calum Wilson	Native psyllid populations and the distribution of *Candidatus* Phytoplasma australiense	Article in Industry Advisory Committee Annual Report, 2012/13, p9.
Calum Wilson	Spotlight on R&D: keeping the psyllids at bay	Article in Potato Australia, December 2012/January2013 edition, p 19.
Calum Wilson and Paul Walker	Monitoring for incursions of the tomato-potato psyllid in Australian potato fields	Potato Industry Extension Program information leaflet, January 2013
Calum Wilson	Monitoring potato crops for psyllids	Article in Potatoes Australia, June/July 2013 edition, pp34-35.
Paul W Walker, Geoff R Allen, Robert S Tegg, Leonie R White and Calum R Wilson	Monitoring for incursions of the tomato potato psyllid, Bactericera cockerelli (Šulc) (Hemiptera: Triozidae), in Australian potato crops	Paper in prep. for submission to Austral Entomology, May 2014.

RECOMMENDATIONS

1. Continuation and extension of surveillance program. The current surveillance program using yellow sticky traps should be continued in order to provide an early-warning of *B. cockerelli* incursions in potato cropping regions. Additional monitoring programs should be implemented to include all other indoor and outdoor commercial solanaceous crops grown in Australia (tomatoes, capsicums, eggplants, chillies and tamarillos) and other non-solanaceous crop host-plants of *B. cockerelli* such as sweet potato.

2. Modelling. There is a need to model the potential distribution of *B. cockerelli* and "*Ca*. L. solanacearum" in Australia. The widespread distribution of host-plants of *B. cockerelli* and "*Ca*. L. solanacearum" in many parts of Australia would assist their spread were they to become established (Plant Health Australia 2011). Also, given the wide geographical distribution of the vector and pathogen in North America, Central America and New Zealand, it is likely that large areas of Australia have climatic conditions suitable for population persistence. Although *B. cockerelli* is not considered to be very cold tolerant, overwintering populations have recently been found in north-western parts of the USA (Idaho, Oregon and Washington) (Jensen *et al*. 2012). In New Zealand, *B. cockerelli* has been collected as far south as Invercargill on the South Island (Vereijssen *et al*. 2012) but it is unclear whether populations are able to persist in this region during winter. Initial studies on the population dynamics of *B. cockerelli* in New Zealand suggest that warm/dry springs and summers result in higher psyllid numbers than wet/cold ones while high rainfall appears to be detrimental to population build-up (Tran *et al*. 2012; Vereijssen *et al*. 2012). A first-guess, *B. cockerelli* CLIMEX model projection for New Zealand has been produced but needs refinement to fully explain where psyllids have been found in the field and to explain differences in populations between regions (Vereijssen *et al*. 2012; D. Logan pers. comm. March 2014).

3. Migration alerts. There is a need to continually monitor for the possible dispersal of *B. cockerelli* into Australia via easterly weather systems originating from New Zealand. A formal system of monitoring such weather systems should be established and to issue warnings to key solanaceous crop industry stakeholders to increase surveillance for *B. cockerelli* incursions when such systems occur. Alerts could be published on electronic media via the Ausveg and TIA websites.

ACKNOWLEDGMENTS

We thank all growers who participated in the trapping program, and all personnel from the industry partners (Simplot Australia, McCain Foods, Smiths Snackfood Company and Snack Brands) and TIA who organised the placement and retrieval of traps. We also thank industry partners and HAL for funding; Dr Iain Kirkwood (Eurogrow Potatoes Ltd, New Zealand) for formulating the original grant application; Dr Gary Taylor (University of Adelaide), Dr Alan Yen (Biosciences Research Division, Department of Primary Industries, Knoxfield) and Mr Lionel Hill (Department of Primary Industries, Parks, Water & Environment, Tasmania) for assisting in the identification of psyllids; and Dr Fiona Constable (DPI Victoria) for assisting in Phytoplasma bioassays and workshops.

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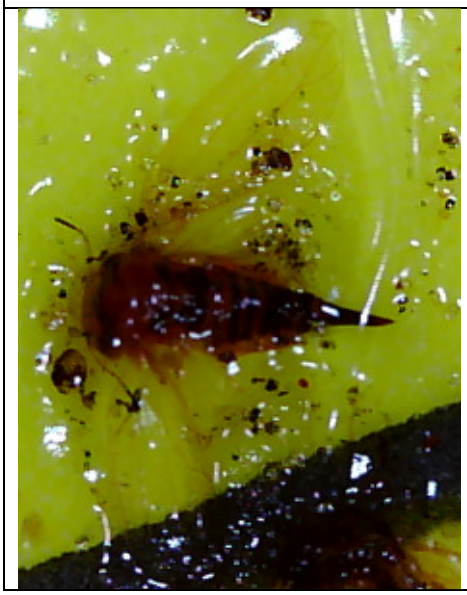
APPENDICES

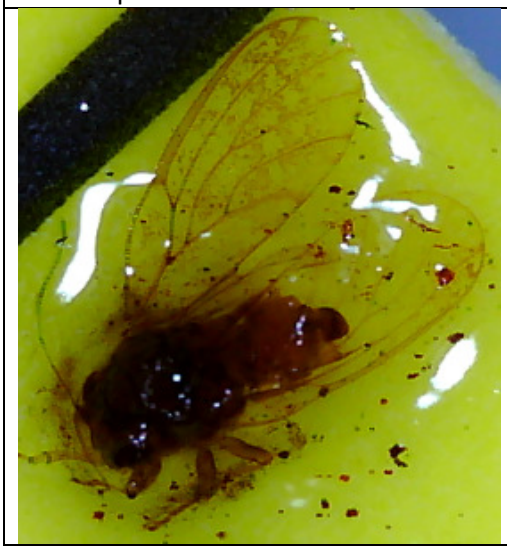
Appendix 1: Psylloidea genera caught on yellow sticky traps

The following photos illustrate the main Psylloidea genera and morpho-species caught on yellow sticky traps placed in potato growing regions of eastern Australia. Identification of all psyllids was verified by Dr Gary Taylor, University of Adelaide, who also gave likely plant associations.

Family: Psyllidae

1. *Ctenarytaina* sp.1	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	NW Tasmania SE Tasmania Victoria South Australia Queensland

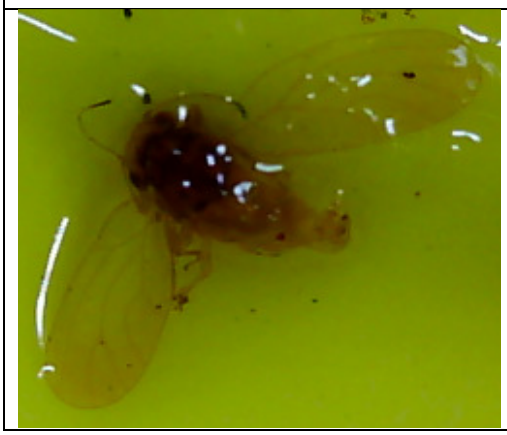
Ctenarytaina sp.2	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	SE Tasmania

Acizzia sp. 1	Plant associations	Regions collected
	*Acacia* or other Mimosaceae	NW Tasmania, SE Tasmania, South Australia, Victoria, Queensland

Acizzia sp. 2	Plant associations	Regions collected
	Solanaceae spp.?	NW Tasmania

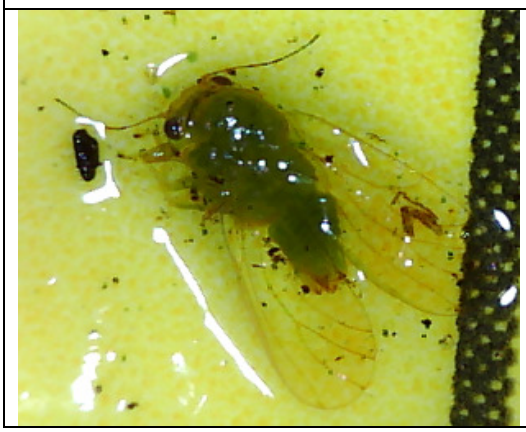
Acizzia sp. 3	Plant associations	Regions collected
	*Acacia* or other Mimosaceae	NW Tasmania

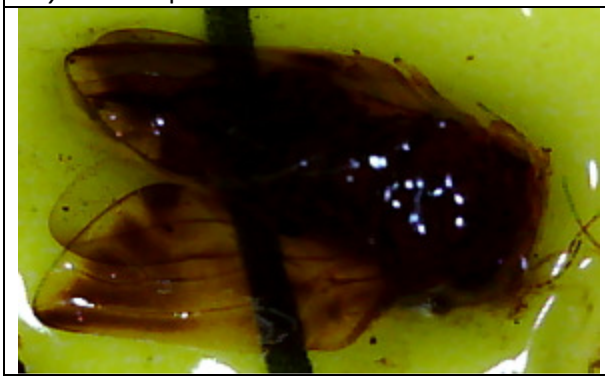
Acizzia sp. 4	Plant associations	Regions collected
	*Acacia* or other Mimosaceae	NW Tasmania

Acizzia sp. 5	Plant associations	Regions collected
	*Acacia* or other Mimosaceae	NW Tasmania

Acizzia sp. 6	Plant associations	Regions collected
	*Acacia* or other Mimosaceae	SE Tasmania

Acizzia sp. 7	Plant associations	Regions collected
	*Acacia* or other Mimosaceae	SE Tasmania

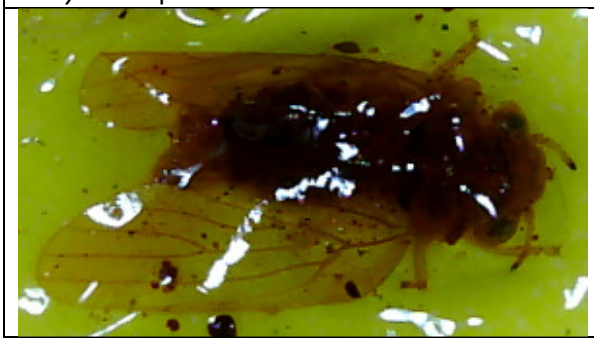
Creiis sp.	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	SE Tasmania

Phylloloma sp.	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	NW Tasmania

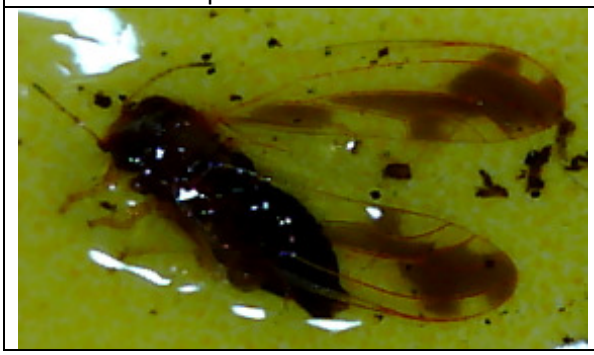
Blastopsylla sp.	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	NW Tasmania

