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Literature review: Tuber transmission of *Candidatus* Liberibacter solanacearum

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1 SUMMARY

The aim of this literature review is to address two main questions:

- What is the relationship between foliar symptoms of *Candidatus* Liberibacter solanacearum (CLso) and tuber infection?
- What happens to CLso titre and Zebra Chip (ZC) symptoms in tubers while in storage?

The majority of the work reviewed was conducted in the USA. It is important to keep in mind that the effects of soil type, climate, and other environmental variables on CLso and ZC symptoms are not well understood. Potato cultivars also display different responses to environmental factors and to CLso infection. The combination of factors are likely to influence the outcome of the experiments.

Following potato plant infestation with CLso-positive tomato potato psyllids (TPP) it takes approximately 3 weeks for foliar and tuber ZC symptoms to develop. However, CLso can move from leaf tissue into tubers within 4 days after infection, and low titres of CLso may not result in ZC symptoms in tubers at harvest. The research in this area implies that control of TPP is important throughout the growing season right up until harvest.

Several peer-reviewed papers have made recommendations about plant tissue sampling for CLso testing. These plant tissue recommendations vary from young leaves to stem material. However, this area needs to be investigated in more detail. CLso titres may vary depending on the method of infection (mother tuber or TPP), the plant age, and the timing of infection.

CLso titres and ZC symptoms have been shown to increase during storage, with the research simulating commercial storage and post-storage conditions. Tubers may be asymptomatic at harvest but develop ZC symptoms while in storage. Post-storage tuber assessment may be required to measure the impact of ZC on the harvested tubers accurately.

While ZC symptom development during storage is recognized as an important issue for process potatoes, it is not seen as a priority for seed potatoes in the USA. A large proportion of infected tubers do not emerge, and those that do are often stunted and delayed compared with uninfected plants. In the USA, it is thought that uninfected plants outcompete the stunted plants, preventing them from becoming a CLso hotspot within a field. A single publication from New Zealand provided evidence that the emergence issue is different in this country.

2 RELATIONSHIP BETWEEN CLSO FOLIAR SYMPTOMS AND TUBER INFECTION

Early work by Henne et al. (2010) reported emergence rates from ZC tubers of 44% for 'Ranger Russett' in North Dakota, USA, and 18% for 'Russet Norkotah' in Texas, USA. The plants that emerged from ZC tubers were stunted and slow growing. This led to the conclusion that they would not be a focal point for disease spread in the field, as uninfected plants would outcompete these diseased plants. Part of Henne and colleagues' work was completed before identification of CLso as the putative causal agent for ZC, and when molecular assays and testing standards were still being established. Seed tubers were selected based on visual symptoms, while later studies used molecular techniques for selection.

Emergence rates of CLso-infected tubers reported from New Zealand were higher than those reported in the USA. A total of 58 plants (various PFR breeding lines) emerged from 62 CLso-positive tubers (93.6% emergence) (Pitman et al. 2011). Only two plants showed typical ZC symptoms, with the others described as having "variable above-ground vigour". Over half the plants that emerged tested positive for CLso. Only a small number of daughter tubers had detectable amounts of CLso after harvest. Unfortunately, the effect of storage on the CLso titres in the daughter tubers was not investigated. It would have been interesting to see if CLso present in low amounts would have increased with storage time in these daughter tubers.

To study the effect of CLso infection timing, potato plants were infested at different time points following emergence (Rashed et al. 2015). CLso-positive TPP were released onto caged 'FL1867' potato plants in Texas, USA, to generate CLso-positive tubers. Tubers were put into cold-storage (4.5°C) for 9-10 months then moved to 26-28°C for four days prior to planting. Plants from tubers infected 1 week before harvest emerged at a similar time and rate as the non-infected controls. Plants from tubers infected earlier (3 or more weeks before harvest) took longer to emerge. Information about the CLso titre of the tubers and plants that emerged was not clear in the publication. It is possible that not all the tubers were infected during the short time period between plant infestation and tuber harvest.

Another area of research is the movement and distribution of CLso within a potato plant, including into tubers, following feeding by CLso-positive TPP. This information can be used to inform plant tissue sampling and surveillance strategies.

The movement of CLso in a range of potato cultivars was assessed by Levy et al. (2011), also based in Texas, USA. TPP from a colony known to be ~70% positive for CLso were clip-caged onto leaves for 7 days. Leaves and petioles above and below the infestation point were tested using polymerase chain reaction (PCR) and quantitative PCR (qPCR) weekly. CLso was detected in plants 3 weeks following infestation but no obvious pattern was observed for the movement of CLso. CLso was found most frequently in young leaves. An outcome from this work was the recommendation of sampling of newly developing leaves for CLso testing (Levy et al. 2011).

