Growth and Yield Performance of *Solanum tuberosum* Grown from Seed Potatoes Infected with ‘*Candidatus Liberibacter solanacearum*’ Haplotypes A and B

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Abstract

Zebra chip (ZC) disease of potato (*Solanum tuberosum*) is associated with infection by ‘*Candidatus Liberibacter solanacearum*’ (Lso). Two haplotypes of Lso—A and B—occur in the United States. Lso haplotype B is more virulent than haplotype A, causing greater disease incidence in tubers, more severe symptoms, and greater loss in tuber yield. This study assessed whether tubers from infected plants generate new infected plants the following year. The effects of both Lso haplotypes A and B on tuber resprout were examined on five potato cultivars. When compared with noninfected tubers, overall plant emergence rate from Lso A- or B-infected tubers was lower, plants emerged slower,

and plants generated lower daughter tuber yields in weight and quantity. Plants generally emerged poorly from Lso B-infected tubers and produced lower daughter tuber yields than Lso A-infected tubers. Regardless of Lso treatment, all daughter tubers were asymptomatic, and only 0.3% tested positive for Lso in experiments conducted over 2 years. This suggests that plants generated from Lso A- and Lso B-infected seed potatoes with severe ZC symptoms are likely not a significant source of Lso in potato fields.

Keywords: Lso, potato, zebra chip

During the past two decades, zebra chip (ZC) disease of potato has caused economic losses by reducing tuber quality throughout the western and central United States. The causal agent of the disease is ‘*Candidatus Liberibacter solanacearum*’ (Lso), which is transmitted to potato and other solanaceous plants including tomato by the potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae). ZC disease was first identified in the early 2000s in southern Texas (Munyaneza et al. 2007a, 2007b; Secor et al. 2009). The geographic range of Lso radiated outward and first occurred in the Pacific Northwest (U.S. states of Idaho, Oregon, and Washington) in 2011 (Munyaneza 2012; Crosslin et al. 2012a, 2012b). Nearly all U.S. states west of the Mississippi river, and even Alberta, Canada, have now had reports of ZC disease (Henrickson et al. 2019; Munyaneza 2012). There are no methods to directly control Lso, so ZC disease is

managed using insecticides to suppress populations of the insect vector. Growers in the Pacific Northwest and Texas have spent an estimated \$11 and \$26 million, respectively, annually on potato psyllid management following the initial outbreaks in these regions (CNAS 2006; Greenway and Rondon 2018).

The high cost for psyllid control is due in part to variability in the severity of ZC outbreaks among years and growing regions and to the inability to predict when or where ZC will occur. Proposed sources of ZC variability include biological differences among haplotypes of the psyllid vector and the pathogen. Potato psyllid occurs in at least four biologically distinct haplotypes, which differ in size, fecundity, presence of endosymbionts, feeding behavior, and host plant preference (Cooper et al. 2015, 2019; Horton et al. 2014; Liu et al. 2006; Mustafa et al. 2015a, 2015b; Swisher et al. 2012, 2014). Yet regardless of haplotype, psyllids that have already acquired Lso have equal capacity to vector the pathogen to potato (Mustafa et al. 2015b).

Seven haplotypes of Lso have been identified globally, but only three are known to occur in North America. Lso haplotypes A and B are typically associated with infection of solanaceous crops in the United States, Mexico, and Central American countries (Dahan et al. 2017; Nelson et al. 2011, 2013). Lso A also occurs in New Zealand (Liefting et al. 2008; Pitman et al. 2011). Recently, Lso haplotype F has been described in a single potato tuber in southern Oregon, although the risk of this haplotype as a cause of ZC outbreaks has not yet been determined (Swisher Grimm and Garczynski 2019). All other described Lso haplotypes occur in Europe and Northern Africa and infect species within the Apiaceae (Lso haplotypes C, D, and E) or Urticaceae (Lso haplotype U) (Alfaro-Fernández et al. 2012, 2017; Haapalainen et al. 2018a, 2018b; Holeva et al. 2017; Munyaneza et al. 2011; Nelson et al. 2013; Tahzima et al. 2014; Teresani et al. 2014).