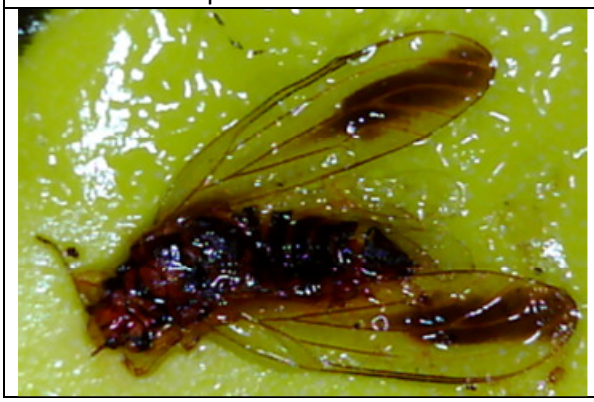
Cardiaspina sp.	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	South Australia

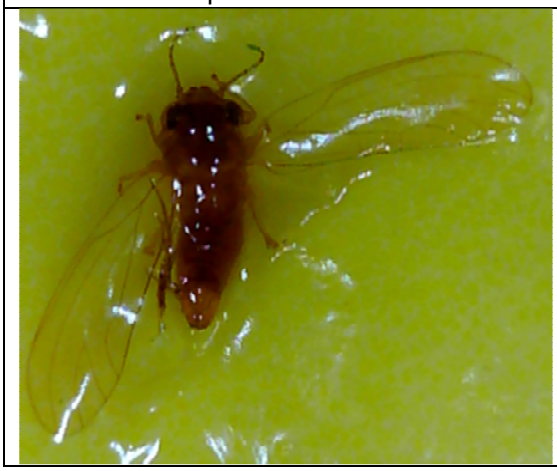
Phellopsylla sp.	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	South Australia

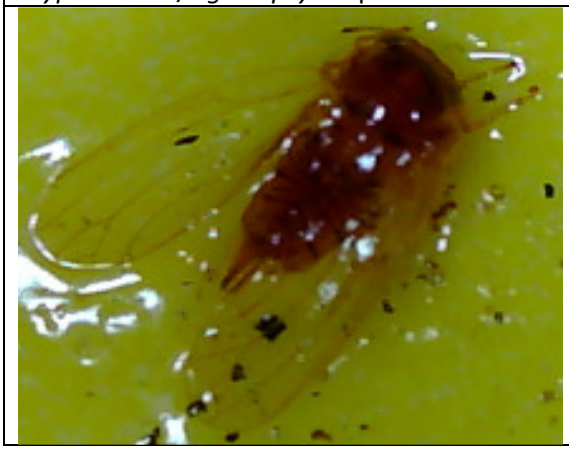
Platyobria sp.	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	NW Tasmania

Anoeconeossa sp.1	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	NW Tasmania, SE Tasmania

Anoeconeossa sp.2	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	SE Tasmania

Anoeconeossa sp.3	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	SE Tasmania

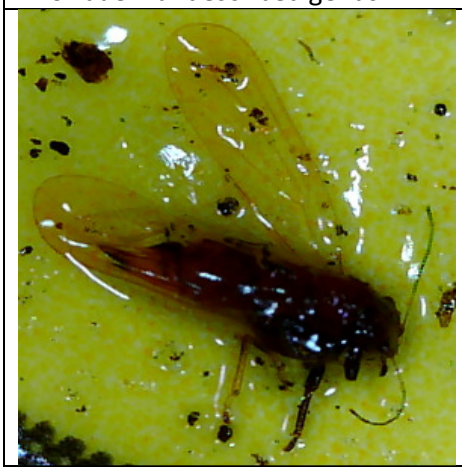
Anoeconeossa sp.4	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	Queensland

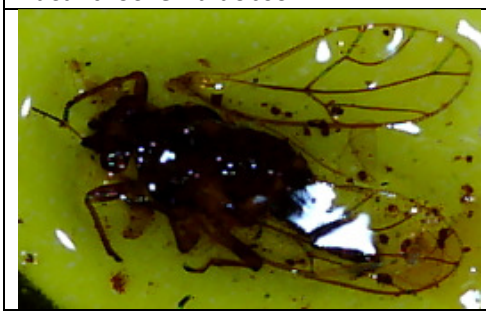
Cryptoneossa/Ageleopsylla sp. 1	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	Queensland

Cryptoneossa/Ageleopsylla sp. 2	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	NW Tasmania

?Eucalyptolyma sp.	Plant associations	Regions collected
	*Eucalyptus* spp. or other Myrtaceae	Queensland

Family: Triozidae

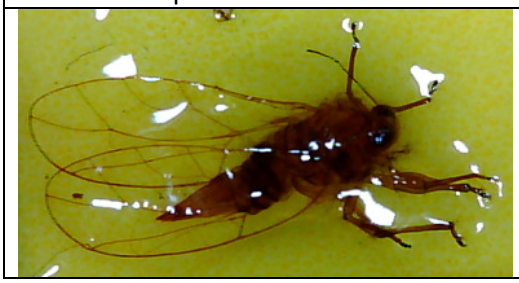
Triozidae – undescribed genus	Plant associations	Regions collected
	*Allocasuarina*	SE Tasmania

Aacanthocnema dobsoni	Plant associations	Regions collected
	*Allocasuarina*	NW Tasmania

Acanthocasuarina tasmanica	Plant associations	Regions collected
	*Allocasuarina*	NW Tasmania

Trioza sp. 1	Plant associations	Regions collected
	Unknown but highly expected to be incidental capture from other host plants and not on potatoes	NW Tasmania

Trioza sp. 2	Plant associations	Regions collected
	Unknown but highly expected to be incidental capture from other host plants and not on potatoes	Queensland

?Cerotrioza sp.	Plant associations	Regions collected
	Unknown but highly expected to be incidental capture from other host plants and not on potatoes	NW Tasmania

A review of the tomato-potato psyllid (*Bactericera cockerelli*) and associated plant diseases

Paul Walker, Robert Tegg & Calum Wilson

Tasmanian Institute of Agriculture, University of Tasmania

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1. Summary

Since arriving in New Zealand the tomato potato psyllid (*Bactericera cockerelli*) and the associated *Candidatus* Liberibacter solanacearum have cost the potato industry at least NZ$120 million. The tamarillo industry has also been devastated with many growers exiting the industry. Tomato and capsicum growers have sustained lesser impacts, but successful IPM programmes have been disrupted.

This literature review aims to provide a current overview of the key aspects of this key horticultural pest and associated diseases. This includes chapters on:

- Detection, spread and economic impact of *B cockerelli* on New Zealand horticulture
- An outline of the biology of *B cockerelli*
- Major disease symptoms induce by *B cockerelli* and the bacteria it vectors
- An overview of control strategies for *B cockerelli*
- An assessment of psyllid monitoring techniques
- A review of phytoplasmas and Liberibacter

We also include a reference list of known *B cockerelli* plant hosts.

2. Detection and spread of *B. cockerelli* in New Zealand

The following account of the detection and spread of *B. cockerelli* and Liberibacter in New Zealand is largely taken from Teulon et al., (2009) with additional notes from published and unpublished sources.

The first authenticated record of *B. cockerelli* in New Zealand was from specimens collected from glasshouse tomatoes in Clevedon, near Auckland, in March 2006, but this sample was not submitted for identification until May 2006 (Teulon et al., 2009). However, several authors believe that *B. cockerelli* may have arrived in New Zealand in 2005. Further psyllid samples from tomatoes were collected by staff from Investigation and Diagnostic Centres, MAF Biosecurity NZ (MAFBNZ), from inside a greenhouse complex in Kumeu, northwest Auckland, in May 2006. MAFBNZ staff identified *B. cockerelli* and this was later validated by US Department of Agriculture Systematic Entomology Laboratory scientists (Gill, 2006). A delimiting survey in the Auckland region showed that *B. cockerelli* was present in several glasshouse facilities around Auckland, including Karaka south of Auckland (Gill, 2006). Retrospective examination (in November 2008) of insect samples from a 7.5 m high suction trap at the Pukekohe Research Centre indicated the presence of *B. cockerelli* outside in Pukekohe, South Auckland, in the week ending 18 April 2006 (Teulon et al., 2009). Additionally, an examination of volunteer potato plants remaining in a field in South Auckland in June 2006 also confirmed this crop to be infested with *B. cockerelli* (Gill, 2006). The wide distribution, lack of linkages between infested sites, and the unknown pathway of entry into New Zealand for TPP led MAFBNZ to conclude that this pest was well established in New Zealand and eradication was not feasible (Gill, 2006). This decision was made before the discovery of the liberibacter vectored by *B. cockerelli* (see below) and the subsequent understanding of its potential negative impact on a range of solanaceous crops in New Zealand and elsewhere (Teulon et al., 2009).

From January to May 2008, disease symptoms similar to 'psyllid yellows' were observed in several TPP-infested commercial glasshouse tomato crops in Auckland with associated crop losses of up to NZ$1 million (Liefting et al., 2009a). Initial extensive diagnostic testing of symptomatic plants for a range of known plant pathogens (i.e. fungi, bacteria and viruses) all proved negative (Liefting et al., 2009a). However, in April 2008, similar symptoms appeared in a glasshouse capsicum crop on the same property as one of the infected tomato crops. Capsicum is not usually known to be susceptible to psyllid yellows (Liefting et al., 2009a). Additional investigation using transmission electron microscopy, polymerase chain reaction (PCR) and sequence analysis led to the discovery of a bacterium new to science that was named 'Candidatus Liberibacter solanacearum' (Liefting et al., 2009a, Liefting et al., 2009b). Hansen et al., (2008) had also reported Candidatus Liberibacter psyllaurous on potato and tomato with 'psyllid yellows' in the USA and shown TPP to be its vector. The 16S rRNA sequences of the 'Candidatus Liberibacter' species from USA (Hansen et al., 2008) and New Zealand (Liefting et al., 2009a, Liefting et al., 2009b) were found to be identical. In June 2008, New Zealand notified its trading partners that a new "Candidatus Liberibacter sp." had been confirmed to be affecting tomato and capsicum crops grown in greenhouses in the North Island (Biosecurity Australia, 2009).

Samples from potato tubers harvested from a breeding trial in May 2008 at Pukekohe showing symptoms of zebra chip (see below) also tested positive for liberibacter (Anderson, 2008, Liefting et al., 2008). Subsequently, Abad et al., (2009) found the same liberibacter species in potato plants infected with zebra chip disease in the United States. In October 2008, some commercial growers also began to notice unusual leaf curling symptoms in Ranger Russet potato crops at about 3-4 weeks post emergence. The symptoms resembled Potato Leaf Roll virus but with purpling. Over the next few months, several more main crop potatoes developed these symptoms and as the season progressed other disease symptoms (yellowing of leaves, aerial tubers, death of stems) were noticed. In March 2009, the Simplot processing factory in Auckland began noticing lower than normal dry matters as well as darkened vascular rings and burning of tubers. A low percentage of tubers also displayed dark streaking, consistent with zebra chip disease seen in the USA and Mexico.

