Interspecific Interactions in the Zebra Chip Pathosystem: Identifying Host Plant Resistance, Vector Cold Tolerance, and Multipartite Interactions with *Potato virus Y*

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Authorization to Submit Dissertation

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Abstract

Zebra chip (ZC) disease is a threat to the potato industry because it reduces production and quality of tubers. The pathogen is associated with the bacteria "*Candidatus* Liberibacter solanacearum" (Lso) that is transmitted by the potato psyllid *Bactericera cockerelli* Šulc (Hemiptera: Triozidae). Since its detection in the United States in 2000, studies have been conducted to address different aspects of the ZC pathosystem. Sources of resistance has been investigated but characterization of resistance has been studied based on the interaction between the potato psyllid and potato or based on ZC symptom severity in fresh tubers at harvest. Moreover most of those studies have been conducted under controlled conditions. However, there are still critical questions to address that are directly relevant to ZC management and epidemiology. The overall objective of this dissertation was to study interactions between the potato psyllid and a range of potato genotypes, Lso and host genotypes and the potato psyllid and Lso. Although host plant resistance has been proposed as an effective approach in integrated pest management (IPM) program to ZC, none of the commercial potato cultivars in the U.S. are known to be resistant or tolerant consistently. In the first chapter we present a literature review of the zebra chip pathosystem. In chapter second chapter we studied Lso development and ZC symptoms in eight selected potato genotypes in the field, and in the third chapter we evaluated the progress of Lso development and ZC symptom progress post-harvest. Potato genotypes from the A07781 family exhibited relative resistance and/or tolerance to ZC because they showed low susceptibility to Lso and expression of ZC symptoms at harvest and after storage. Therefore, they were proposed as potential source of resistance to ZC. The fourth chapter was aimed at studying the effect of cold temperatures on potato psyllid mortality in relation to their Lso status and the vector haplotype. Lso may promote its vector's

ability to acclimate to cold temperatures but it was not influenced by the potato psyllid genotype (haplotype). We also evaluated the effect of Lso on potato psyllid respiration and we have found that Lso affects the respiration of potato psyllids negatively. Finally, *Potato virus Y* (PVY) and Zebra Chip (ZC) pathosystems can coexist in within fields, and they affect the potato industry productivity. Monitoring of potato fields indicates that potato psyllids arrive later in the growing season, when PVY may be already established. The objective was to evaluate the impact of PVY on the ZC pathosystem. Pre-existing PVY infection did not affect Lso development, but it negatively impacted Lso vectors by reducing their oviposition. Therefore, PVY may limit the spread of Lso by affecting its vector. Findings from the present dissertation contribute to our understanding of the ZC pathosystem, and represent the first reported study of its interaction with another pathosystem (PVY) in potato host.

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Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

In the United States, potato production in 2018 totaled 23064 million kg. Idaho has the highest annual production with 7112 million kg, followed by Washington (5324 million kg) and Wisconsin (1443 million kg). Idaho, Washington, and Oregon, otherwise known as the Pacific Northwest (PNW) has increased its potato production in the last several eighteen of years, producing 57% of the U.S. production in 2000 to 60.2% in 2018 (Guenthner 2010, USDA-NASS 2018). Therefore, the PNW has been considered the leader in potato production in the United States. Eigthy seven percent of the potatoes produced in the US are utilized for processing and table stock markets (USDA-NASS 2018).

Zebra chip (ZC) disease negatively impacts the potato industry, as it reduces yield and quality of tubers (Munyaneza et al. 2012, Munyaneza et al. 2015). The pathogen is associated with the bacterium "*Candidatus* Liberibacter solanacearum" (Lso), also known as "*Candidatus* Liberibacter psyllaurous". Lso is transmitted by the potato psyllid *Bactericera cockerelli* Šulc (Hemiptera: Triozidae) (Hansen et al. 2008, Liefting et al. 2009). ZC-affected tubers are unsuitable for marketing due to the characteristic tissue discoloration of fresh and fried products (Miles et al. 2010, Munyaneza et al. 2012). Consequently, the potato industry has been forced to increase the use of insecticides to reduce psyllid population in an effort to control ZC spread (Buttler and Trumble 2012, Guenthner et al. 2012, Cooper and Bamberg 2014); frequent insecticide application, however, is a costly and unsustainable approach (Goolsby et al. 2012, Greenway 2014, Prager et al. 2013). Frequent use of insecticides can induce resistance in the potato psyllid (Prager et al. 2013) because potato psyllids have a high fecundity rate and short generation times (Goolsby et al. 2012). Also, chemical applications have negative effects on beneficial insects (Buttler and Trumble 2012).

