Eur J Plant Pathol (2011) 129:389–398
DOI 10.1007/s10658-010-9702-1

Tuber transmission of '*Candidatus* Liberibacter solanacearum' and its association with zebra chip on potato in New Zealand

Andrew R. Pitman · Gabby M. Drayton · Simona J. Kraberger · Russell A. Genet · Ian A. W. Scott

Accepted: 11 October 2010 / Published online: 24 October 2010

Abstract Zebra chip, an emerging disease of potatoes, has recently been associated with '*Candidatus* Liberibacter solanacearum' in New Zealand. The phloem-limited bacterium is known to be vectored by the tomato potato psyllid (*Bactericera cockerelli*). In this study, the role of tuber transmission in the spread of *Ca.* L. solanacearum was investigated by replanting potato tubers infected with *Ca.* L. solanacearum in the absence of the psyllid. Nested PCR demonstrated that *Ca*. L. solanacearum could be transmitted from the mother tubers both to the foliage of growing plants and to progeny tubers, resulting in symptomatic and asymptomatic plants. Of 62 *Ca*. L. solanacearum-infected tubers four did not sprout symptomatic of zebra chip. A further two plants developed foliar symptoms associated with zebra chip during the growing season and died prematurely. Fifty-six of the infected tubers produced asymptomatic plants, although *Ca.* L. solanacearum was detected in the foliage of 39 of them indicative of transmission into asymptomatic progeny plants. At harvest, *Ca.* L. solanacearum was found in the daughter tubers of only five of the 39 asymptomatic plants, and only one of these plants was found to have zebra chip symptoms in the daughter tubers. Our results show that tuber transmission of *Ca.* L. solanacearum could play a role in the life cycle of this pathogen, providing a source for acquisition by *Bactericera cockerelli* and for movement of the pathogen to other regions of New Zealand via transport of seed tubers.

Keywords *Solanum tuberosum* · Zebra chip · Bacterium-like organism · Nested PCR · Phytopathogen

A. R. Pitman (✉) · G. M. Drayton · S. J. Kraberger · R. A. Genet · I. A. W. Scott
The New Zealand Institute for Plant & Food Research Limited,
Private Bag 4704,
Christchurch, New Zealand
e-mail: andrew.pitman@plantandfood.co.nz

A. R. Pitman
Bioprotection Research Centre, Lincoln University,
PO Box 84, Canterbury, New Zealand

Introduction

Zebra chip, an emerging disease of potato (*Solanum tuberosum*), was first described in Mexico in 1994, and subsequently in Central America and the southwest of the United States (Secor et al. 2006; Gudmestad and Secor 2007). In Mexico alone, up to 100% of plants in individual potato crops can be so severely infected with the disease that entire fields are abandoned, leading to severe economic losses. Zebra chip has also been recorded in New Zealand, where yields can be reduced by up to 60% and harvested tubers may produce less dry matter than normal tubers

resulting in downgrading of their marketable value (Liefting et al. 2008a).

Zebra chip is characterized by dark flecking throughout affected tubers, which is thought to be the result of necrosis of vascular tissue. Occasionally, severe streaking of the medullary ray tissues occurs that becomes more pronounced in tubers after frying, making them unsuitable both for fresh market sale and processing. Foliar symptoms of plants affected by zebra chip vary widely, but include an upward rolling of the basal portion of young leaves, chlorosis, purple top, shortened internodes, small leaves, enlargement of the stems, swollen auxillary buds, aerial tubers and early plant senescence. These symptoms are similar to psyllid yellows (PY), a disease first observed on potatoes following feeding by the tomato potato psyllid *Bactericera cockerelli* (formerly *Paratrioza cockerelli*) (Richards 1928).

Bactericera cockerelli was found to be associated with zebra chip in potato when controlled exposure of potato plants to *B. cockerelli* under greenhouse and field conditions produced characteristic foliar and tuber symptoms (Munyaneza et al. 2007). Plants with zebra chip, however, continued to show symptoms even following relief from psyllid feeding pressure (JE Munyaneza, USDA-ARS Wapato, personal communication), suggesting that *B. cockerelli* was a vector for an unknown etiological agent of zebra chip.

In 2008, evidence emerged that a bacterium could be the causative agent of PY and zebra chip. Hansen et al. (2008) identified a new species, tentatively designated *Candidatus* Liberibacter psyllaurous, which was found to infect *B. cockerelli* and its solanaceous host plants potato and tomato, potentially causing PY. Phylogenetic analyses showed that *Ca.* L. psyllaurous was closely related to several *Ca*. Liberibacter species implicated in Huanglongbing (HLB), presently considered the most serious and destructive disease of citrus in the world (Bové 2006). Thus far, three pathogens in the genus *Ca*. Liberibacter have been associated with HLB: *Candidatus* Liberibacter asiaticus (Jagoueix et al. 1994; Jagoueix et al. 1996), *Candidatus* Liberibacter africanus (Jagoueix et al. 1994; Planet et al. 1995; Jagoueix et al. 1996; Garnier et al. 2000) and *Candidatus* Liberibacter americanus (Teixeira et al. 2005).

At the same time as *Ca.* L. psyllaurous was identified, a new disease of tomato resembling PY was observed in commercial greenhouse tomato crops in New Zealand (Liefting et al. 2009a). Similar symptoms associated with the invasive psyllid, *B. cockerelli*, were also detected in other solanaceous crops such as pepper, tamarillo and potato (Liefting et al. 2008a; Liefting et al. 2008b). The tubers of infected potato plants were found to exhibit dark flecking and streaking characteristic of zebra chip rather than PY. Transmission electron microscopy of infected plants identified bacterium-like organisms (BLOs) in the phloem of symptomatic tomato and pepper. PCR amplification of DNA from infected material using primers modified for detecting the well characterised BLOs of citrus, belonging to the genus *Ca*. Liberibacter, led to the tentative identification of a new *Ca*. Liberibacter species, *Ca*. L. solanacearum, associated with solanaceous species including potato (Liefting et al. 2009a).