3. Economic impact of *B. cockerelli* and Liberibacter in New Zealand

The following account of the impact of *B. cockerelli* and Liberibacter in New Zealand is also largely taken from Teulon et al. (2009) with additional notes from published and unpublished sources.

3.1 Impact on greenhouse crops

The potential for *B. cockerelli* to become a major pest in greenhouse crops was realised early by industry leaders and it became a major feature of greenhouse crops research initiatives at Plant & Food Research and Horticulture New Zealand. The research emphasis has been on the effective use of insecticides (see section 7) and exploration for biological control agents within New Zealand and overseas (see section 7). Effective psyllid management has now become critical for maximising crop production and quality and to ensure market access for export fruit. Many growers are forced to use pesticides toxic to beneficial insects, thus jeopardising the ongoing development of Integrated Pest Management and biological control in New Zealand greenhouse crops.

3.2 Impact on potato crops

Retrospective analysis of trial data by Anderson (2008) attributed poor potato seedling establishment in potato breeding trials at Pukekohe in March 2007 and March 2008 at least in part to psyllid damage. Additionally, potatoes from main-crop trials harvested in late May 2008 at Pukekohe had extremely poor tuber quality, yields, dry matter levels and a range of symptoms, including indications of zebra chip, typical of psyllid infestation (Anderson 2008). In the 2007-08 season, Anderson (2008) considered that potato growers may have been protected from serious psyllid damage by regular applications of insecticide to control tuber moth.

Industry initiatives for sustainable psyllid control began in November 2008 with the publication of an interim resistance management strategy (Anon., 2008a). In 2008-09, symptoms including leaf curling, purpling and stunting of potato crops in the presence of psyllids were common in some North Island potato crops, but these symptoms were not seen in South Island crops even with psyllids present.

In 2008-09, the estimated cost of the potato psyllid/Liberibacter/Phytoplasma complex to the New Zealand potato industry was NZ$46m through reduced yields, rejects from processing and additional control costs (too late to save crops) (M. Spencer, unpublished PowerPoint presentation). Experience from this season suggested that the only effective option to minimize the effect was the regular application of insecticides (Psyllid News, November 2009). Accordingly, in 2009-10 and 2010-11, while there were no devastating crop losses there was a considerable rise in production costs due to the increased amount of insecticides applied to crops in order to reduce psyllid numbers (weekly or fortnightly applications, often exceeding NZ$1,200/ha). Although the increased spraying regimes (see section 7.2) appeared to provide good control of psyllids, the effect of the psyllids/Liberibacter complex on yields and tuber quality was still evident in the 2010-11 season. Furthermore, additional costs were incurred through the Auckland Simplot processing factory having to employ extra staff to handle the situation and for grading out infected tubers. In the 2011-12 season the wetter conditions have contributed to a better season with reduced psyllid numbers and a lesser requirement for insecticide, although still approximately an average of seven insecticide sprays have been typically used (Frank Mulchay, Simplot Australia, *pers. com.*).

3.3 Impact on outdoor tomato crops

During 2007-08 major problems with *B. cockerelli* were first noticed on outdoor tomatoes in Hawke's Bay, with severe yield effects (Teulon et al., 2009). Significant impacts on yields and production costs were also observed in the processing tomato industry in 2008-09. The need for intensive agrichemical control programmes for *B. cockerelli* control on outdoor tomatoes, until new biological control practices are developed, is likely to severely disrupt the very effective IPM programmes established for this crop in New Zealand (Cameron et al., 2009, Teulon et al., 2009).

3.4 Impact on export trade

On 4 June 2008, after the discovery of liberibacter in greenhouse tomatoes and capsicums in New Zealand, MAFBNZ withdrew its phytosanitary certification for New Zealand fresh tomatoes and capsicums. This resulted in the immediate cessation of trade for these and other solaneceous fruits to key export countries such as Japan, Australia and the Pacific Islands (Teulon et al., 2009). On 17 July 2008, MAFBNZ reinstated its phytosanitary certification for New Zealand-grown tomato and capsicum export produce. Japan reopened its market for New Zealand export tomatoes and capsicums on 12 August 2008. The Australian market was not re-opened until early December 2008 and then with a range of new requirements to ensure that *B. cockerelli* did not enter Australia (MAFBNZ, 2008). The revised emergency measures allowed imports of tomato and capsicum from New Zealand, subject to demonstrated control of the psyllid population in production sites (greenhouses) and mandatory methyl bromide fumigation. Access for tomatoes and capsicums was still closed for Fiji in March 2009. With the discovery of the liberibacter in potato in New Zealand, MAFBNZ continued to issue phytosanitary certification (with the exception of French Polynesia) for potatoes, citing a greater understanding of the risks associated with *B. cockerelli* /liberibacter on potato. Fiji suspended import permits for potatoes from New Zealand for most of July 2008 subject to New Zealand potatoes meeting their specified conditions to ensure *B. cockerelli* did not enter Fiji. Additionally, exports of potatoes to French Polynesia were halted for just over 1 week.

Reduced export receipts of New Zealand capsicums of around $NZ5.22m in 2008 were attributed primarily to the effects of the psyllid/liberibacter incursion on (1) closing the main export markets and (2) the time needed to regain access to them (Teulon et al., 2009). Additionally, Robertson (2009) considered that the 2008 closure of New Zealand's principle export markets as a result of TPP/liberibacter cost the greenhouse tomato industry in the order of $NZ3 million in lost exports alone.

3.5 Other impacts

Another impact was the need to generate additional funding, resources and infrastructure to support research on the emerging psyllid/Liberibacter problem. In the 2009/10 financial year, over $NZ1.2 million was spent on psyllid research in New Zealand via two main research streams: the Sustainable Psyllid Management project under the MAF Sustainable Farming Fund; and the Plant & Food Research's internal research programme (Psyllid News, July 2010). Additional research projects were also funded by Plant & Food Research via other funding sources eg Potatoes NZ, Lincoln University, Massey University, agrichemical companies and supply companies (Psyllid News, July 2010).

In 2010, Potatoes NZ initiated a voluntary fund for research on the *B. cockerelli* and Liberibacter problem. They requested that everyone in the potato value chain (growers, merchants, retailers, processors and exporters) contribute $NZ1 per tonne of potatoes harvested or handled in 2010 to the voluntary fund. It was estimated that $NZ5 million of research funding might be needed in the following five years. It was hoped that the voluntary funds could be used to leverage additional research funds from NZ Government agencies (Psyllid News, February 2010). By January 2011, over $NZ296K had been raised for the Psyllid voluntary contribution (Psyllid News, January 2011).

4. Biology of *Bactericera cockerelli*

4.1 Taxonomy and nomenclature

Order:	Hemiptera
Suborder:	Sternorrhyncha
Superfamily:	Psylloidea
Family:	Triozidae
Subfamily:	Bactericerinae
Genus:	*Bactericera*
Species:	*cockerelli*
Synonyms:	*Paratrioza cockerelli, Trioza cockerelli*

The superfamily Psylloidea contains six families (Carsidaridae, Phacopteronidae, Psyllidae, Calophyidae, Homotomidae and Triozidae). There are approximately 40 species of Triozidae in Australia (A. Yen pers. comm.) but none belong to the genus *Bactericera*. Worldwide, there are approximately 28 species of *Bactericera*.

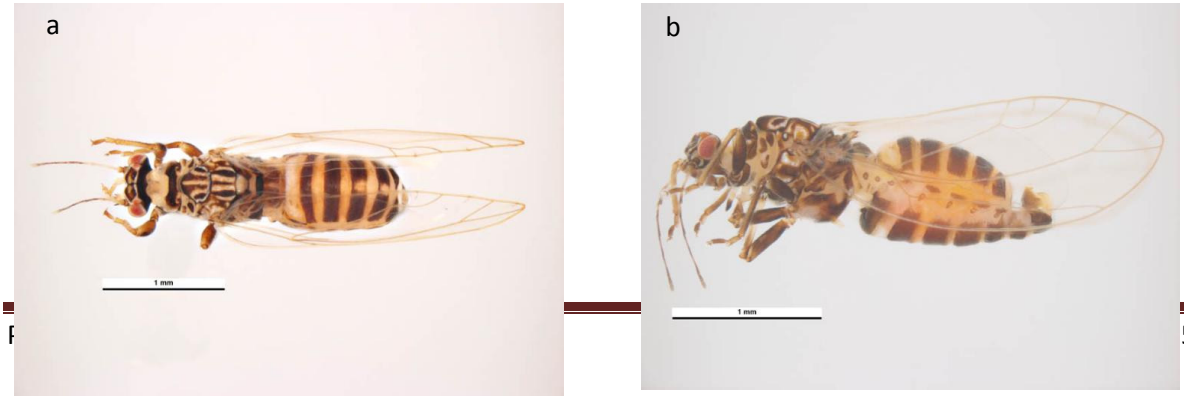
T. D. Cockerell collected the first specimen of *B. cockerelli* on cultivated capsicums in Boulder, Colorado, USA, in 1909, which was subsequently described by Sulc as *Trioza cockerelli* (Pletsch, 1947). Crawford (1911) later assigned the species to the genus *Paratrioza* that he proposed in 1910. Burckhardt & Lauterer (1997) synonymised the genus *Paratrioza* with *Bactericera*.

Common names include: 'tomato psyllid', 'potato psyllid', 'tomato/potato psyllid', 'potato/tomato psyllid' and 'potato and tomato psyllid'. Currently only the first two are formally recognized in the Common Names of Insects & Related Organisms produced by the Entomological Society of America (Stoetzel, 1989). In New Zealand it is commonly referred to as the 'potato psyllid', 'tomato potato psyllid' or TPP for short.

4.2 Identification

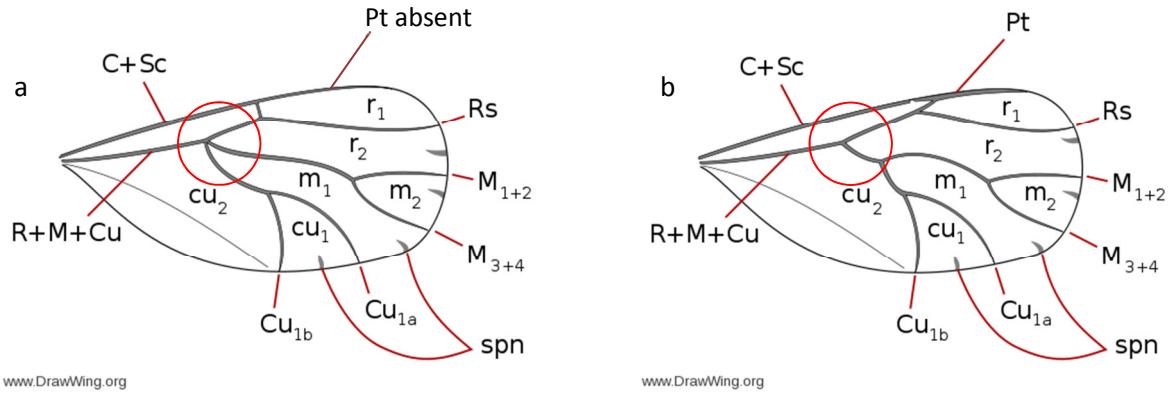
A key to distinguish *B. cockerelli* from other psyllid species occurring in Australia is currently being written (A. Yen pers. comm.). Images of *B. cockerelli* adults are available on the Pest and Disease Image Library (PaDIL) website (http://www.padil.gov.au/). A poster published by Dale and Nielsen, giving the main characteristics for distinguishing TPP from other psyllids in New Zealand, is available on the New Zealand Institute for Plant & Food Research Ltd website (URL).