Lso haplotypes A and B, which are both associated with ZC disease in North America, differ in virulence and disease severity (Harrison et al. 2018; Swisher Grimm et al. 2018). Two independent studies on Lso infection of potato demonstrated that compared with infection by Lso haplotype A, haplotype B caused more severe symptoms, higher rates of symptom development, and greater yield reductions (Harrison et al. 2018; Swisher Grimm et al. 2018). A similar study in tomato also showed that symptoms caused by Lso haplotype

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B were more severe than those caused by haplotype A (Mendoza-Herrera et al. 2018). Commercial potatoes are grown not from true seed but by vegetative propagation of tubers (seed potatoes) from the previous growing season. Although several studies have examined the effects of Lso infection on the regrowth of seed tubers, no study has compared the effects caused by Lso haplotypes A and B. Two studies conducted in the United States before the identification of Lso haplotypes or with mixed infection showed that $\leq 50\%$ of infected seed potatoes sprouted, and those that did produce new plants were apparently not infected with Lso (Henne et al. 2010; Rashed et al. 2015). However, another study conducted in New Zealand indicated that seed potatoes generated by plants infected with Lso haplotype A develop new plants infected with the pathogen (Pitman et al. 2011).

The goal of this study was to compare the growth and yield of potato plants grown from noninfected seed potatoes or from seed potatoes infected by Lso haplotypes A or B. This was achieved by infecting plants of five potato cultivars, storing the harvested tubers using standard industry methods, replanting the tubers the following growing season, and recording plant emergence rates and time, symptom development, presence of Lso, and tuber quality and quantity.

Materials and Methods

Sources of potato seed pieces. Generation 2, noninfected seed potatoes and seed potatoes infected at the tuber initiation stage (approximately 12 to 13 weeks prior to harvest) of the Atlantic, Ranger Russet, Russet Burbank, Russet Norkotah, and Umatilla Russet cultivars were produced in 2014 and 2015 at the USDA-ARS research farm near Moxee, WA (Swisher Grimm et al. 2018). Here, plants were grown in field cages and were infested with potato psyllids released at the base of each plant ($n = 3$ for colonies with 80 to 100% infection rate, and $n = 5$ for colonies with 50 to 65% infection rate) from laboratory colonies infected with either Lso haplotype A or haplotype B (Swisher Grimm et al. 2018). Noninfested plants grown in cages were used to produce noninfected seed tubers. The mature tubers were harvested by hand in autumn of each year and stored at 10°C. The following spring, each seed potato was bisected at the stem end prior to planting to verify that ZC symptoms were present in the inoculated tubers and that no symptoms were present in the healthy tubers. Symptom severity was ranked on a scale of 0 (no symptoms) to 3 (severe symptoms) as previously described (Rashed et al. 2013; Swisher Grimm et al. 2018). In both years of this study, nearly all tubers produced by plants infected with either Lso haplotype A or B exhibited severe symptoms, whereas tubers produced by noninfected plants were asymptomatic (Table 1).

Field design and experimental protocol. The field assays were conducted at the USDA-ARS research farm located near Moxee, WA, in 2015 and 2016 using seed potatoes produced during the previous growing season. In each year, hilled rows were generated with a buried irrigation drip line, and potato seed tubers were planted by hand at a depth of 15 cm in late May. Eight potato seed tubers of either ZC symptomatic Lso haplotype A or Lso haplotype B seed, or healthy potato seed, were planted in each field cage, at a space of roughly 20 cm. Field cages were utilized to keep out insect pests, preventing any in-season spread of Lso and other insect-transmitted pathogens. Treatment combinations (cultivar by infection status) were arranged in a completely randomized design during both years. The number of cages included for each cultivar by treatment combination was dependent upon the number of available symptomatic seed potatoes produced during the previous growing season (Table 1).