Phylogenetic analyses of the 16S rRNA gene, the 16S/23S rRNA spacer region and the *rpl*KAJL-*rpo*BC of *Ca*. L. solanacearum confirmed the close relationship of the organism to the other *Ca*. Liberibacter infecting citrus, but revealed that the bacterium was a distinct species (Liefting et al. 2009c). Interestingly, the 16S rRNA DNA sequences of *Ca*. L. solanacearum isolates from New Zealand (e.g. accession number EU849020) and those of the tentatively described '*Ca*. L. psyllaurous' from the United States (e.g. accession number EU812557) are identical and the species are considered to be closely related bacteria, if not the same (Wen et al. 2009). Further comparison of the 16S rRNA genes of *Ca*. L. solanacearum from New Zealand with isolates collected from potato plants affected with zebra chip in Central America, Mexico and the United States confirmed the association of *Ca*. L. solanacearum in zebra chip disease worldwide (Secor et al. 2009).

Although *Ca*. L. solanacearum can be successfully transmitted to potato plants by grafting (Secor et al. 2009), the role of seed tuber transmission in the spread of *Ca*. L. solanacearum and zebra chip remains unknown. When seed tubers affected by PY are planted, tubers may fail to germinate or result in a poor stand of weak stems or hair sprouts (Snyder et al. 1946). Tubers affected by zebra chip similarly either fail to sprout or produce weak plants and hair sprouts (Secor et al. 2009). In some instances, however, tubers with zebra chip symptoms produce apparently healthy plants that give rise to daughter

tubers, either with or without zebra chip symptoms (Gudmestad and Secor 2007).

In the present study, nested PCR was used to follow the transmission of *Ca*. L. solanacearum from mother tubers affected by zebra chip into the foliage and into the daughter tubers of resulting plants, in the absence of *B. cockerelli*. The ability of infected tubers to sprout and produce symptoms in the developing foliage and daughter tubers was also investigated. We discuss these results in relation to the potential spread of *Ca*. L. solanacearum through tuber transmission and the associated risks to the potato industry in New Zealand.

Materials and methods

Collection of potato samples

Potato tubers and foliage (New Zealand Institute for Plant and Food Research advanced breeding lines) used in this study were collected from plants with zebra chip symptoms and from symptomless plants. Samples were obtained after invasion of potato fields by *B. cockerelli* in Pukekohe, New Zealand, in the 2007–2008 growing season. Zebra chip was confirmed by the presence of foliar symptoms and the darkening of the medullary rays in tubers characteristic of this disease.

Tuber transmission experiments

To study the transmission of *Ca*. L. solanacearum from mother tubers to progeny tubers in the absence of *B. cockerelli*, symptomatic and asymptomatic tubers were each cut in half (longitudinally) and the presence of *Ca*. L. solanacearum in one half was confirmed by nested PCR. It was assumed a negative PCR result from a symptomless tuber indicated that the bacterium was not present. The second half of each tuber was then stored over winter, prior to planting in Canterbury, New Zealand, in the 2008–2009 season. Sixty-two infected and 38 *Ca*. L. solanacearum negative tubers were planted in a netted enclosure to prevent invasion of plants by the psyllid, and the development of symptoms was monitored between planting of the mother tubers and harvest of the resulting crop (November 2008 to May 2009). At the end of the season (or before if shoots failed to emerge or plants became symptomatic), two DNA samples were extracted both from leaf tissue and from mother tubers. Where daughter tubers were produced, two DNA samples were also extracted from at least one daughter tuber from each plant. The nested PCR, specific for detecting *Ca*. L. solanacearum, was performed on each sample to confirm the movement of *Ca*. L. solanacearum from the mother tuber into the leaves of developing plants as well as its transmission into progeny tubers.

DNA isolation

Total DNA was extracted from foliage and tubers of potato using a DNeasy Plant Mini Kit (Qiagen). For foliage, approximately 100 mg was used from the mid-veins of leaves. For tubers, 150–200 mg was taken from the vascular ring of each tuber. Initially, 650 μl AP1 buffer (DNeasy Plant Mini Kit) was added to the plant tissue in a screw cap vial containing 1 gS300-sized metal beads. Samples were then beaten for approximately 3 min using a Mini Bead Beater 8 (Biospec) and centrifuged at 8,000 rpm for 20 s to pellet the cell debris. Approximately 400 μl of the supernatant was added to a new tube and the sample was then treated according to manufacturer's instructions. Finally, samples were eluted in 50 μl EB buffer and stored at −20°C until required. Freeze-dried material from tomato plants infected with *Ca*. L. solanacearum was used as a positive control in each extraction.

PCR amplification

(i) Single step PCR. *Ca*. L. solanacearum-specific PCR reactions were performed using primers OA2 (5′ - GCGCTTATTTTTAATAGGAGCGGCA - 3′) and OI2c (5′ -GCCTCGCGACTTCGCAACC CAT - 3′) to amplify a 1,160-bp fragment from the 16 S rRNA gene, as previously described by Liefting et al. (2008a). Each reaction was performed in a 25 μl reaction containing 1x GoTaq Green Master Mix (Promega) and 0.2 μM of each primer. The following amplification conditions were used: Initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 66°C for 30 s, and extension at 72°C for 1 min and a final extension step of 72°C

for 10 min in a GeneAmpR PCR system 9,700 thermocycler (Applied Biosciences). To confirm the efficiency of template amplification, a separate PCR was performed using plant internal control primers, 28Sf (5′ - CCCTGTTGAGCTTGACTC TAGTCTGGC - 3′) and 28Sr (5′ - AAGAGCC GACATCGAAGGATC - 3′) (Werren et al. 1995), which produced a 600-bp fragment under identical reaction conditions.