Despite the impact of ZC on the potato industry and efforts to address this disease, the development of an effective Integrated Pest Management (IPM) program is still in progress. As host plant resistance can play an effective role in any IPM strategy, several studies have been conducted to screen for sources of resistance and tolerance against Lso and/or the potato psyllid (Butler et al. 2011, Diaz-Montano et al. 2014, Levy et al. 2014, Rubio-Covarrubias et al. 2017, Rashidi et al. 2017). However, characterization of resistance in these studies has been addressed based on the interaction between the potato psyllid and potato (Butler et al. 2011, Diaz-Montano et al. 2014, Levy et al. 2015), or based on the evaluations of ZC symptom severity in fresh tubers at harvest (Munyaneza et al. 2011, Rubio-Covarrubias et al. 2017). Rashidi et al. (2017) characterized resistance to ZC by symptom development in relation to Lso titer under greenhouse conditions. While some promising results have been reported by Rashidi et al. 2017, there is still a need for evaluating promising genotypes for susceptibility to Lso under field conditions and/or post-harvest. Moreover, given that recent studies have shown that the pathogen-plant interaction is not limited to the growing season and that Lso continues to multiply within infected tubers after harvest and during storage (Rashed et al. 2015, Rashed et al. 2018), it is important to extend evaluations of genotype susceptibility to infection post-harvest, until marketing.

The insect vector of Liberibacter has an important role in the spread of disease; studies have identified four haplotypes of this insect vector in the United States (Swisher et al. 2012, 2013, 2014a). For now, the Northwestern haplotype, which is known to overwinter in the PNW, has been mainly reported on some wild solanaceous species and potato (Swisher et al. 2013a, Horton et al. 2016 and Thinakaran et al. 2017), the majority of the field-infesting Northwestern haplotype do not carry Lso (Swisher et al. 2013a). It is possible that the Lso-

infected Northwestern psyllid haplotype fail to survive the harsh winters in Idaho because Lso causes reduction in fitness in potato psyllids (Nachappa et al. 2012, Yao et al. 2016, Frias et al. 2018). Recent studies have reported that the potato psyllids have several wild hosts to develop on (Murphy et al. 2013, Murphy et al. 2014, Horton el at. 2014, Horton et al. 2015, Horton et al. 2016, Wenninger et al. 2019). In the PNW, to date, a broader range of wild hosts have been identified for the Western haplotype than for the Northwestern haplotype (Cooper et al. 2019, Kaur et al. 2018, Thinakaran et al. 2017, and Wenninger et al. 2019). Therefore, it is important to investigate overwintering success of the different potato psyllid haplotypes and whether Lso-status of the potato psyllid might have an influence on overwintering success.

Finally, different components of the ZC pathosystem continuously interact with their surrounding environment, including other plant pathogens that may affect the potato host. In the Pacific Northwest, the main potato production region of the United States, *Potato virus Y* (PVY) is a widespread viral pathogen that impacts seed potato production (Karasev and Gray 2013). PVY can be transmitted by several species of aphids in a non-persistent manner (Mondal et al. 2016, Karasev and Gray 2013). In central and eastern Idaho, several years of monitoring the potato fields indicated that potato psyllids that carry Lso are likely to arrive later in the growing season (Rondon 2012, Dahan et al. 2019 *in press*), which is when PVY may already be established in potato fields. Thus, it becomes interesting to quantify the impact of PVY on Lso development and vector behavior to determine whether PVY infection could influence ZC epidemiology.