Plant emergence dates were recorded from each potato seed tuber, and days to emergence were calculated for the individual plants at the end of the season, with the day planted as day 0 and the first day after planting as day 1. Following plant emergence, the health of the above-ground foliage was recorded if it did not appear normal or healthy, or died prior to harvest. After emergence and initial plant growth (roughly 30 to 60 cm in size), terminal leaf tissue was collected from approximately 88% of plants that emerged from Lso

haplotype A and B potato seed tubers and from approximately 37% of plants that emerged from the healthy potato seed tubers (limit was made due to large number of noninoculated control plants). All plant material was taken back to the laboratory for molecular analyses.

Harvest and processing of daughter tubers. At the end of both seasons, daughter tubers generated from each noninfected (healthy), Lso haplotype A-infected, or Lso haplotype B-infected seed potato tuber were harvested by hand and collected into harvest bags by individual plant. Plants were harvested approximately 125 and 152 days after planting for 2015 and 2016, respectively. Following harvest, total yield (kg) and total number of tubers generated from each mother tuber were recorded. A subset of up to three daughter tubers from each plant (however, not all plants produced three daughter tubers) were assessed for the presence of ZC disease symptoms by bisecting the stem end of each tuber and determining if ZC symptoms were absent (score = 0) or if ZC symptoms were present (score varied from 1 to 3) (Rashed et al. 2013; Swisher Grimm et al. 2018). The stem-end piece (~1 g) from one of the three cut tubers was rinsed in water and saved for subsequent molecular analysis in the laboratory.

Molecular detection of Lso in tuber and leaf tissue. All leaf tissue and tuber samples were processed using the same extraction and polymerase chain reaction (PCR) methods. Briefly, total nucleic acids were extracted using a modified cetyltrimethylammonium bromide protocol (Munyaneza et al. 2010). All nucleic acid samples were resuspended in 100 μ l of water prior to all PCR analyses.

For the detection of Lso in leaf and tuber samples, conventional PCR was used with primers OA2 and OI2c (Crosslin et al. 2011). To distinguish between the specific Lso haplotype, A or B, conventional PCR with primers SSR-1F and SSR-1R was used (Lin et al. 2012; Wen et al. 2013). For both PCR reactions, the total volume was 25 μ l, which included 5 μ l of GoTaq Green PCR buffer, 0.25 μ l of 10mM dNTPs, 0.5 μ l of each primer (20 μ M), 17.65 μ l of H₂O, 0.1 μ l of GoTaq DNA polymerase (Promega, Madison, WI), and 1 μ l of the nucleic acid extraction. Thermal cycle conditions for PCR with primers OA2/OI2c were 94°C for 2 min, followed by 40 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 1 min, and a

Table 1. Seed potato treatments for five cultivars in the 2-year study^a

Treatment	N	2015	N	2016
		Mean symptom severity of seed tubers		Mean symptom severity of seed tubers
Atlantic				
Noninfected	3	0	3	0
Lso haplotype A	9	2.86	3	2.96
Lso haplotype B	9	3	9	2.83
Ranger				
Noninfected	3	0	3	0
Lso haplotype A	9	2.93	5	2.85
Lso haplotype B	9	2.89	9	3
Umatilla				
Noninfected	3	0	3	0
Lso haplotype A	9	2.72	6	1.65
Lso haplotype B	9	2.97	9	1.83
Burbank				
Noninfected	3	0	3	0
Lso haplotype A	8	2.78	9	2.88
Lso haplotype B	9	3	9	2.99
Norkotah				
Noninfected	3	0	3	0
Lso haplotype A	8	2.39	6	2.96
Lso haplotype B	9	2.92	9	2.92

^a The number (N) of cages planted with either noninfected, '*Candidatus* Liberibacter solanacearum' (Lso) haplotype A-infected, or Lso haplotype B-infected seed varied for cultivar and year depending upon availability of tubers. The mean symptom severity of seed potatoes was generated from scoring each bisected seed potato for zebra chip symptom severity (scale of 0 to 3).