By using potato plants with only two stems coming from one mother tuber, Levy et al. (2011) also showed vascular transfer of CLso from one stem to the other. This could happen relatively quickly, in some cases within 3 weeks of TPP feeding. Comparing the movement of CLso to that of rhodamine B dye showed that the vascular architecture of potatoes partially limits CLso movement (Cooper et al. 2015). Leaves with a direct vascular connection to the point of TPP feeding were more likely to be positive for CLso. However, CLso was also detected in areas without direct connections, indicating that it can move independently from the phloem. Information about the bicollateral (containing two types of phloem in the stem) vascular system of solanaceous plants should be incorporated into future work and sampling strategies.

It took 3-4 weeks for foliar symptoms to develop following infestation of potato plants with CLso-positive TPP in the USA (Buchman et al. 2012; Levy et al. 2011; Rashed et al. 2014). The time for foliar symptom development did not change with plant age (Rashed et al. 2014). Rashed et al. (2014) also reported that it took approximately 3 weeks before CLso can be detected by qPCR in young leaf material. However, they were able to detect CLso in stem material sooner, just 14-20 days after infestation. This study suggested that stem material may be a better sampling point for CLso testing.

While foliar symptoms and CLso in leaf material take approximately the same time to be detected, symptoms in tubers are quite different. Fresh-cut tuber symptoms can reliably be seen in as little as 3 weeks for susceptible varieties such as 'Atlantic' (Rashed et al. 2014). However, if tuber infection happened 2 weeks before harvest, visual symptoms were not reliable as symptomless tubers could have detectable amounts of CLso. Molecular testing can be used to assess the rate of CLso tuber infection more accurately. For example, 74.3% of daughter tubers from plants infested with TPP 2 weeks before harvest tested positive for CLso at harvest, but only 1.3% of those daughter tubers showed ZC symptoms at harvest (Rashed et al. 2014).

3 ROLE OF STORAGE ON CLSO TITRE AND TUBER SYMPTOMS

A range of storage regimes have been used in studies investigating CLso and ZC disease in the USA. However, studies have only recently started to address the role of storage and storage temperature in the development of ZC disease. While early CLso infection can negatively affect the yield and tuber size (Buchman et al. 2012), the majority of studies have focused on tubers infected 1-2 weeks before harvest. This is because of observations when ZC and CLso were first recognised as a major problem that tubers without symptoms at harvest could develop ZC symptoms during storage.

The majority of this work was done in Texas, USA. A couple of early studies were performed in Washington State, USA. No published work from New Zealand was found.

CLso-infected tubers generally showed increased concentrations of phenolic compounds, reducing sugars and certain amino acids compared with non-infected tubers (Buchman et al. 2012; Rashed et al. 2013; Wallis et al. 2012; Wallis et al. 2015; Wallis et al. 2014). These responses have been measured across several varieties such as 'Atlantic', 'Red La Soda', 'FL1867', and 'Russett Norkotah', as well as breeding lines with low ZC symptoms when infected. Tuber ZC symptoms increased in severity over time. However, CLso titres peaked 3 weeks after infection and then decreased (Rashed et al. 2013). ZC symptoms did not directly correspond to CLso titre in these studies. This suggests that CLso infection initiates a host defence response that continues to affect the quality of the tuber.

Rashed et al. (2015) investigated the role of different storage regimes on CLso titre. 'FL1867' plants in Texas were infested with CLso-positive TPP 1 or 2 weeks prior to harvest. Tubers were put into cold-storage (5.5°C) for 2, 4, or 6 months followed by 5 weeks at room temperature. Multiple samples were taken from tubers over time to track the CLso titre. The number of tubers that tested positive for CLso increased with each week of ambient storage, regardless of the cold-storage period. The CLso titres also increased during room temperature storage. Tubers from plants infested 2 weeks before harvest had higher CLso titres than plants infested 1 week before harvest. Tubers stored for 6 months had higher CLso titres than tubers stored for 2 months. Following storage, the tubers were planted out and emergence was measured. Approximately half the plants emerged; however, CLso was detected in only 2% of

the emerged plants when they were sampled at the base of the above-ground stem. This result again reinforced the idea that seed tuber infection is unlikely to generate disease hotspots in fields for US growers.