(ii) Nested PCR. Nested PCR (16 S rRNA) was carried out according to Liefting et al. (2009b). The first round of the *Ca*. L. solanacearum-specific nested PCR reactions was performed using the primers OA2 and OI2c as described above for the single step PCR. A second round of PCR (nested PCR) was then carried out using Lib16S01F (5′ - TTCTACGGGATAACG CACGG - 3′) and Lib16S01R (5′ - CGTCAG TATCAGGCCAGTGAG - 3′) to produce an amplicon of 580 bp. The 25 µl reaction mixtures contained 1x GoTaq Green Master Mix (Promega), 0.2 µM of each primer and 1 µl (of a 1 in 20 dilution) from the first reaction as a template. The amplification conditions for the second round were: Initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 66°C for 30 s, and extension at 72°C for 1 min and a final extension step of 72°C for 10 min.

(iii) Multiplex PCR. Multiplex PCR was performed using the *Ca*. L. solanacearum-specific primers LsoF (5′ - CGAGCGCTTATTTTTAATAGGAG C - 3′) and OI2c as well as primers Fbtub-1 (5′ - TGATTTCCAAGGTAAGGGAGGA - 3′) and Rbtub-1 (5′ –CATGTTGCTCTCGGCTTCAG - 3′) to generate 1,160-bp (Liberibacter 16 S rRNA) and 881-bp (potato ß-tubulin internal amplification control) fragments, respectively (Wen et al. 2009). PCR reactions were performed in a 25 µl reaction containing 1x buffer, 0.2 mM dNTPs, 2 mM $MgSO_4$, 0.4 µM of the LsoF/OI2c primers, 0.13 µM of the Fbtub-1/ Rbtub-1 primers, 1 unit Platinum Taq (Invitrogen) and 2 µl of template DNA. Reactions conditions were: Initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 2 min and a final extension step of 72°C for 10 min.

Results

Pre-testing potato tubers prior to planting demonstrates the benefits of nested PCR for detection of *Ca*. L. solanacearum

A nested PCR protocol previously designed to detect *Ca*. L. solanacearum in solanaceous plants (Liefting et al. 2009b), was tested for detection of the bacterium in potato plants collected from fields infested by *B. cockerelli* in New Zealand and showing zebra chip symptoms. After the first round of amplification with primers OA2 and OI2c, a fragment of 1,160 bp was amplified using DNA from plants showing zebra chip symptoms. Subsequent nested PCR using primers Lib16S01F and Lib16S01R resulted in the amplification of a 580-bp product in samples derived from symptomatic foliage and tubers (Fig. 1). No fragment was amplified from DNA of symptomless potato plants.

To compare the efficacy of the nested PCR with existing single-step PCR and multiplex PCR protocols for detecting *Ca*. L. solanacearum, nine potato tubers harvested from a field heavily infested by *B. cockerelli* were tested for the presence of *Ca*. L. solanacearum using the three diagnostic techniques (Fig. 2). First, DNA from each sample was used as a template for amplifying the potato 28S gene using

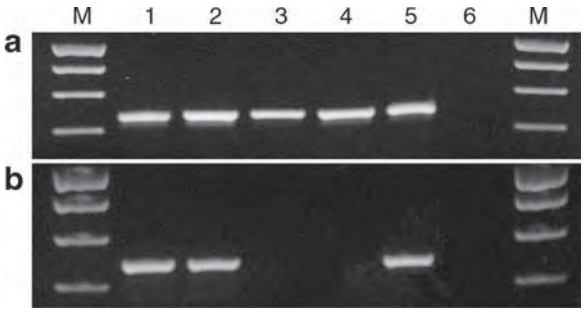

Fig. 1 Specific detection of *Ca*. L. solanacearum (Lso) from zebra chip-affected potato plants using nested PCR. **a**. Internal positive control PCR using primers 28Sf and 28Sr. **b**. Lso-specific nested PCR. The agarose gels show amplification products of 600 bp and 580-bp using the 28Sf and 28Sr primers and nested PCR, respectively. PCR products were generated upon amplification of DNA extracted from: Lane 1, symptomatic foliage; Lane 2, symptomatic tuber; Lane 3, uninfected foliage; Lane 4, uninfected tuber; Lane 5, freeze-dried positive control from tomato; Lane 6, negative PCR control. M, DNA size marker