final elongation step of 72°C for 5 min. PCR products were run on a 1.5% agarose gel with ethidium bromide staining for visualization of the 1,168-base pair band. Thermal cycle conditions for PCR with primers SSR-1F/SSR-1R were 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s, and a final elongation step of 72°C for 10 min. PCR products were run on a 2% agarose gel with ethidium bromide staining for visualization of the 240- and

180-base pair bands for Lso haplotypes A and B, respectively. Control Lso-positive nucleic acid extractions with Lso haplotype A and/or haplotype B were included in each PCR reaction, as well as a no-template, H₂O negative control.

Statistical analysis. All statistical analyses were performed using the GLIMMIX procedure of SAS 9.4 (SAS Institute, Cary, NC). Response variables for analyses included emergence rate, emergence

Fig. 1. Infection with '*Candidatus* Liberibacter solanacearum' (Lso) haplotype A and B effects plant emergence and daughter tuber formation compared with noninfected control seed potato tubers. The proportion ($\pm$ standard error) of tubers to have plants emerge (A), mean ($\pm$ standard error) number of days to emergence (B), mean ($\pm$ standard error) tuber yield (kg) per plant (C), and mean ($\pm$ standard error) number of tubers produced per plant (D) were analyzed from noninfected, Lso A-infected, and Lso B-infected seed potatoes of five cultivars in a 2-year trial. Uppercase letters indicate significant differences in Lso treatments for each cultivar.

time (days), weight (kg) of tubers produced by plants, and number of tubers produced by plants. The mean values from the eight plants in each cage were included in the analyses. Emergence rate was examined as a logistic variable using the number of emerged plants divided by the total number of seed tuber plants in a given cage. Days to emergence and tuber weight were examined assuming a normal distribution (DIST = G of the MODEL statement), whereas tuber number or tubers per plant was examined assuming a negative binomial distribution for count data (DIST = NB of the MODEL statement). The fixed effects in each analysis included potato cultivar, infection status, and the main effect interaction, and year was included as a random variable. When the interaction term was significant, differences among means were estimated using the SLICEDIFF option of the LSMEANS statement with a Tukey's adjustment for multiple comparisons (ADJUST = TUKEY) to compare means among treatments of each cultivar.

Results

Plant emergence. There was a significant main effect interaction for plant emergence rate, indicating that the effects of Lso infection on tuber sprouting were not consistent among cultivars ($F_{8,177} = 10.68$; $P < 0.0001$). Identical trends in emergence rates were observed for all cultivars, except Atlantic. For these cultivars (Norkotah, Umatilla, Burbank, and Ranger), a greater proportion of

noninfected tubers produced plants compared with the Lso-infected tubers, regardless of Lso haplotype, and a greater proportion of tubers infected with Lso haplotype A successfully emerged compared with tubers infected with Lso haplotype B (Fig. 1A). For the Atlantic cultivar, emergence rates did not differ significantly between the Lso haplotype A and haplotype B seed potatoes, but only a small proportion of noninfected tubers produced plants due to a secondary infection.

A significant cultivar by Lso treatment interaction was also observed for emergence time ($F_{8,147.1} = 3.49$; $P = 0.0010$). Noninfected tubers produced aboveground plants in 20 to 30 days regardless of cultivar, which was significantly shorter than tubers infected with Lso regardless of haplotype, for all cultivars except Atlantic (Fig. 1B). In all cultivars except Norkotah, tubers infected with Lso haplotype A produced plants 20 to 30 days earlier than those infected with Lso haplotype B (Fig. 1B). The delay in number of days to emergence for tubers infected with Lso haplotype B compared with Lso haplotype A, and even more so with the uninoculated control tubers, was evident in plant size at the time of harvest (Fig. 2).