Figure 1. *B. cockerelli* : a) female dorsal view; b) male lateral view (source: PaDIL web site, http://www.padil.gov.au/).



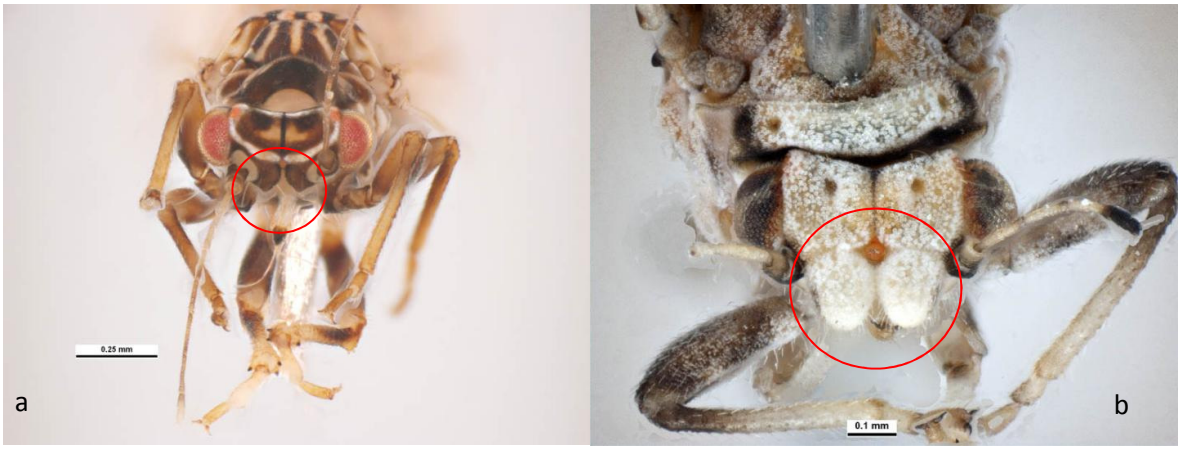
Species belonging to the same family as *B. cockerelli* (Triozidae) can readily be distinguished from other psyllid families by the presence of trifurcate branching on the basal vein of the forewing and the absence of a pterostigma (Fig. 2a). In other psyllid families the basal vein is always bifurcate and the pterostigma is usually present (Fig. 2b).

Figure 2. a) typical *Triozidae* forewing with trifurcation of veins R+M+Cu1 (red circle) and absence of pterostigma; b) typical *Psyllidae* forewing with bifurcation of veins R+M+Cu1 (red circle) and presence of pterostigma (Pt) (source: www.DrawWing.org).



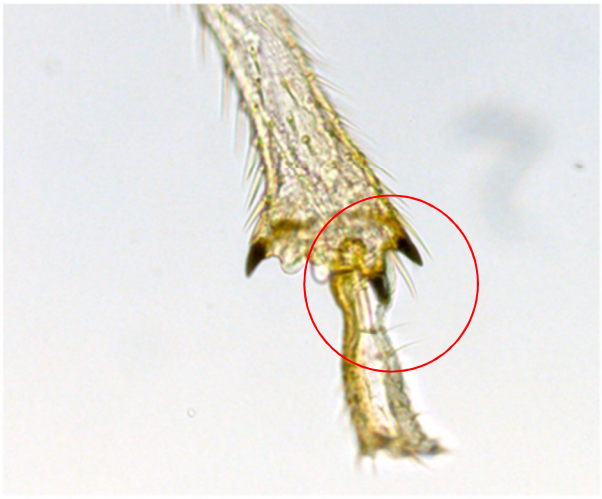
Within the Triozidae, the subfamily Bactericerinae (absent from Australia) have short genal cones (Fig. 3a) compared to the Triozinae and several other species of psyllids which have well developed genal cones (Fig. 3b).

Figure 3. a) short genal cones on *B. cockerelli* (circled); b) well developed genal cones on *Diaphorina citri* (Psyllidae).



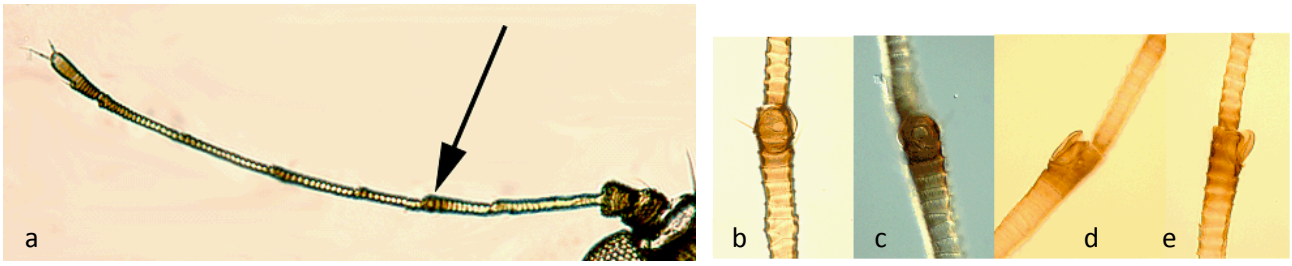
An additional distinguishing feature of the genus *Bactericerca* is the presence of only 2 of inner apical spurs on the tibia of the hind legs as opposed to 3 in other genera (Fig. 4).

Figure 4. *B. cockerelli* hind-leg tibia with 2 inner apical spurs (source: New Zealand Institute of Plant & Food Research).



Another unique feature of *B. cockerelli* is the presence of a specialised disk-shaped organ (rhinarium) on the fourth antennal segment (Fig. 5). Also, the wing membrane of *B. cockerelli* is transparent with no colour patterns and the cubital cell (Cu) is short and compact.

Figure 5. Antenna of *B. cockerelli* showing disk-shaped rhinarium on the fourth segment: a), b and c) dorsal view; d) and e) lateral view (source: Alan Yen, DPI Victoria).



4.3 Description and life history

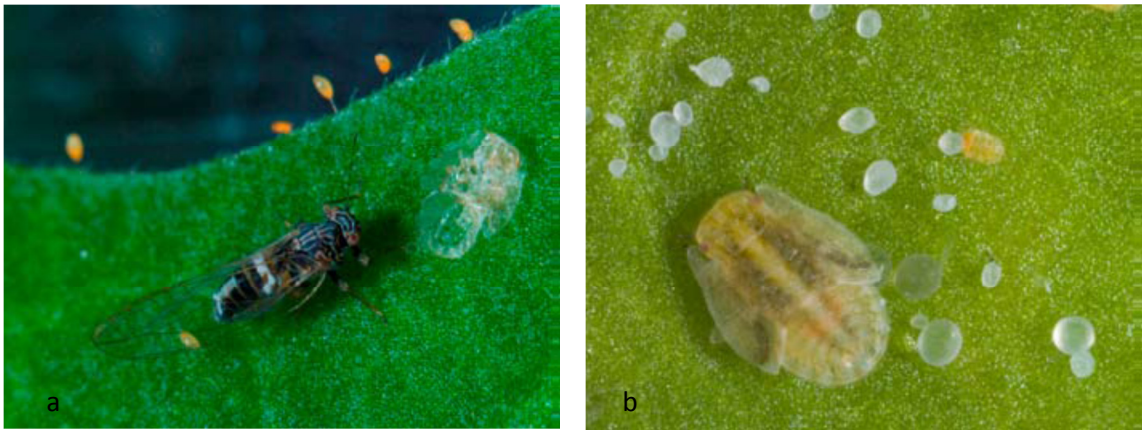
Adult *B. cockerelli* are about the size of an adult aphid but look like tiny cicada under magnification (Fig. 6a). The female lays yellow eggs that are attached by short stalks to plant leaves, usually to the leaf edges (Figure 6a). Psyllid nymphs are flat scale-like insects (Fig. 6b) which are usually inactive but move when disturbed. The nymphs moult five times before reaching the adult stage. Both nymphs and adults feed by sucking plant juices, which is how they are thought to spread Liberibacter.

Developmental times, reproductive performance and longevity of *B. cockerelli* vary according to the host plant and rearing temperature. Psyllid development occurs between 15.5°C and 32.2°C with optimum development occurring at 26.6°C. In the laboratory, development was faster on potato plants than eggplant or capsicum (Yang et al., 2010, Yang & Liu, 2009) (Table 1).

Table 1. Mean life history parameters for *B. cockerelli* reared in the laboratory on three host plants at 26.7 ± 2°C constant. Data from Yang & Lui (2009) and Yang et al. (2010).

	Host plant		
Parameter	Potato	Capsicums	Eggplant
Egg development (d)	4.4	5.9	5.0
Nymphal development (d)	15.2	20.2	19.1
1st instar	3.1	5.7	5.0
2nd instar	2.4	3.1	3.2
3rd instar	2.7	3.0	3.1
4th instar	2.7	3.1	3.2
5th instar	4.3	5.3	4.7
Egg to adult (d)	22.4	26.2	24.1
Preoviposition period (d)	8.4	8.0	8.8
Oviposition period (d)	43.9	47.0	53.4
Fecundity (n)	399.7	403.6	338.1
Male longevity (d)	35.3	53.9	39.4
Female longevity (d)	62.1	55.0	62.2

Figure 6. a) Adult *B. cockerelli* and eggs laid on leaf margin; b) Nymphs and psyllid sugars



Nymphs and adults secrete plant sap as white granules called 'psyllid sugars' (Figs. 6b & 7). In humid conditions and where there are large numbers of psyllids, black sooty mould fungi can grow on the sugars. Dense sooty mould on leaves may reduce photosynthesis, but this is rarely a problem on outdoor plants as the psyllid sugars are usually removed by wind and rain.

Figure 7. Psyllid sugars on glasshouse grown capsicum

4.4 Distribution of *B. cockerelli*

Bactericera cockerelli is thought to have originated in North America. In the USA it is found in Arizona, California, Colorado, Idaho, Kansas, Minnesota, Montana, Nebraska, Nevada, New Mexico, North Dakota, Oklahoma, South Dakota, Texas, Utah and Wyoming (Blood et al., 1933, Carter, 1954, EPPO, 2009, Ferguson et al., 2003, Pletsch, 1947). Recently it has been documented in south-central Washington and northern Oregon (Munyaneza 2010). It has also been reported in Canada in Alberta, British Columbia, Ontario, Quebec and Saskatchewan (Ferguson et al., 2003). The psyllid has been reported from Mexico in Durango, Tamaulipas, and Michoacán and as far south as Mexico City and Rio Frio in Puebla (Cranshaw, 1993, Pletsch, 1947) and in Guatemala and Honduras (Abdullah, 2008). *B. cockerelli* was first detected in New Zealand in 2006 (Gill, 2006, Teulon et al., 2009).