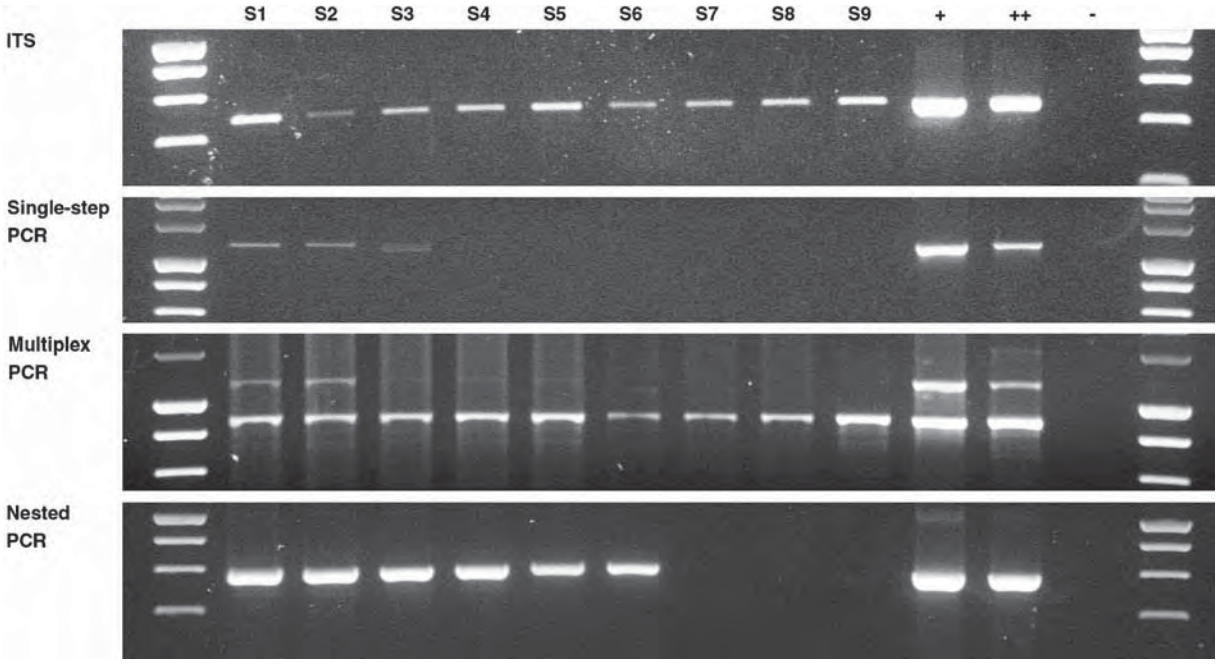

Fig. 2 Comparison of single-step PCR, multiplex PCR and nested PCR for sensitive detection of *Ca*. L. solanacearum (Lso) in potato tubers. The agarose gels show amplification products of 600 bp for the ITS PCR, 1,160 bp for the single-step PCR, 881 bp (internal control) and 1,160-bp (Lso-specific) for the multiplex PCR and 580 bp for the nested PCR. PCR products were amplified using DNA extracted from: S1–S9, potato tubers from a field infested by B. cockerelli; lane +, a freeze-dried positive control from tomato; lane + +, a positive control tuber; lane -, negative PCR control. M, DNA size marker

primers 28Sf and 28Sr. All samples produced the appropriate 600-bp amplicon, confirming the ability to amplify DNA from each sample. Subsequent single step PCR using the *Ca*. L. solanacearum-specific primers OA2 and OI2c generated an expected amplicon of 1,160-bp from DNA extracted from three of the nine tubers. Multiplex PCR, using the primers Fbtub-1 and Rbtub-1 as internal control primers for the amplification of the plant β-tubulin gene and the primers LsoF and OI2c for the specific detection of *Ca*. L. solanacearum, produced the expected bands in only two of the samples. The bacterium was detected in six of the nine potato tubers tested using nested PCR, demonstrating the improved detection of *Ca*. L. solanacearum using this protocol.

Tuber transmission of *Ca*. L. solanacearum is associated with development of tuber and foliar symptoms in progeny plants

Tuber transmission studies demonstrated that *Ca*. L. solanacearum was transmitted from mother tubers both to the emerging foliage and to progeny tubers in the following season in the absence of *B. cockerelli* (Table 1). The transmission of *Ca*. L. solanacearum was associated with the development of foliar and tuber symptoms previously linked to zebra chip (Fig. 3). Of the 62 tubers known to be infected by *Ca*. L. solanacearum prior to planting, four failed to sprout. In one instance, the failure to sprout was followed by the development of small tuber-like structures on the surface of the mother tuber (Fig. 3a). Furthermore, several tubers formed marble-like progeny tubers indicative of zebra chip (Fig. 3b). Subsequent nested PCR of two DNA samples extracted from each non-sprouting tuber confirmed the presence of *Ca*. L. solanacearum. Only one mother tuber, in which *Ca*. L. solanacearum was not detected by nested PCR, did not sprout. That tuber was found to have been affected by a soft-rot, indicating that its failure to sprout was unlikely to have been caused by infection with *Ca*. L. solanacearum.

A large proportion (58) of the mother tubers known to be infected by *Ca*. L. solanacearum sprouted, showing variable above-ground vigour similar to that observed in uninfected plants. During

Table 1 Numbers and respective *Ca*. L. solanacearum (Lso) status for seed tubers and their resulting plants and daughter tubers after cultivation from mother tubers infected with Lso, in the absence of the tomato potato psyllid *B. cockerelli*.