The role of storage temperature on ZC symptom development was investigated by Wallis et al. (2017). CLso-positive TPP were released onto 'Russet Norkotah' and 'Red La Soda' plants 2 weeks before harvest. Tubers were sampled from the stolon end before storage at 3, 6, or 9°C for 12 weeks. Samples were taken from the tubers repeatedly, to measure CLso titres and for biochemical analyses. At 12 weeks postharvest, the tubers were sliced to assess fresh-cut ZC tuber symptoms. ZC scores and phenolic compound concentrations were higher in tubers from both cultivars stored at 3°C than at 6°C or 9°C. However, considerable variation was observed in the CLso titres. The cold storage regime used may not reflect what is used commercially, as it contained no ramp-down phase.

Recent research in Texas has simulated temperatures of commercial storage and post-storage retail chain movement to track the development of CLso titres and ZC symptoms in tubers using the cultivar 'Russett Norkotah' (Rashed et al. 2018). This research was carried out over 2 growing seasons in 2013 and 2014. Plants were infested with CLso-positive TPP 14, 10 or 4 days before harvest to assess the risk of late-season infection. The temperature regime included 3 weeks at 13°C for wound healing before decreasing 0.3°C/day until the cool storage temperature of 7.2°C was reached. Tubers were maintained at this temperature for 8-9 weeks. The retail chain period was 13°C for 4 weeks. This study confirmed much of the previous work. Tubers from plants that were infested earlier showed higher titres of CLso, and had higher ZC severity scores compared to tubers from plants infected 4 days before harvest. CLso titres increased for all tuber groups during storage, including during the retail chain period. Following storage, ZC symptoms did not develop in the majority of tubers from plants infested 4 days before harvest, but some of the tubers did have detectable amounts of CLso. The number of tubers infected and the CLso titres differed significantly between the 2 seasons. The authors concluded that post-storage screening for ZC symptoms and CLso was more reliable than screening at harvest.

4 POINTS FOR CONSIDERATION

The majority of the research reviewed was conducted in Texas, USA, using a few US potato cultivars. There is a large amount of variation in the research results, as is expected for this type of biological study. However, it makes it difficult to compare results from different years and from different researchers in different locations. The effects of soil type, climate, and other environmental variables are not well understood, but are likely to influence the results. A single paper from researchers at PFR differs in its conclusions about emergence of CLso-infected tubers compared with data reported by others from Texas.

Experiments carried out by Wallis, Rashed, Rush and colleagues (Rashed et al. 2018; Rashed et al. 2014; Rashed et al. 2015; Wallis et al. 2014) in Texas used a TPP colony that was a Central biotype. It had a mixed infection of CLso haplotypes A and B. However, the data supporting this were not published. It would be of interest to know if one CLso haplotype was present in a greater proportion than the other, and if both haplotypes were transferred to the potato plants and tubers. It is not known how the TPP biotypes or CLso haplotypes may have affected the outcome of these experiments.

Numerous publications discuss the uneven distribution and low titre of CLso as a potential issue (Pitman et al. 2011; Wallis et al. 2017). The methods used to detect CLso have improved over

time (e.g. the use of qPCR instead of PCR) and this may lower the causes of the variation seen in some of the earlier work. In addition, the tuber sampling method most commonly used may also have contributed to the variation. The description of tuber sampling generally given suggests that 0.1 g of a tuber slice was used for DNA extraction and subsequent CLso testing. Restricting the sample to vascular tissue, as described in Pitman et al. (2011), may also reduce this variation. However, a better understanding of CLso distribution in plants and tubers could improve the sampling strategies used in these sorts of studies.

Temporal sampling also creates challenges. Re-sampling the same tuber leads to multiple openings/wounds in the tuber. This could affect the tuber physiology and defence responses. There is also no available evidence that CLso is uniformly distributed around the area being sampled. The alternative of sampling multiple tubers also has drawbacks, as tubers differ in individual composition and fitness. There is also no method to ensure the tubers are infected at the same time or with the same titre of CLso.

In the experiments performed by Rashed et al. (2015), a large number of tubers from infested plants did not test positive for CLso and did not result in infected plants. This observation has not been followed up. Was the low titre in these tubers insufficient to establish infection in emerging plants? Could these plants have become disease hotspots and infected TPP, although not showing symptoms themselves?

Further research should be conducted in order to understand the impact of New Zealand environmental conditions and potato cultivars on the issue of tuber-based CLso transmission. It is also important to address the issues relating to the distribution of CLso, particularly in tubers.