4.5 Biotypes of *B. cockerelli* and migration in North America

Two genetically distinct populations of *B. cockerelli* have been identified in North America; a native and an invasive biotype (Liu et al., 2006). The native biotype overwinters in eastern Mexico and Texas and is thought to migrate annually northward on warm monsoon winds from the Gulf of Mexico to Colorado, Nebraska, Montana and other northern States, and possibly into southern parts of British Columbia, Alberta and Saskatchewan, in Canada (Davidson et al., 2008, Liu et al., 2006). Each year, the high summer temperatures in Arizona and New Mexico and the cold winter temperatures in central North America are thought to prevent survival of psyllid migrants, requiring populations to re-establish from their native range in southern Texas/north-eastern Mexico (Lui & Trumble, 2007).

The invasive biotype is associated with the western expansion of the species' distribution in North America since 1999-2000, into southern and central California from Baja, Mexico (Liu et al., 2006). Movement of psyllids from Baja is associated with a weather condition known as the Catalina Eddy, a frequently occurring low-pressure system centred near Catalina Island, CA, that creates winds that sweep north from western Mexico to southern California (Liu et al., 2006). Until 1999-2000, infestations in western North America by *B. cockerelli* were historically rare. Sporadic populations were reported in relatively small geographic areas within California during the 1930s and 1940s but these rarely persisted for more than a year or two before disappearing (Lui & Trumble, 2007). The recent invasions have been unusual both for the duration of the infestation and for the extent of the

geographic range invaded but no explanations have been provided as to why or how they may have originated (Lui & Trumble, 2007).

Liu & Trumble (2007) compared the fitness of native (Texas) and invasive (California) populations. When reared on tomato and capsicums, the native biotype had higher survivorship, growth indices and shorter developmental times than psyllids from the invasive populations. The fecundity of the native biotype was also significantly higher than the invasive biotype on tomato, but not on capsicums. However, the invasive biotype was found to be resistant to two (out of three) insecticides tested whereas the native biotype was not. Lui & Trumble (2007) were unable to determine whether the invasive biotype was pre-adapted to pesticide resistance, or if the resistance developed after the range expansion and is simply a contributing factor to maintaining the expansion.

It is not known which biotype is present in New Zealand.

4.6 Host plants of *B. cockerelli*

Although *B. cockerelli* is mostly found on members of the Solanaceae, in North America it has been reported to feed on species belonging to the Amaranthaceae, Asclepiadaceae, Asteraceae, Brassicaceae, Chenopodiaceae, Convolvulaceae, Fabaceae, Lamiaceae, Lycophyllaceae, Malvaceae, Menthaceae, Pinaceae, Poaceae, Polygonaceae, Ranunculaceae, Rosaceae, Salicaceae, Scrophulariaceae, Violaceae and Zygophyllaceae (Pletsch, 1947; Wallis, 1951; Anon. 2009). These hosts include a range of cultivated and non-cultivated plants that are widely distributed in Australia. However, it is thought that in New Zealand, *B. cockerelli* only reproduces on nine species of Solanaceae and Convolvulaceae (Table 2) (Anon. 2008b), although Cape Gooseberry (*Phalaris peruviana*), which is now known to be susceptible to Liberibacter infection in New Zealand (Liefting et al. 2008b), should also be included in this list. In other countries, *B. cockerelli* has been recorded being able to reproduce on several other species of Solanaceae and Convulvulaceae as well as 2 species of Lamiaceae (Appendix A). While black nightshade (*Solanum nigrum*) (Solanaceae) is listed as a host in the USA, in New Zealand adults may lay eggs on the plant, but all nymphs die (Anon. 2008b). Geographical differences in which host-plants are suitable for reproduction may be due to variation between biotypes of *B. cockerelli* and this requires further investigation.

Table 2. Plants on which *B. cockerelli* can breed in New Zealand. Plants that support large populations of the psyllid are indicated with an asterisk (adapted from Anon. 2008b and Biosecurity Australia 2009).

Family Name	Crop/weed	Common name	Scientific name
Convulvulaceae	Crop	Kumara	*Ipomoea batatas*
Solanaceae	Crop	Capsicum*	*Capsicum annuum*
	Crop	Egg plant*	*Solanum melongena*
	Weed	Poroporo	*Solanum aviculare* and *probably S. laciniatum*
	Crop	Potato*	*Solanum tuberosum*
	Crop	Tamarillo	*Solanum betaceum*
	Crop	Tomatoes*	*Solanum lycopersicum*
	Crop	Cape gooseberry	*Physalis peruviana*
	Weed	Thorn apple	*Datura stramonium*
	Weed	Apple of Peru	*Nicandra physalodes*

5. Zebra chip disease and the link with *B. cockerelli* and "*Candidatus* Liberibacter solanacearum"

5.1 Discovery of Zebra chip disease

Zebra chip disease (ZC) was first documented in 1994 in potato fields around Saltillo, Mexico, and was originally called 'papa manchada' (stained potato) (Munyaneza et al., 2007). The new disease was named zebra chip due to the characteristic striped pattern of necrosis in tuber cross-section which becomes more prominent when chips from infected tubers are fried (Fig.). ZC was first detected in the USA in 2000 in commercial potato fields in Texas. At first the disease was only sporadically important economically until the 2004-2006 growing seasons when it caused millions of dollars of losses to both potato producers and processors in numerous locations in the USA and Mexico, often causing the abandonment of entire potato fields (Munyaneza et al., 2007). ZC is also serious in potato production areas of Guatemala and Honduras (Munyaneza et al., 2007). Zebra chip disease in solanaceous plants has now been documented in Arizona, California, Colorado, Idaho, Kansas, Nebraska, Nevada, New Mexico, Montana, North Dakota, Texas, Utah and Wyoming in the USA; Alberta in Canada; Coahuila and Nuevo León in Mexico; Guatemala; Honduras; and New Zealand (Abdullah, 2008, Carter, 1939, EPPO, 2009, MAFBNZ, 2008, Munyaneza et al., 2007, Munyaneza, 2010). Symptoms resembling ZC were first observed in New Zealand in potato tubers harvested from a breeding trial in South Auckland in May 2008 (Liefting et al., 2008).

It was observed that ZC-infected potato plants exhibited a range of plant symptoms that resembled those of the potato purple top wilt syndrome caused by the Columbia Basin potato phytoplasma and transmitted by the beet leafhopper, *Circulifer tenellus* (Munyaneza et al., 2007). However, the exact causal agent (s) and vector (s) of ZC remained unknown until preliminary surveys to look for insects associated with potato crops in south-western USA indicated the *B. cockerelli* was the most common and abundant insect in all of the ZC infected potato fields (Munyaneza et al., 2007). Subsequent experiments confirmed that *B. cockerelli* was the vector of ZC in potatoes and that "Ca. L. solanacearum" was the casual agent of the disease (Liefting et al., 2008, Munyaneza et al., 2007). Hanson et al. (2008) determined that the causal agent of "psyllid yellows" in tomatoes and potatoes was "Ca. L. psyllarous" which was also transmitted by *B. cockerelli* and later found to be identical to "Ca. L. solanacearum" (Lin et al., 2009).

Subsequent research has shown that the *B. cockerelli* can acquire Liberibacter after feeding on infected potato plants in less than 1 hour and they can inoculate other plants within two hours (Munyaneza, unpublished report). The optimum temperature for the development of ZC in potatoes is 22-32^{o}C with low (below 13^{o}C) or high temperatures (above 35^{o}C) slowing development.

Since 2008, significant ZC-like crop losses in potatoes have occurred in southern/eastern Europe and Russia. However, the causal agent was identified by Acs et al. (2010) as a different type of phytoplasma (Stolbur) and to date no ZC has been detected. Transmission of the Stolbur phytoplasma in potatoes has been associated with several species of psyllids (mainly *Reptalus* spp.), but not *B. cockerelli* which is currently absent from Europe.

In 2008, "*Ca.* L. solanacearum" was reported in psyllid affected commercial carrot fields in Finland (Munyaneza et al., 2010). This was the first report of "*Ca* L. solanacearum" associated with a non-solanaceous species and the first report of the pathogen outside of North and Central America and New Zealand. Transmission was associated with the carrot psyllid (*Trioza apicalis*).

6. Symptoms of psyllid feeding, psyllid yellows and Liberibacter

For many years the salivary secretion of *B. cockerelli* was known to cause a debilitating yellowing plant condition in tomatoes and potatoes called “psyllid yellows” (Hansen et al., 2008). The factor in psyllid secretions that caused psyllid yellows was originally thought to be a toxin produced by the psyllid. However, Hansen et al. (2008) demonstrated that the causative agent of psyllid yellows was the bacterium “Ca. L. psyllaurous” (named “Ca. L. solanacearum” by (Liefting et al., 2008)) which was vectored by *B. cockerelli*. Psyllid salivary toxins are no longer implicated in the aetiology of the disease as it was successfully transmitted by grafting in greenhouse trials (De Boer et al., 2007).

6.1 Tomatoes

Symptoms of psyllid feeding damage/disease transmission in tomatoes include: ‘spiky’ and chlorotic apical growth, leaf curling, mottling, plant stunting, and in some cultivars fruit deformation. Symptoms of psyllid yellows are the yellowing and stunting of the growing tip and a cupping or curling of the leaves (Figure 8). Many flowers may fall off the trusses of infected plants and fruit may be small and misshapen.

Figure 8. Symptoms of *B. cockerelli* feeding damage and psyllid yellows in tomatoes (source: Biosecurity Australia 2009)

6.2 Potatoes

Fresh tuber symptoms of ZC include: discolouration of modullary rays, ranging from mild to severe, which affects the entire length of the tuber. Potato chips made from ZC infected tubers present dark stripes which become markedly more visible after frying (hence the disease name 'Zebra chip'), leading to rejection from the potato chip industry. When infected tubers are boiled they are mushy with an earthy taste. When planted, infected tubers may not produce plants.

ZC affected potato plants may also show vascular browning and flecking of stems, enlarged lenticels of underground stem and visibly coloured stolon attachment. Foliar symptoms of ZC resemble purple top wilt and fusarium wilt. Symptoms also include: swollen nodes, proliferation of auxiliary buds, aerial tubers, zigzag stems, leaf curl, stunting, yellowing, scorching and wilt.

Potatoes affected by *B. cockerelli* but without Liberibacter infection, show a number of symptoms including chaining and some internal discolouration of the flesh, typically browning of the vascular ring and/or brownish streaks of the medullary rays. Vascular symptoms such as these can arise from many other disorders and diseases such as water stress and Verticillium.