Daughter tuber yield and count. Analysis of the mean daughter tuber yield (kg) per plant identified a significant cultivar by Lso treatment interaction in this 2-year study ($F_{8,176} = 4.55$; $P < 0.0001$). Plants grown from noninfected seed potatoes produced greater yield than did plants grown from Lso-infected tubers, but this trend was significantly different only for the cultivars Atlantic, Norkotah, and Ranger (Fig. 1C). Greater yields were also produced by Atlantic and Ranger plants grown from seed potatoes infected with Lso haplotype A compared with those infected with Lso haplotype B, and Norkotah tubers infected with Lso haplotype A produced yields intermediate to noninfected seed potatoes or seed potatoes infected with Lso haplotype B (Fig. 1C).

A significant cultivar by Lso treatment interaction was identified for the mean number of daughter tubers produced per plant in the 2-year study ($F_{8,147} = 2.13$; $P = 0.0360$). For all cultivars except Norkotah, plants grown from seed potatoes infected with Lso haplotype B produced significantly fewer daughter tubers than did noninfected plants or plants infected with Lso haplotype A (Fig. 1D).

Lso testing of leaf tissue and daughter tubers. Although Lso A- and Lso B-infected tubers tended to have a slower emergence rate compared with noninfected tubers, plants emerging from Lso-infected seed potato tubers were healthy looking (Fig. 2). None of

Fig. 2. Plants generally emerged faster from noninfected seed potatoes compared with plants emerging from seed infected with '*Candidatus Liberibacter solanacearum*' (Lso) haplotype A and those infected with Lso haplotype B. Images capture plant size and health at harvest in 2015 for the noninfected, Lso A, and Lso B treatments in the Umatilla cultivar.

Table 2. Detection of '*Candidatus Liberibacter solanacearum*' (Lso) in leaf tissue and daughter tubers^a

Treatment	2015		2016	
	Lso detection in leaf tissue	Lso detection in daughter tuber tissue	Lso detection in leaf tissue	Lso detection in daughter tuber tissue
Atlantic				
Noninfected	0/2	0/2	0/4	0/3
Lso haplotype A	0/30	0/25	0/11	0/16
Lso haplotype B	0/10	0/8	0/30	0/43
Ranger				
Noninfected	0/3	0/15 ^b	0/4	0/12
Lso haplotype A	0/12	0/9	0/10	0/10
Lso haplotype B	0/2	0/1	0/6	0/6
Umatilla				
Noninfected	0/5	0/15 ^b	0/14	0/20
Lso haplotype A	0/37	0/28	0/18	0/22
Lso haplotype B	0/10	0/5	0/18	0/18
Burbank				
Noninfected	0/3	0/15 ^b	0/4	0/24
Lso haplotype A	0/28	0/21	0/37	0/38
Lso haplotype B	0/13	1/8	0/27	0/26
Norkotah				
Noninfected	0/4	0/15 ^b	0/5	0/5
Lso haplotype A	0/19	0/14	0/13	0/16
Lso haplotype B	0/8	0/8	0/16	0/16

^a Table indicates the number of positive samples of leaf tissue or daughter tuber tissue collected from plants that emerged from noninfected, Lso haplotype A-infected, or Lso haplotype B-infected seed potatoes out of the total number of samples tested for Lso by conventional PCR.

^b For some noninfected controls, only a subset of daughter tubers from the total number of emerged plants were tested for Lso.

the plants that emerged from Lso A- or Lso B-infected seed potatoes produced aboveground symptoms characteristic of ZC. Plant tissues that emerged from the noninfected control, Lso A-infected, and Lso B-infected seed potatoes were tested for Lso by conventional PCR in 2015 and 2016 (Table 2). In both years, no plants tested positive from any Lso treatment.

Daughter tubers from all plants were scored for ZC symptoms of vascular darkening, spotting, and discoloration. No ZC symptoms were observed from any of the daughter tubers from Lso haplotype A- or haplotype B-infected seed potato tubers (Fig. 3). At least one tuber per plant was sampled from Lso haplotype A- or haplotype B-infected treatments to detect Lso using conventional PCR methods (Table 2). In 2015, a single daughter tuber (Burbank cultivar, Lso B-infected seed potato tuber) from a total of 127 Lso haplotype A- or haplotype B-infected plants was positive for Lso (0.8%). In 2016, no Lso haplotype A- or haplotype B-infected tubers from a total of 211 were positive for Lso using conventional PCR.