Preplant status of mother tuber		Lso detected	Lso not detected
		62	38
Tuber sprouting	Sprouting tubers	58	37[a]
	Non-sprouting tubers: Lso detected	4	N/T
	Non-sprouting tubers: Lso not detected	0	N/T
Foliar development	Sprouting tubers showing no foliar symptoms (asymptomatic)	56	37
	Asymptomatic plants: Lso detected	39	0
	Asymptomatic plants: Lso not detected	17	37
	Sprouting tubers showing foliar symptoms	2	0
	Symptomatic plants: Lso detected	2	0
	Symptomatic plants: Lso not detected	0	0
Daughter tubers	Asymptomatic plants producing progeny tubers	56	37
	Asymptomatic plants producing progeny tubers: Lso detected	5	0
	Asymptomatic plants producing progeny tubers: Lso not detected	51	37
	Asymptomatic plants producing progeny tubers with zebra chip symptoms	1	0
	Symptomatic plants producing progeny tubers	1 (marble tubers)	0
	Symptomatic plants producing progeny tubers: Lso detected	Tubers not produced	0
	Symptomatic plants producing progeny tubers: Lso not detected	Tubers not produced	0
	Symptomatic plants producing progeny tubers with zebra chip symptoms	Tubers not produced	0

[a] A single tuber did not sprout due to soft-rot

N/T not tested

the season, only two plants exhibited leaf cupping, curling, and purple colouration typical of plants affected by zebra chip (Table 1; Fig. 3c). Nested PCR of duplicate DNA samples extracted from leaf tissue of each symptomatic plant showed that *Ca*. L. solanacearum was present in both plants expressing foliar symptoms (Table 1). These symptomatic plants subsequently died prematurely, consistent with previous reports of early senescence.

Of 62 mother tubers, 56 produced progeny with no foliar symptoms suggesting that *Ca*. L. solanacearum had not been transmitted to the foliage in these plants. However, asymptomatic plants were subsequently found to be positive for *Ca*. L. solanacearum by nested PCR (Table 1). Duplicate DNA samples extracted from a representative leaf from 39 (70%) of the symptomless plants produced a *Ca*. L. solanacearum-specific fragment indicative of the presence of the bacterium. *Ca*. L. solanacearum could not be detected in the two DNA samples extracted from each of the remaining 17 plants. Failure to amplify a PCR product from these plants confirmed that *Ca*. L. solanacearum had not been transmitted into the progeny or that its titre was below detection levels. Plants grown from tubers in which *Ca*. L. solanacearum was not detected did not show symptoms associated with zebra chip, nor did they produce a *Ca*. L. solanacearum-specific amplicon upon testing of leaf tissue immediately prior to haulm destruction by nested PCR.

At the end of the growing season, progeny tubers harvested from the trial were screened for zebra chip symptoms and tested for the presence of *Ca*. L. solanacearum using nested PCR. The severe darkening of the medullary rays in fresh tubers from fields infested by *B. cockerelli* was not detected in any progeny tubers, while moderate flecking of the vascular tissue characteristic of zebra chip was only apparent in progeny tubers from a single infected plant. No symptoms were found in the remaining daughter tubers tested prior to frying. Symptoms of zebra chip in symptomatic daughter tubers were enhanced by frying, and produced mild discolouration of tubers from other infected plants (Fig. 3d). How-

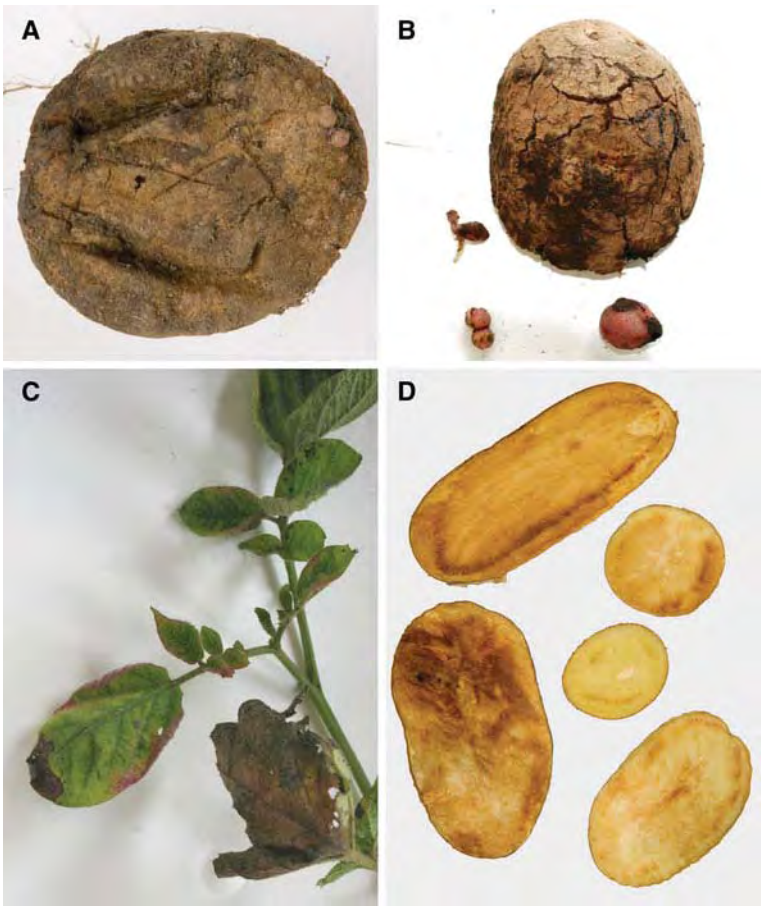

Fig. 3 Foliar and tuber symptoms associated with tuber transmission of *Ca*. L. solanacearum (Lso): **a**. A non-sprouting mother tuber infected with Lso; **b**. A Lso-infected mother tuber with marble-like progeny; **c**. Foliar symptoms associated with a Lso-infected progeny plant; **d**. Fried daughter tubers (Lso-positive) derived from Lso-infected mother tubers showing little or no expression of zebra chip-like symptoms

ever, none of the fried progeny tubers showed the severe symptoms observed in the mother tubers at harvest after infestation by *B. cockerelli*. Nested PCR of duplicate DNA samples extracted from a representative tuber from each harvested plant confirmed that *Ca.* L. solanacearum was present in the daughter tubers showing mild zebra chip symptoms (Table 1). *Candidatus* L. solanacearum was also detected in daughter tubers from a further four plants originating from infected mother tubers. No symptoms of zebra chip were observed, and *Ca*. L. solanacearum was not detected by nested PCR in daughter tubers produced by *Ca*. L. solanacearum negative plants.