Observational studies suggest that the symptoms of psyllid yellows are different from ZC. While the foliar symptoms of psyllid yellows strongly resemble those of ZC, the tuber symptoms are different. Psyllid yellows cause a stunting and yellowing of the growing tip, and the edges of the curled leaves often have a pink blush (Figure 8a). Similar to ZC, the stem may have swollen nodes and show a browning of the vascular tissue. After a while, infected potatoes develop a scorched appearance and plants collapse prematurely. Potato plants that are infected at an early stage develop numerous small tubers. However, plants affected by ZC tend to die quickly in contrast to those with psyllid yellows. Also, plants infected with psyllid yellows can recover if the psyllids are removed but ZC infected plants do not recover.

Figure 9. Symptoms of: a) psyllid yellows/zebra chip disease in potato foliage; b) zebra chip disease in a fresh tuber; c) zebra chip disease in cooked tubers (source: Biosecurity Australia 2009)

6.3 Capsicums

Capsicums affected by psyllid feeding damage/disease develop: chlorotic or pale green leaves, sharp tapering of leaf apex, upward leaf curling, shortened internodes and petioles, necrosis of apical meristem, flower abortion, and plant stunting (Fig. 9a, b).

Figure 10. Symptoms of *B. cockerelli* feeding and disease transmission in capsicum (source: Biosecurity Australia 2009)

6.4 Tamarillos

Tamarillo (*Solanum bectaceum*) is a subtropical tree crop native to central America. They are grown commercially in Ecuador, Colombia, Western Australia and on the north island of New Zealand. In New Zealand, tree losses of 50% or more have been reported due to *B. cockerelli* feeding and transmission of Liberibacter. The following description is from Watson (2009) (in: Nelson 2009 Proceedings of the 7th World Potato Congress, Christchurch, New Zealand, 26 March 2009). Liberibacter in tamarillo was first detected in Whangarei, New Zealand, in December 2008 (Liefting

et al 2008b). Early symptoms of Liberibacter infection in tamarillos include leaf cupping and pink colouration of new growth. Mature trees show yellowing of leaves and eventually peripheral burning leading to leaf drop, branch dieback and tree death over 2-4 months. Symptoms can look similar to phytophora infection but as the roots of Liberibacter infected trees are not affected, they remain firm in the ground. Pruned trees also show poor bud break and have small shoots with small leaves. This can progress to shoot dieback and tree death over 1-4 months. If shoots are produced they are often multiple shoots from a single bud that remain thin with small leaves and no fruit trusses develop. Symptoms can be restricted to particular branches and can become more significant as crop load increases through the season.

6.5 Carrots

In 2008, "*Ca*. L. solanacearum" was discovered in commercial crops of carrots in Finland that were infested with carrot psyllids (*Trioza apicalis*) (Munyaneza et al., 2010). Symptoms of affected plants included leaf curling, yellow and purple discolouration of leaves, stunted growth of shoots, and proliferation of secondary roots.

7. Control of *B. cockerelli*

7.1 Biological control

7.1.1 Parasitoids

In North and Central America the most important parasitoid of B. cockerelli is *Tamarixia trioza.* In Mexico *T. triozae* occurs naturally in crops and achieves up to 80% parasitism of *B. cockerelli* in unsprayed capsicum crops.

In 2006-7, Plant & Food Research (PFR) in New Zealand began searching for biological control agents of *B. cockerelli* but no native agents were found that could control psyllids on covered tomatoes (R. Gardner-Gee, unpublished report 2011). Therefore, in 2008 the tomato industry gained approval to import *T. triozae* into New Zealand. In 2009, colonies of this parasitoid were established in PFR quarantine facilities for host specificity testing. It is hoped that this parasitoid will have a role in greenhouse *B. cockerelli* control and also in suppressing psyllid populations in the general environment (S. Ogden, unpublished report).

However, *T. triozae* attacks 9 psyllid species, belonging to 6 genera, in North America. Therefore, PFR tested a broad range of native psyllids (6 species) as possible hosts of *T. triozae*. The results to date of no-choice bioassays suggest that *T. triozae* could lay eggs and develop on at least one native psyllid species (*Trioza panacis*) and further tests are being conducted. Lui et al. (2012) conducted a risk assessment of insecticides on *T. triozae* and found that several of those used to control *B. cockerelli* were highly toxic to the parasitoid.

7.1.2 Predators

Recent research within both Australia (Horne & Page, 2012) and New Zealand (Larsen et al., 2011, MacDonald FH et al., 2010, Walker et al., 2011) has identified key predators that play a role in controlling key insect pests. The damsel bug (*Nabis kinbergii*), brown lacewing (*Micromus tasmaniae*) and 11- spotted ladybird (*Coccinella undecimpunctata*) already play key roles in controlling potato moth, aphids and western flower thrips, respectively. These three key predators have also been shown to eat all three lifestages of the psyllid, i.e. eggs, nymphs and webbed adults; this suggests they may play a key role in controlling psyllid numbers (Horne & Page, 2012). Other predators have also been identified include the small hoverfly (*Melanostoma fasciatum*), mites, sheet web spiders (Linyphiidae) and *Orius* species (Walker et al., 2011). Indeed, in NZ, hoverflies are the second most important species for psyllid control becoming the dominant predators in summer. Whilst the NZ hoverfly species is not found in Australia there are different hoverfly species that exist that may play a beneficial role in controlling psyllid numbers. In developing an IPM control strategy compatible in potato for targeting psyllids the recommended beneficial combination would include the four key predators: damsel bug, brown lacewing, common spotted ladybirds and hoverflies (Horne & Page, 2012).

Reports of 14 plus (or weekly) insecticides (including non-selective insecticides) being used on the potato crop over the growing season is not compatible with IPM (Horne & Page, 2012); hence the need to use selective insecticides.

7.1.3 Diseases – entomopathogens

In New Zealand, a range of commercially available entomopathogens and unique isolates are being evaluated for B. cockerelli control in the greenhouse and for potential use in potato production, although the utility of these products in potato crops may be reduced in areas where frequent fungicide applications are required for blight control (S. Ogden, unpublished report). During trials at the Pukekohe Research Station in New Zealand, an unidentified entomopathogenic fungus was noticed infecting *B. cockerelli* nymphs in the field (G. Walker et al. 2011, unpublished report).

7.2 Chemical control

Research on the most efficacious insecticides to use for controlling *B. cockerelli* infesting indoor and outdoor crops is on-going in New Zealand and the USA. Insecticide options and best spraying practices used to maximise *B. cockerelli* control were recently published by Page-Weir et al. (2011), Potatoes New Zealand (Psyllid News, December 2011), Peracto New Zealand Limited (Anonymous, 2011) and by the New Zealand greenhouse tomato and capsicum industries (Anonymous, 2008). Several new products are expected to be registered in New Zealand over the next 1-3 years. Abamectin and imidacloprid have been shown to be particularly efficacious against *B. cockerelli* (Page-Weir et al., 2011) but abamectin is effective only immediately after application (Gharalari et al., 2009). The latter authors achieved higher adult mortality with abamectin when potato plants were treated with ground application rather than aerial application, perhaps due to greater leaf coverage. Unfortunately, both abamectin and imidacloprid, along with many others commonly used for *B. cockerelli* control, is also highly toxic to many beneficial insects, including the parasitic wasp *T. triozae* (Lui et al., 2012). Currently, a range of 'soft' insecticides are being evaluated for B. cockerelli control for potential use in greenhouse crops and some of these are being selected for testing in potato crops (S. Ogden, unpublished report).

Low rates of insecticides may also reduce the attractiveness of plants to psyllids but only if sufficient coverage of leaves is achieved (Gharalari et al., 2009). Butler et al. (2011) tested the residual effects of several insecticides over time on the behaviour of adult psyllids and the transmission of ZC. All insecticides tested significantly reduced probing durations and increased the amount of time spent on leaves, suggesting that they act as deterrents to feeding as well as repellents. The insecticides imidacloprid and abamectin significantly lowered transmission of ZC by psyllids compared to untreated controls (Butler et al., 2011).

Resistance to some insecticides has developed in invasive populations of *B. cockerelli* in the USA (Lui & Trumble, 2007). Invasive (California) populations were shown to have significantly higher LC_{50} values (concentrations causing 50% mortality) for imidacloprid and spinosad, but not spiromesifen, than native (Texas) populations of psyllids. Accordingly, in New Zealand the rotation of insecticide groups is advocated to lower the risk of resistance developing (Psyllid News, December 2011). It is recommended that insecticides falling within the same mode of action group are applied for no

longer than one generation of *B. cockerelli* (about 1 month in summer) before switching to an insecticide with a different mode of action.

7.3 Cultural control

Heavy rain is known to reduce the activity and presence of psyllids with wet seasons reducing subsequent outbreaks of Zebra Chip infestations (F. Mulchay, Simplot Australia; pers. comm.). Anecdotal evidence suggests that targeted irrigation, more specifically irrigation that has a high physical impact on foliage such as gun irrigation may be a useful deterrent to psyllid activity (Horne & Page, 2012).

Also, Walker et al. (2011) suggested that planting early season potato crops in the New Zealand district of Pukekohe may avoid damaging infestations of *B. cockerelli*.

The usage of border strips has been successfully demonstrated to reduce psyllid infestations in potato crops on the South Island of NZ (Horne & Page, 2012). Grassy strips, a couple of metres in width, were planted around the edge of potato crops. The psyllids tended to occupy these areas which could then be targeted with insecticides. This would enable beneficial predators to maintain populations in the middle of the crop, away from insecticides. This strategy of utilising border sprays has restricted psyllids to the edge of the crop and it was only towards the end of crop growth that psyllids were observed on the edge (up to 50 metres inside crop) of the potato fields, suggesting a role for border strips in an IPM strategy for psyllid control (Horne & Page, 2012).

7.4 Genetic engineering

The *Mi-1.2* gene, identified from wild varieties of tomato (*Solanum peruvianum*), has been incorporated into commercial varieties of tomato and has been shown to confer resistance to several species of phloem feeding insects including *B. cockerelli* (Casteel et al., 2006). In choice studies, tomato plants bearing this gene were less preferred by psyllids resulting in fewer eggs laid. Also, psyllid survival rates from egg to adult were lower on plants containing the gene. To date, no potato cultivars resistant to Zebra chip have been found but different cultivars do appear to have different levels of attractiveness to B. cockerelli (Psyllid News, February 2010).

8. Monitoring and detection methods

Methods used to assess the presence of *B. cockerelli* in crops include: (1) indirect sampling of adults with yellow sticky traps or water pan traps; (2) direct sampling of nymphs based on sweep net or beat trays, and (3) visual inspection of eggs, nymphs, and adults on crop leaves (Martini et al., 2012). Water pan traps, sweep nets and beat trays have been used rarely. Sticky traps (adults) and the visual inspection of leaves (nymphs) are the main sampling methods used and will be discussed here.