Discussion

The purpose of this 2-year study was to compare the germination success, growth, and yield of plants produced from noninfected seed potatoes to those infected with either Lso haplotype A or B in five common U.S. potato cultivars (including one chipping cultivar and four russet cultivars). Overall, results suggest that seed tubers exhibiting severe ZC symptoms caused by both Lso haplotypes A and B cause lower emergence rates and delayed emergence, leading to lower daughter tuber yield and number of tubers produced when compared with healthy tubers free of ZC symptoms. Lso haplotype B appeared more virulent than Lso haplotype A, with a lower proportion of plant emergence, greater number of days to emergence, lower tuber yield, and lower tuber count generated from Lso B-infected seed potato tubers exhibiting severe ZC symptoms. Variability was predominantly seen with the Atlantic cultivar, where poor emergence of the noninfected controls was due to presence of a secondary bacterial infection destroying the noninoculated control tubers. The Lso-infected tubers were not affected by this secondary bacterial infection, suggesting a difference in tuber physiology, possibly due to the presence of Lso.

These findings support the results from two previous studies by Henne et al. (2010) and Rashed et al. (2015), in which less than 50% of ZC-symptomatic tubers sprouted. Additionally, these findings support the differences in Lso haplotype epidemiology identified in our previous study (Swisher Grimm et al. 2018), in which current-season infection with Lso haplotype B, regardless of the haplotype of the psyllid vector, was associated with a greater incidence of ZC, more severe tuber symptoms, and reduced tuber yield and numbers.

Results from this current study suggest that plant emergence from Lso A- or Lso B-infected seed potatoes exhibiting severe ZC

symptoms is likely not a significant source of Lso infection in potato fields. Lso was not detected from leaf tissue collected from plants that emerged from Lso A- or Lso B-infected seed potatoes in this 2-year study. This absence of detectable Lso in above-ground foliage is in contrast to the results seen from a similar study in New Zealand, in which nearly 70% of asymptomatic leaf tissue that emerged from Lso haplotype A-infected mother tubers (with unknown symptom severity) tested positive for the pathogen (Pitman et al. 2011). It is possible that potato cultivar or environmental conditions such as weather events, soil temperature, or growing degree days could account for differences in the production of Lso-positive foliage between studies, although this is entirely speculative. Regardless, the detection of Lso in only a single daughter tuber generated from an Lso B-infected seed potato in this 2-year study provides further support that Lso movement from infected seed to newly emerging plants (above-ground foliage and daughter tubers) is not a likely source of Lso infection in U.S. potato fields.

The lower emergence rate in Lso A- and Lso B-infected tubers exhibiting severe ZC symptoms, as well as the greater number of days to emergence in these Lso treatments, suggest that commercial potato growers in the Pacific Northwest, where this trial was completed, and perhaps across the United States, should not be concerned with planting a small percentage of Lso-infected seed. As long as the majority of seed planted is Lso-free, any low percentage of plants that do emerge from infected seed will be quickly choked out by the healthy-growing, noninfected plants nearby. In a large commercial potato field, the probability of a very small percentage of symptomatic, Lso-infected plants emerging from seed and generating an adequate, persistent supply of Lso for the potato psyllid vector to transmit is likely negligible, based on the results from this current study. An assessment of the ability of potato psyllids to acquire Lso from plants that emerge from Lso-infected seed would be needed to confirm this.

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Fig. 3. No visual zebra chip (ZC) symptoms were detected in daughter tubers harvested from '*Candidatus* Liberibacter solanacearum' (Lso) haplotype A- and haplotype B-infected seed potatoes. Despite seed potatoes showing severe ZC symptoms for the Lso A- and Lso B-infected tubers, disease symptoms were absent in daughter tubers for all cultivars.

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