Discussion

Until recently the etiological agent of zebra chip remained unknown. The discovery, however, that *Ca.* L. solanacearum was associated with plants affected by zebra chip, and the expression of symptoms upon transmission of *Ca.* L. solanacearum by grafting or psyllid feeding, demonstrated the involvement of this bacterium in the disease (Hansen et al. 2008; Secor et al. 2009). Using nested PCR, we have shown the transmission of *Ca.* L. solanacearum from infected seed potato tubers into progeny plants in the absence of the insect vector *B. cockerelli*. *Candidatus* L. solanacearum was found to be transferred both to the foliage and daughter tubers of resulting plants. A number of these plants developed symptoms associated with zebra chip, although symptoms resulting from tuber transmission were less pronounced than those related to insect-inoculation and feeding. Tuber transmission of *Ca.* L. solanacearum suggests that seed potato tubers from infected plants could provide a secondary source of inoculum for the following crop as well as those in surrounding areas in New Zealand.

Often, tubers harvested from crops affected by *B. cockerelli* do not sprout or produce only hair sprouts (Linford 1928; Metzger 1936; Snyder et al. 1946; Sanford 1952). Seed pieces that fail to produce plants remain dormant (Edmundson 1940). Cultivation of tubers from plants affected by *B. cockerelli* can also produce numerous small daughter tubers (Binkley and Metzger 1929; Richards and Blood 1933). In our transmission trial, we observed both dormancy of seed tubers and development of marble-like daughter tubers when mother tubers affected by zebra chip were planted. However, unlike previous studies in which a high frequency of non-sprouting tubers were observed, only 6.4% of seed tubers infected with *Ca.* L. solanacearum did not produce plants and remained dormant the following season. It is difficult to obtain quantitative data on the sprouting rates of potatoes affected by zebra chip in other studies, but the variable sprouting could be a result of differences in cultivar, abiotic factors or the titre of the pathogen in tubers.

A large proportion (93.6%) of tubers infected with *Ca.* L. solanacearum successfully sprouted. Of these, only 3.4% produced haulms or daughter tubers with zebra chip symptoms, yet 70% of the plants that did not develop foliar or tuber symptoms were shown by nested PCR to be infected with *Ca.* L. solanacearum. In citrus, *Candidatus* Liberibacter asiaticus was detected in 41.7% of seedlings following seed transmission. However, HLB-like symptoms, such as yellow shoot, blotch mottle and vein corky on the leaves were observed in only a low percentage of seedlings. *Candidatus* L. asiaticus remained at a very low titre in seedlings, unlike that in graft- or psyllid-transmitted HLB-affected citrus plants, and most, if not all, of the seedlings did not develop typical HLB disease over a 3 year period (Benyon et al. 2009). Perhaps the factors that modulate the titre of *Ca*. L. asiaticus and disease development in citrus are similar to those involved in the low level of *Ca.* L. solanacearum-related symptoms observed upon tuber transmission in potatoes in New Zealand.

The low level of disease expression in our study is also consistent with the earlier observations of Shapovalov (1929), who, when studying PY, found that in the absence of the psyllid plants grown from potato tubers affected by *B. cockerelli* exhibited different stages of PY and that some appeared to be free from the disease altogether. Richards (1931) also recorded that normal progeny could be obtained from tubers harvested from psyllid-infested plants and that, under greenhouse conditions, disease symptoms were not induced uniformly.

Shapovalov (1929) speculated that *B. cockerelli*-induced PY symptoms were very much more pronounced in dry and hot conditions than when artificially shaded. Development of zebra chip symptoms also appears to be dependent on light intensity (JE Munyaneza, USDA-ARS Wapato, personal communication). To ensure that tubers infected with *Ca.* L. solanacearum were not affected by *B. cockerelli* during our trial, plants were contained within a netted enclosure that also gave artificial shading. It is possible that shading reduced the incidence of disease symptoms in plants carrying *Ca.* L. solanacearum and that foliar and tuber symptoms are not a good indicator of transmission of the pathogen under these conditions. Furthermore, the majority of our experiments were conducted in Canterbury, an area where *B. cockerelli* has been found but zebra chip has not yet appeared in commercial potato crops. It is possible that environmental conditions in Canterbury are less conducive to the expression of zebra chip than those in more northerly potato-growing areas of New Zealand.

Alternatively, the low levels of symptom expression in our study maybe due to the absence of an additional etiological agent involved in the zebra chip disease complex. In addition to *Ca*. L. asiaticus, a phytoplasma related to *Candidatus* Phytoplasma asteri was recently detected in Citrus showing HLB symptoms in Guangdong, P. R. China (Chen et al. 2009). In New Zealand, *Candidatus* Phytoplasma australiense has been detected in potatoes showing zebra chip symptoms (Liefting et al. 2009b). *Candidatus* Phytoplasma australiense is associated with Australian grapevine yellows and Papaya dieback (Andersen et al. 2006).