8.1 Sticky traps

Yellow sticky traps (or cards) are an easy to use method for monitoring adult *B. cockerelli* but expensive when deployed in high numbers and the combination of wind and dust can make traps unreadable (Martini et al., 2012). Studies have also shown poor and inconsistent correlations between the number of adult psyllids on traps and in the corresponding vegetation. However, in New Zealand, sticky traps are thought to give a good indication of when the first *B. cockerelli* arrive in an area and of seasonal changes in numbers (Walker et al., 2011). They are currently being used in Australia as one of several methods to detect possible incursions of *B. cockerelli* into potato fields and glasshouse crops. Since November 2010, Plant & Food Research in New Zealand have operated a network of sticky traps across the North and South islands in commercial potato, tomato and tamarillo sites. The traps have been useful in documenting the spread of *B. cockerelli* in New Zealand and seasonal population changes. The phenology of *B. cockerelli* has been similar between years with numbers increasing significantly in late December/early January and declining in early April in most regions. The number of psyllids caught in 2011/2012 was lower than in previous years.

8.2 Visual inspection of leaves

Visual inspection of leaves, buds or fruits is the standard method employed by crop consultants to assess the presence of *B. cockerelli* in crops (Martini et al., 2012). While it is probably the most accurate method to use, it is tedious and time consuming as it requires that at least 50-100 leaves are inspected from individual fields. Ideally leaves should be inspected under a binocular microscope to score the number of psyllids present. Martini et al. (2012) developed a leaf washing method to speed up the process of inspecting leaves for *B. cockerelli* nymphs. Using this method, they showed that psyllid numbers were most predominant in the middle portion of the plant canopy and that the spatio-temporal distribution of nymphs varied among potato varieties. Accordingly, in New Zealand it is now recommended that 2 middle leaves from 50 plants are inspected to give a reliable estimate of psyllid numbers in potato crops (Psyllid News, December 2011, Walker et al., 2011).

9. Biology of phytoplasmas and their transmission by insects

Phytoplasmas (originally called mycoplasma-like organisms) are obligate, intracellular prokaryotic parasites of plant phloem tissue and transmitting insect vectors. They cause more than 700 diseases, ranging from mild yellowing to death of infected plants, in hundreds of plant species (Weintraub & Beanland, 2006). Like liberibacters, they cannot be cultured *in vitro* in cell-free media. They are characterised by their lack of a cell wall, being instead bounded by a triple layered membrane, a pleiomorphic or filamentous shape, normally with a diameter less than 1 micrometer, and their very small genomes (Lee et al., 2000, Liefting et al., 2011).

Three known mechanisms introduce phytoplasmas into the vulnerable tissue of host plants: (a) vegetative propagation or grafting of infected plant material, (b) vascular connections made between infected and non-infected host plants by parasitic plants such as dodder (*Cuscuta* spp.) and (c) vector insects feeding on non-infected host plants. Recent reports suggest a fourth possible source of phytoplasma: seed transmission. However, there are more described phytoplasmas than there are identified mechanisms of infection or insect vector species. Insects are the main method vectors of phytoplasmas, particularly leafhoppers, in which they are also able to replicate.

Common symptoms of phytoplasma infection in plants are: phyllody (production of leaf-like structures in place of flowers); yellowing of leaves (thought to be due to effect on movement of carbohydrates in the phloem) and virescence (development of green flowers due to loss of pigment in the petals). Many plants infected with phytoplasma gain a bushy or witch's broom appearance due to changes in normal growth patterns caused by infection. Most plants also show apical dominance but phytoplasma infection can cause the proliferation of auxiliary shoots and an increase in the size of internodes.

Because phytoplasmas are phloem-limited, only phloem-feeding insects can potentially acquire and transmit the pathogen. However, within the groups of phloem-feeding insects only a small number, primarily in three taxonomic groups, have been confirmed as vectors of phytoplasmas. Most vectors belong to the Hemiptera. The Hemipteran superfamily containing the largest number of vector species is the Membracoidea, within which all known vectors to date are confined to the family Cicadellidae. The second largest group is the Fulgoromorpha, in which four families of vector species are found (Cixiidae, Delphacidae, Derbidae, and Flatidae). The smallest suborder is Sternorrhyncha, in which only two genera in the superfamily Psylloidea are confirmed vectors (see next section).

The interaction between insects and phytoplasmas is complex and variable. The complex sequence of events required for an insect to acquire and subsequently transmit phytoplasmas to plants suggests a high degree of fidelity between insect vector species and the phytoplasmas that they transmit. However, numerous phytoplasmas are transmitted by several different insect species. In addition, a single vector species may transmit two or more phytoplasmas, and an individual vector can be infected with dual or multiple phytoplasma strains.

Vector–host plant interactions also play an important role in determining the spread of phytoplasmas. Polyphagous vectors have the potential to inoculate a wider range of plant species,

depending on the resistance to infection of each host plant. The phytoplasma-insect relationship can be beneficial, deleterious, or neutral in terms of its impact on the fitness of the insect host.

Phloem-feeding insects acquire phytoplasmas passively during feeding in the phloem of infected plants. The feeding duration necessary to acquire a sufficient titre of phytoplasma is the acquisition access period (AAP). The AAP can be as short as a few minutes but is generally hours, and the longer the AAP, the greater the chance of acquisition. The AAP may also depend on the titer of phytoplasmas in the plants.

The time that elapses from initial acquisition to the ability to transmit the phytoplasmas is known as the latent period (LP) and is sometimes called the incubation period. The LP is temperature dependent and ranges from a few to 80 days. During the LP the phytoplasmas move through and replicate in the competent vector's body. Phytoplasmas can pass intracellularly through the epithelial cells of the midgut and replicate within a vesicle, or they can pass between two midgut cells and through the basement membrane to enter the hemocoel. Phytoplasmas circulate in the hemolymph, where they may infect other tissues such as the Malpighian tubules, fat bodies and brain, or reproductive organs; replication in these tissues, albeit not essential for transmission, may be indicative of a longer co-evolutionary relationship between host and pathogen.

To be transmitted to plants, phytoplasmas must penetrate specific cells of the salivary glands and high levels must accumulate in the posterior acinar cells of the salivary gland before they can be transmitted. At each point in this process, should the phytoplasmas fail to enter or exit a tissue, the insect would become a dead-end host and would be unable to transmit the phytoplasmas.

Transovarial transmission of phytoplasmas was first confirmed in the leafhopper species *Hishimonoides sellatiformis* as a vector of the mulberry dwarf phytoplasma (Kawakita et al ., 2000), and with *Matsumuratettix hiroglyphicus* as a vector of the sugarcane white leaf phytoplasma (Hanboonsong et al., 2002). In laboratory studies, Tedeschi et al (2006) also confirmed the transovarial transmission of "*Candidatus* Phytoplasma prunorum", through the psyllid vector *Cacopsylla pruni*.

Transmission of phytoplasmas by psyllids

Confirmed psyllid vectors of phytoplasma are: *Cacopsylla* spp. (Psyllidae), which transmits AP group (16SrX) phytoplasmas to pome and stone fruit trees, and *Bactericera trigonica* Hodkinson (Triozidae), which transmits a stolbur (Sr16XII) phytoplasma to carrots.

9.1 Phytoplasmas in potatoes

Phytoplasma diseases of potato have become increasingly important in recent years, due to the epidemic appearance and geographical spread of the diseases, as well as significant yield losses in potato production and low quality of produced tubers (Ember et al., 2011, Jovic et al., 2011). Different phytoplasma 16Sr groups are infecting potato worldwide, however many are causing similar symptoms (Jovic et al., 2011). Potato phytoplasma diseases in Europe were for a long time diagnosed only on the basis of visual symptoms. Based on visual symptoms the diseases caused by phytoplasmas can be classified into two groups: aster yellows and potato witches'-broom. The aster yellows group has many different names including purple top wilt, haywire, apical leafroll, bunch top,

purple dwarf, yellow top, potato hair sprouts, stolbur, potato phyllody, and potato marginal flavescence (Ember et al., 2011). The aster yellow phytoplasmas occur worldwide and are economically more important than the potato witches' broom disease which is usually of minor economic importance (Ember et al., 2011, EPPO/CABI, 1996).

However, this approach is not very reliable and the use of modern molecular techniques such as polymerase chain reaction (PCR) is required in order to accurately determine the etiology of these phytoplasma diseases (Ember et al., 2011). Recent molecular advances detailed for delineating phytoplasmas are summarised in Marcone (2012) which describes the benefits of utilising multi-locus sequence analysis for delineating genetically close but pathologically and/or ecologically distinct strains. This optimisation of molecular techniques is critically important within Australia for differentiating between phytoplasmas that are already within Australia and potential new biosecurity threats that may arrive, '*Ca*. L. solanacearum' (F. Constable, DPI Vic, pers. comm.).

10. Biology of Liberibacter

The genus "*Candidatus* Liberibacter" (*Alphaproteobacteria*) was originally described from bacteria associated with plants suffering citrus huanglongbing (greening disease) (Hansen et al., 2008). Three species were known to cause the disease, primarily in citrus and related plants in the Rutaceae: "*Candidatus* Liberibacter asiaticus", "*Candidatus* Liberibacter africanus" and "*Candidatus* Liberibacter" americanus. Huanglongbing is known to be transmitted by two psyllid species, *Diaphorina citri* and *Trioza erytreae*. "*Candidatus* Liberibacter psyllaurous" was the fourth species in this genus to be described by Hansen et al. (2008). They determined that it was the bacterium vectored by *B. cockerelli* which caused "psyllid yellows" in potato and tomato. At a similar time, (Liefting et al., 2008) published a paper describing the same organism causing zebra chip in potatoes but named it "*Candidatus* Liberibacter solanacearum". This synonym is now more often used in preference to "psyllaurous" demonstrated by the 42 papers presented at the 2011 SCRI Zebra Chip Annual Reporting Session where all but 2 used the terminology "*Candidatus* Liberibacter solanacearum". A fifth species, "*Ca*. Liberibacter europaeus" was recently described from pear trees where it seems to cause no specific disease symptoms and is vectored by the psyllid *Cacopsylla pyri* (Raddadi et al., 2011).

The Candidatus part of the bacterium's name indicates that it cannot be maintained in a Bacteriology Culture Collection, as it is unculturable. Detection of Liberibacter relies on PCR amplification of their 16S rRNA gene with specific primers, with detection recorded from both the psyllid and potato material (including shoot and tuber material) (Lin et al., 2009). New primers targeting a conserved intergenic region between the 16S and 23S rDNA genes are potentially more sensitive and offer better differentiation between 'Ca. Liberibacter solanacearum' and closely related 'Ca. Liberibacter' species (Ravindran et al., 2011). In Australia, nested and semi-nested PCR assays have been developed and validated that differentiate between "*Candidatus* Liberibacter solanacearum" and other known phytoplasmas with similar symptoms (Fiona Constable, pers. comm.). Liberibacters are phloem-restricted, gram negative bacteria (Jagoueix et al., 1994), mainly transmitted by insects. As the bacterium multiplies, it can restrict the movement of nutrients, weakening and stunting the plant and may eventually kill it. Liberibacter in potatoes reduces nitrogen, causes conversion of starches to sugars, reduces the size of chloroplasts and causes chloroplast distortion. Dodder, a parasitic plant, has also been demonstrated to transmit Liberibacter. Pruning and other mechanical transmission methods do not appear to be important in transmission, but human movement of host material contributes to the spread of the vectors and the disease. Indeed 'Ca. L. solanacearum' could be transmitted from the mother tubers both to the foliage of growing plants and to progeny tubers indicating that seed tubers may play a key role in the life cycle of this pathogen (Pitman et al., 2011).