5 ABBREVIATIONS

CLso	*Candidatus* Liberibacter solanacearum; the bacterium vectored by the tomato potato psyllid and the putative causal agent of zebra chip disease in potato tubers
TPP	Tomato potato psyllid, *Bactericera cockerelli*, a miniature cicada affecting solanaceous crops and a vector for CLso
ZC	Zebra chip
PCR	Polymerase chain reaction; a molecular biological method for amplifying (creating multiple copies of) a short, well-defined part of a DNA strand, which are visualised as bands on a gel. This can be a single gene, or just a part of a gene.
qPCR	Quantitative polymerase chain reaction; a specialised technique that allows the results of a PCR reaction to be visualised in real time as the reaction progresses. It allows the measurement of minute amounts of DNA sequences (the amplified, well-defined part of a DNA strand – see PCR) in a sample; no gel is required for visualisation. It also allows for the titre of a sample to be determined by comparing to standards of a known amount.
PFR	The New Zealand Institute for Plant & Food Research Limited

6 REFERENCES

Buchman JL, Fisher TW, Sengoda VG, Munyaneza JE 2012. Zebra chip progression: from inoculation of potato plants with *Liberibacter* to development of disease symptoms in tubers. American Journal of Potato Research 89(2): 159-168.

Cooper WR, Alcala PE, Barcenas NM 2015. Relationship between plant vascular architecture and within-plant distribution of '*Candidatus* Liberibacter solanacearum' in potato. American Journal of Potato Research 92(1): 91-99.

Henne DC, Workneh F, Wen A, Price JA, Pasche JS, Gudmestad NC, Rush CM 2010. Characterization and epidemiological significance of potato plants grown from seed tubers affected by Zebra Chip disease. Plant Disease 94(6): 659-665.

Levy J, Ravindran A, Gross D, Tamborindeguy C, Pierson E 2011. Translocation of '*Candidatus* Liberibacter solanacearum', the Zebra Chip pathogen, in potato and tomato. Phytopathology 101(11): 1285-1291.

Pitman AR, Drayton GM, Kraberger SJ, Genet RA, Scott IAW 2011. Tuber transmission of '*Candidatus* Liberibacter solanacearum' and its association with zebra chip on potato in New Zealand. European Journal of Plant Pathology 129(3): 389-398.

Rashed A, Olsen N, Wallis CM, Paetzold L, Woodell L, Rashidi M, Workneh F, Rush CM 2018. Postharvest development of '*Candidatus* Liberibacter solanacearum' in late-season infected potato tubers under commercial storage conditions. Plant Disease 102(3): 561-568.

Rashed A, Wallis CM, Paetzold L, Workneh F, Rush CM 2013. Zebra chip disease and potato biochemistry: tuber physiological changes in response to '*Candidatus* Liberibacter solanacearum' infection over time. Phytopathology 103(5): 419-426.

Rashed A, Workneh F, Paetzold L, Gray J, Rush CM 2014. Zebra chip disease development in relation to plant age and time of '*Candidatus* Liberibacter solanacearum' infection. Plant Disease 98(1): 24-31.

Rashed A, Workneh F, Paetzold L, Rush CM 2015. Emergence of 'Candidatus Liberibacter solanacearum'-infected seed potato in relation to the time of infection. Plant Disease 99(2): 274-280.

Wallis CM, Chen JC, Civerolo EL 2012. Zebra chip-diseased potato tubers are characterized by increased levels of host phenolics, amino acids, and defense-related proteins. Physiological and Molecular Plant Pathology 78: 66-72.

Wallis CM, Munyaneza JE, Chen J, Novy R, Bester G, Buchman JL, Nordgaard J, Hest Pv 2015. '*Candidatus* Liberibacter solanacearum' titers in and infection effects on potato tuber chemistry of promising germplasm exhibiting tolerance to zebra chip disease. Phytopathology 105(12): 1573-1584.

Wallis CM, Rashed A, Wallingford AK, Paetzold L, Workneh F, Rush CM 2014. Similarities and differences in physiological responses to '*Candidatus* Liberibacter solanacearum' infection among different potato cultivars. Phytopathology 104(2): 126-133.

Wallis CM, Rashed A, Workneh F, Paetzold L, Rush CM 2017. Effects of holding temperatures on the development of zebra chip symptoms, '*Candidatus* Liberibacter solanacearum' titers, and phenolic levels in 'Red La Soda' and 'Russet Norkotah' tubers. American Journal of Potato Research 94(4): 334-341.

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