Candidatus L. solanacearum was not detected in 27.4% of the plants grown from seed tubers infected with the bacterium, suggesting that the pathogen had not been transmitted from the mother tubers in these plants or that the titre of *Ca.* L. solanacearum was too low to detect by nested PCR. *Candidatus* Liberibacter species associated with citrus HLB have different geographical distributions associated with temperature sensitivity (Schwarz and Green 1972). Indeed, '*Ca.* L. americanus'-infected sweet orange trees do

not develop HLB blotchy mottle leaf symptoms and become '*Ca.* L. americanus'-negative when maintained under warm conditions (Lopes et al. 2009). We observed more severe symptoms in progeny grown in Pukekohe, New Zealand (data not shown), than in Canterbury, where (according to nested PCR) loss of *Ca.* L. solanacearum was relatively high in growing foliage and daughter tubers. Environmental factors such as temperature may affect the viability of *Ca.* L. solanacearum, tuber transmission and the development of zebra chip in New Zealand. Measurement of the persistence of this bacterium in plants grown in other geographical locations and monitoring of the development of symptoms in plants under different conditions of temperature, light intensity and soil moisture may help to explain differences in symptom expression.

Replicate sampling was required to ensure nested PCR identified plant tissue infected by *Ca.* L. solanacearum, especially when plants were asymptomatic. These data suggest that the titre of *Ca.* L. solanacearum in potato plants maybe low or that distribution of the pathogen maybe uneven. This is consistent with the difficulty in detecting *Ca*. Liberibacter species causing Huanglongbing, which has been attributed to either low concentration or uneven distribution of the bacteria in citrus plants (Benyon et al. 2008). Even in symptomatic citrus plants, the distribution of *Ca*. L. asiaticus has been shown to vary between different tissues using quantitative PCR (Li et al. 2009). The titre of *Ca*. Liberibacter species in asymptomatic citrus has not been established, but other bacterial pathogens of potato have been found to elicit symptoms only after reaching threshold cell densities (Pérombelon 2002). Pathogen population density maybe important for the development of HLB symptoms in citrus as well as zebra chip in potato.

The transmission of *Ca.* L. solanacearum from mother tubers to progeny plants, and the presence of the bacterium in asymptomatic plants grown from infected tubers are likely to have a significant impact on New Zealand's seed potato industry. Seed potatoes are produced in areas inhabited by *B. cockerelli* in this country, yet symptoms of zebra chip have yet to be observed in commercial crops. Our results indicate that asymptomatic infection of seed crops with *Ca.* L. solanacearum and the subsequent distribution of harvested seed tubers to other growing areas could enhance the rate of spread of zebra chip. It could also reduce the yield of marketable potatoes for both the fresh and processing industries if infected seed potatoes are planted under environmental conditions conducive to the development of more severe symptoms.

Acknowledgements We thank Mr Warrick Nelson, Professor Richard Falloon and Dr Bob Fullerton for helpful critique of the manuscript as well as Mr John Anderson from the New Zealand Institute for Plant & Food Research Limited and Dr Lia Liefting from the Plant Health and Environment Laboratory at MAF Biosecurity, New Zealand, for the provision of infected plant material. This research was supported by the New Zealand Institute for Plant & Food Research Limited.

References

Andersen, M. T., Newcomb, R. D., Liefting, L. W., & Beever, R. E. (2006). Phylogenetic analysis of "*Candidatus* Phytoplasma australiense" reveals distinct populations in New Zealand. *Phytopathology, 96*, 838–845.

Benyon, L., Zhou, L., Weathersbee, A., & Duan, Y. (2008). Nested PCR is essential for the detection of extremely low titer of *Candidatus* Liberibacter asiaticus from citrus and its vector psyllid *Diaphorina citri*. *Phytopathology, 98*, S21.

Benyon, L., Zhou, L., Duan, Y., McCollum, G., Powell, C., Hall, D., et al. (2009). Seed transmission of '*Candidatus* Liberibacter asiaticus' in citrus without typical huanglongbing. *Phytopathology, 99*, S11.

Binkley, A. M., & Metzger, C. H. (1929). Psyllid yellows (cause undetermined). United States Department of Agriculture, Bureau of Plant Industry. *Plant Disease Reporter, 68*(suppl.), 29.

Bové, J. M. (2006). Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology, 88*, 7–37.

Chen, J., Pu, X., Deng, X., Liu, S., Li, H., & Civerolo, E. (2009). A Phytoplasma related to '*Candidatus* Phytoplasma asteri' detected in citrus showing Huanglongbing (Yellow Shoot Disease) symptoms in Guangdong, P. R. China. *Phytopathology, 99*, 236–242.

Edmundson, W. (1940). The effect of psyllid injury on the vigor of seed potatoes. *American Potato Journal, 17*, 315–317.

Garnier, M., Jagoueix-Eveillard, S., Cronje, P., Le Roux, H., & Bové, J. M. (2000). Genomic characterisation of a liberibacter present in an ornamental Rutaceous tree, *Calodendrum capense*, in the Western Cape province of South Africa. Proposal for a "*Candidatus* Liberibacter africanus subsp. capensis". *International Journal of Systematic and Evolutionary Microbiology, 50*, 2119–2125.

Gudmestad, N. C., & Secor, G. A. (2007). Zebra chip: A new disease of potato. *Nebraska Potato Eyes* (University of Nebraska–Lincoln) 19, PHREC 07-05a.

Hansen, A. K., Trumble, J. T., Stouthamer, R., & Paine, T. D. (2008). A new Huanglongbing species, "*Candidatus*

Liberibacter psyllaurous", is vectored by the Psyllid *Bactericera cockerelli* (Sulc). *Applied and Environmental Microbiology, 74*, 5862–5865.

Jagoueix, S., Bové, J. M., & Garnier, M. (1994). The phloem-limited bacterium of greening disease of citrus is a member of the α subdivision of the Proteobacteria. *International Journal of Systematic Bacteriology, 44*, 397–386.