10.1 "*Candidatus* Phytoplasma australiense"

In January 2009, considerable confusion arose when "*Candidatus* Phytoplasma australiense" was found infecting potatoes in New Zealand where *B. cockerelli* and Liberibacter were also present (Liefting et al., 2009b). Plants in a commercial crop in the Waikato Region were observed to have upward rolling and purpling of leaves; symptoms similar to those infected with Liberibacter

("*Candidatus* Liberibacter solanacearum"). DNA analysis of plant tissues revealed infections of '*Ca*. P. australiense', sometimes together with the Liberibacter.

"*Candidatus* Phytoplasma australiense" had been known to exist in New Zealand for a long time but at the time of its discovery in potatoes the only other known hosts were strawberries and some native plant species. However, recently another four, diverse, hosts of '*Ca*. P. australiense' in New Zealand have been identified: Jerusalem cherry (*Solanum pseudocapsicum*), swan plant (*Gomphocarpus fruitcosa*), celery (*Apium graveolens*) and boysenberry (*Rubus* hybrid) (Liefting et al., 2011).

The discovery of "*Ca*. P. australiense" in potatoes was of concern as this phytoplasma is associated with several lethal plant diseases both in New Zealand and in Australia. In New Zealand it causes: lethal yellows in strawberry, sudden decline in cabbage tree (*Cordyline australis*), lethal decline in *Coprosma robusta* and yellow leaf disease in *Phormium tenax* and *P. cookianum* (New Zealand flax). In Australia "*Ca*. P. australiense" is associated with Australian grapevine yellows in South Australia and with papaya dieback in Queensland (and occasionally in the Northern Territory and Western Australia), as well as diseases in a range of other hosts including Paulowinia trees, strawberry, lucerne, pumpkin and bean (EPPO data sheet;(Liefting et al., 2011)).

In potatoes, reported symptoms of "*Ca*. P. australiense" infection are similar to psyllid yellows/Liberibacter: leaf yellowing and purpling, leaf and stem necrosis, aerial tubers and tuber discolouration. However, tuber discoloration is not as severe as Liberibacter infected plants.

In other countries, several species of insects (mainly leafhoppers and planthoppers) are known to transmit Phytoplasmas to plants (Weintraub & Beanland, 2006). However, despite searches for the presence of Phytoplasmas in endemic and introduced leafhoppers and planthoppers, the vector of "*Ca*. P. australiense" in Australia and in several host plants in New Zealand is unknown. Currently, the only known vectors of '*Ca*. P. australiense' are two species of Cixidae planthoppers (*Zeoliarus* (*Oliarus*) *atkinsoni* and *Z. oppositus*) which are endemic to New Zealand (Charles et al., 2002, Liefting et al., 2011). The former is restricted to *Phormium* and therefore is unlikely to transmit the phytoplasma to other plant species but *Z. oppositus* is more polyphagous and may be responsible for spreading the disease to other plant species. In strawberries in New Zealand, (Charles et al., 2002) confirmed the presence of "*Ca*. P. australiense" in two native species of Cicadellidae (*Arawa variegata* and *Recilia hospes*) caught on sticky traps set in crops. Whether these insects transmit the phytoplasma in strawberries is yet to be demonstrated. While it is suspected that *B. cockerelli* may have been involved in the spread of "*Ca*. P. australiense" into New Zealand potatoes, this is yet to be proven.

Current thoughts on the presence of "*Ca*. P. australiense" in potatoes in New Zealand are that while it can be occasionally found, symptoms do not appear to have a serious effect on yield and tuber quality unlike the Liberibacter (M. Spencer, unpublished). A report on a grant transmission trial in New Zealand was inconclusive. No disease transmission was observed, but difficulties in phytoplasma detection were noted (Smith et al, 2011).

Appendix A: Known hosts of the tomato–potato psyllid (*Bactericera cockerelli*)

(Source: DAFF Final PRA report for "*Candidatus* Liberibacter psyllaurous", 2009)

Host	Common name	Host association	Present in Australia	ICON conditions for Nursery stock
Convolvulaceae				
Convolvulus arvensis L.	Field bindweed	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**No** (C10285)
Ipomoea batatas (L.) Lam.	Sweet potato, Kumara	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7383, C7384, C7300)
Ipomoea purpurea (L.) Roth	Morning glory	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**NA**
Lamiaceae				
Mentha spicata L.	Spearmint	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7328, C7329, C7300)
Micromeria chamissonis (Benth.) Greene		Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**No record**	**NA**
Solanaceae				
Atropa belladonna L.	Deadly nightshade, Bella Donna	Unknown	**Yes** (Randall 2007)	**Yes** (C7301, C7302, C7300)
Capsicum annuum L.	Capsicum, Pepper	Breeding host. This species supports large populations of the psyllid (Horticulture New Zealand 2008b)	**Yes** (AVH 2009)	**No**
Capsicum frutescens L.	Chilli	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**No**
Datura fastuosa L.		Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**No record**	**No** (C7172)
Datura inoxia Mill.		Oviposit, complete its nymphal development and emerge as normal	**Yes** (AVH 2009)	**No** (C7172)

		adult (Knowlton and Thomas 1934)		
Datura stramonium L.	Jimsonweed, Thornapple	Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**Yes** (AVH 2009)	**No** (C7172)
Hyoscyamus albus L.		Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**Yes** (AVH 2009)	**Yes** (C7301, C7302, C7300)
Hyoscyamus niger L.	Henbane	Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**Yes** (AVH 2009)	**Yes** (C7301, C7302, C7300)
Lycium andersonii A. Gray		Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**NA**
Lycium exsertum A. Gray		Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**NA**
Lycium fremontii A. Gray		Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**NA**
Lycium halimifolium Mill.	Matrimony vine	Breeding host (Wallis 1955)	**No record**	**NA**
Lycium macrodon A. Gray		Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**NA**
Lycium pallidum Miers		Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**NA**
Lycium parishii A. Gray		Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**NA**
Lycium quadrifidum Moc. & Sessé ex Dunal		Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**NA**
Lycium torreyi A. Gray		Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**NA**

Lycopersicon esculentum Mill. [synonyms: *Solanum lycopersicum* L., *Lycopersicon lycopersicum* (L.) H. Karst.]	Tomato	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**No**
Lycopersicon pimpinellifolium (L.) Mill.	Currant tomato	Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**Yes** (AVH 2009)	**No**
Nicandra physalodes (L.) Gaertn.	Apple of Peru	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7301, C7302, C7300)
Nicotiana affinis Moore	Flowering tobacco	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**No** (C6066)
Nicotiana glutinosa L.		Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**No record**	**No** (C6066)
Nicotiana tabacum L.	Tobacco	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**No** (C6066)
Nicotiana texana Maxim.		Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**No record**	**No** (C6066)
Nierembergia hippomanica Miers	Cup flower	Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**Yes** (AVH 2009)	**Yes** (C7301, C7302, C7300)
Physalis angulata L.	Cut leaf ground-cherry	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7427, C7300, C18152
Physalis comata Rydb.	Wild ground-cherry	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**NA**
Physalis alkekengi L.	Chinese lantern	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7427, C7300, C18152)
Physalis heterophylla Nees	Clammy ground-cherry	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**No**

Physalis ixocarpa Brot. ex Hornem. *[synonym: Physalis philadelphica Lam.]*	Tomatillo	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7427, C7300, C18152)
Physalis lanceolata Michx.		Breeding host (Wallis 1955)	**No record**	**No**
Physalis lobata Torr.	Purple ground-berry	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**Yes** (C7427, C7300, C18152)
Physalis longifolia Nutt.	Longleaf ground-cherry	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**NA**
Physalis mollis Nutt.	Longleaf ground-cherry	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**NA**
Physalis peruviana L.	Cape gooseberry	Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**Yes** (AVH 2009)	**Yes** (C7427, C7300, C18152)
Physalis pruinosa L.	Husk tomato	Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**No record**	**Yes** (C7427, C7300, C18152)
Physalis rotundata Rydb.	Longleaf ground-cherry	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**No record**	**NA**
Solanum aviculare G. Forst.	Bullibulli	Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**Yes** (AVH 2009)	**Yes** (C7436,) C18152
Solanum baylisii Geras.		Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**No record**	**Yes** (C7436, C18152)
Solanum betaceum Cav. [synonym: *Cyphomandra betacea* (Cav.) Sendtn.]	Tamarillo	Breeding host (Horticulture New Zealand 2008b)	**Yes** (AVH 2009)	**Yes** (C7436, C18152)
Solanum carolinense L.	Ball nightshade, Bull nettle, Horse nettle, Devil's tomato	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7436, C18152)
Solanum chenopodioides Lam.	Velvety nightshade, Whitetip nightshade	Oviposit, complete its nymphal development and emerge as normal	**Yes** (AVH 2009)	**No**

		adult (Knowlton and Thomas 1934)		
Solanum citrullifolium A. Braun		Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**No record**	**Yes** (C7436, C18152)
Solanum elaeagnifolium Cav.	White horse-nettle, Silver-leaf nightshade	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7436, C18152)
Solanum jamesii Torr.	Wild potato	Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**No record**	**Yes** (C7436, C18152)
Solanum melongena L.	Eggplants, Aubergine	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7436, C18152)
Solanum mexicanum Moc. & Sessé ex Dunal		Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**No record**	**Yes** (C7436, C18152)
Solanum nigrum L.	Wonderberry, Black nightshade, Blackberry nightshade, Garden huckleberry	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7436, C18152)
Solanum pseudocapsicum L.	Jerusalem cherry	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7436, C18152)
Solanum pyracanthos Lam.	Porcupine tomato	Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**No record**	**Yes** (C7436, C18152)
Solanum racemigerum Zodda		Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**No record**	**Yes** (C7436, C18152)
Solanum rostratum Dunal	Buffalo-bur	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**No**
Solanum sanitwongsei Craib		Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**No record**	**Yes** (C7436, C18152)

Solanum sisymbriifolium Lam.	Viscid nightshade, Sticky nightshade	Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**Yes** (AVH 2009)	**Yes** (C7436, C18152)
Solanum triflorum Nutt.	Wild tomato	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7436, C18152)
Solanum tuberosum L.	Potato	Oviposit, complete its nymphal development and emerge as normal adult (Wallis 1955)	**Yes** (AVH 2009)	**Yes** (C7322, C7323, C7300)
Solanum villosum Mill.	Hair nightshade	Oviposit, complete its nymphal development and emerge as normal adult (Knowlton and Thomas 1934)	**Yes** (AVH 2009)	**Yes** (C7436, C18152)

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