Jagoueix, S., Bové, J. M., & Garnier, M. (1996). PCR detection of the two '*Candidatus*' liberobacter species associated with greening disease of citrus. *Molecular and Cellular Probes, 10*, 43–50.

Li, W., Levy, L., & Hartung, J. S. (2009). Quantitative distribution of '*Candidatus* Liberibacter asiaticus' in citrus plants with citrus huanglongbing. *Phytopathology, 99*, 139–144.

Liefting, L. W., Perez-Egusquiza, Z. C., Clover, G. R. G., & Anderson, J. A. D. (2008). A new '*Candidatus* Liberibacter' species in *Solanum tuberosum* in New Zealand. *Plant Disease, 92*, 1474.

Liefting, L. W., Ward, L. I., Shiller, J. B., & Clover, G. R. G. (2008). A new '*Candidatus* Liberibacter' species in *Solanum betaceum* (tamarillo) and *Physalis peruviana* (cape gooseberry) in New Zealand. *Plant Disease, 92*, 1588.

Liefting, L. W., Sutherland, P. W., Ward, L. I., Paice, K. L., Weir, B. S., & Clover, G. R. G. (2009). A new '*Candidatus* Liberibacter' species associated with diseases of Solanaceous crops. *Plant Disease, 93*, 208–214.

Liefting, L. W., Veerakone, S., Ward, L. I., & Clover, G. R. G. (2009). First report of 'Candidatus Phytoplasma australiense' in Potato. *Plant Disease, 93*, 969.

Liefting, L. W., Weir, B. S., Pennycook, S. R., & Clover, G. R. G. (2009). '*Candidatus* Liberibacter solanacearum', associated with plants in the family Solanaceae. *International Journal of Systematic and Evolutionary Microbiology, 59*, 2274–2276.

Linford, M. (1928). Plant diseases in Utah in 1927. *Plant disease reporter. Supplement, 59*, 95–99.

Lopes, S. A., Frare, G. F., Bertolini, E., Cambra, M., Fernandes, N. G., Ayres, A. J., et al. (2009). Liberibacters associated with citrus huanglongbing in Brazil: '*Candidatus* Liberibacter asiaticus' is heat tolerant, '*Candidatus* Liberibacter americanus' is heat sensitive. *Plant Disease, 93*, 257–262.

Metzger, C. (1936). Some preliminary notes on the effect of psyllid yellows on seed stock from infected plants. *American Potato Journal, 13*, 277–285.

Munyaneza, J. E., Crosslin, J. M., & Upton, J. E. (2007). Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with "zebra chip", a new potato disease in Southwestern United States and Mexico. *Journal of Economic Entomology, 100*, 656–663.

Pérombelon, M. C. M. (2002). Potato diseases caused by soft rot erwinias: an overview of pathogenesis. *Plant Pathology, 51*, 1–12.

Planet, P., Jagoueix, S., Bové, J. M., & Garnier, M. (1995). Detection and characterization of the African citrus greening liberobacter by amplification, cloning and sequencing of the rplKAJL-rpoBC operon. *Current Microbiology, 30*, 137–141.

Richards, B. L. (1928). A new and destructive disease of potato in Utah and its relation to the potato psylla. *Phytopathology, 18*, 140.

Richards, B. L. (1931). Further studies with psyllid yellows of the potato. *Phytopathology, 21*, 103.

Richards, B. L., & Blood, H. L. (1933). Psyllid yellows of the potato. *Journal of Agricultural Research, 46*, 189–216.

Sanford, G. (1952). Phloem necrosis of potato tubers associated with infestation of vines by *Paratrioza cockerelli* Sulc. *Scientific Agriculture, 32*, 433–439.

Schwarz, R. E., & Green, G. C. (1972). Heat requirements for symptom suppression and inactivation of the greening pathogen. In: *Proceedings of the fifth Conference of the International Organisation of Citrus Virologists*, 1972. Gainesville, Florida, United States of America, 44 –51.

Secor, G. A., Lee, I., & Bottner, K. (2006). First report of a defect of processing potatoes in Texas and Nebraska associated with a new Phytoplasma. *Plant Disease, 90*, 377.

Secor, G. A., Rivera, V. V., Abad, J. A., Lee, I. M., Clover, G. R. G., Liefting, L. W., et al. (2009). Association of '*Candidatus* Liberibacter solanacearum' with the zebra chip disease of potato established by graft and psyllid transmission, electron microscopy and PCR. *Plant Disease, 93*, 574–583.

Shapovalov, M. (1929). Tuber transmission of psyllid yellows in California. *Phytopathology, 19*, 1140.

Snyder, W. C., Thomas, H. E., & Fairchild, S. J. (1946). Spindling or hair sprout of potato. *Phytopathology, 36*, 897–904.

Teixeira, D. D. C., Saillard, C., Eveillard, S., Danet, J. L., da Costa, P. I., Ayres, A. J., et al. (2005). "*Candidatus* Liberibacter americanus", associated with citrus huanglongbing (greening disease) in São Paulo State, Brazil. *International Journal of Systematic and Evolutionary Microbiology, 55*, 1857–1862.

Wen, A., Mallik, I., Alvarado, V. Y., Pasche, J. S., Wang, X., Li, W., et al. (2009). Detection, distribution and genetic variability of '*Candidatus* Liberibacter' species associated with zebra complex of Potato in North America. *Plant Disease, 93*, 1102–1115.

Werren, J. H., Windsor, D., & Guo, L. (1995). Distribution of *Wolbachia* among neotropical arthropods. *Proceedings of the Royal Society of London. Series B, 262*, 197–204.