Zebra Chip Pathosystem

Zebra chip is associated with Lso, which is an uncultured phloem-limited bacterium. So far, six haplotypes of Lso have been described based on single nucleotide polymorphisms (SNPs) of 16S, ISR-23S and 50S ribosomal gene regions (Nelson et al. 2011). These haplotypes have been associated with different crops. Haplotypes A, B (Lso A, Lso B), and the recently reported haplotype F (Lso F) (Swisher and Garczynski 2019) have been associated with solanaceous species. Haplotypes C and D (Lso C and Lso D) have been associated with carrots (Nelson et al. 2013). The haplotype E (Lso E) is known to affect celery (Teresani et al. 2014) and the haplotype U is associated with carrots (Haapalainen et al. 2018). Lso is primarily found in solanaceous crops such as potatoes, tomatoes (*Solanum lycopersicon*), eggplants (*Solanum melongena*), peppers (*Capsicum annuum*) and also in some wild species from the family Solanaceae (Abad et al. 2009, Hansen et al. 2008, Munyaneza et al. 2012, Murphy et al. 2013). The cellular morphology of Lso has been proposed to be similar to "*Candidatus* Liberibacter asiaticus" (Las) (Secor et al. 2009), another Liberibacter that is responsible for Huanglongbing (HLB) disease in citrus (Bove 2006). In potatoes, the Lso B is mostly distributed in the US, whereas the Lso A is found in Central America, Mexico, New Zealand, in addition to the United States. Both haplotypesare found in Texas, Kansas, Nebraska (Nelson et al. 2011) and Idaho (Dahan et al. 2017). Lso B has been associated with more severe symptoms than Lso A (Mendoza-Herrera et al. 2018 and Swisher et al. 2018). Both Lso A and B are efficiently transmitted by potato psyllids from Central, Western, and Northwestern haplotypes (Swisher et al. 2018). The vector haplotype does not have influence on the Lso transmission because incidents of ZC is observed similarly regardless of the psyllid haplotype

transmitting the pathogen (Swisher et al. 2018). Therefore, the haplotype of the potato psyllid does not affect the spread of the pathogen.

Lso is transmitted from plant to plant by the potato psyllid *Bactericera cockerelli* (Sulc) (Hansen et al. 2008). The potato psyllid belongs to the order Hemiptera, Suborder Sternorrhyncha. Insect vectors such as hemipterans represent a key group of vectors that transmit plant pathogens (e.g., viruses and bacteria). They possess specialized "piercing-sucking" mouthparts which allow them to penetrate the epidermal tissue and access the phloem sap (Perilla-Henao and Casteel 2016, Howe and Jander 2008). The adult potato psyllids measure about 2.5 mm long and they exhibit characteristic white lines on the head and throrax (Munyaneza et al. 2012). Moreover, four haplotypes of the potato psyllid have been identified in the United States. They have been described as Central, Western, Northwestern and Southwestern haplotypes (Swisher et al. 2012, 2013, 2014a). These populations have been identified by High-Resolution Melting (HRM) analysis, a method which is based on the identification of SNPs on an amplicon from mitochondrial cytochrome oxidase subunit I gene (COI) of *B. cockerelli* (Swisher et al. 2012). Although four haplotypes have been reported, Central and Western haplotypes are the most widely distributed genotypes in the United States. The Central haplotype is distributed from eastern Mexico throughout Texas, Kansas, Colorado, Nebraska, Wyoming, North Dakota and Idaho (Swisher et al. 2012, 2013, Dahan et al. 2017), whereas the Western haplotype is found in California, New Mexico to Washington, Oregon and Idaho (Swisher et al. 2012, 2013). The Northwestern haplotype has been found on potato fields in Washington, Oregon, and Idaho (Swisher et al. 2013, 2014, Dahan et al. 2017). The Southwestern haplotype has been first reported in New Mexico and Southern Colorado (Swisher et al. 2014a), but has also been reported in Idaho (Dahan et al. 2017). Although most

haplotypes can transmit Lso (Swisher et al. 2018), the non-Lso-positive psyllids from the Southwestern haplotype have been reported. In the PNW, the predominant potato psyllid genotype found in the ZC-affected fields is the Western haplotype (Swisher et al. 2013, 2014, Dahan et al. 2017). Recent studies have shown that the abundance of potato psyllid haplotypes and the prevalence of Lso genotypes can change during seasons (Dahan et al. 2017, Dahan et al 2019). Moreover, the dynamic of psyllids and their haplotypes vary along season (Wenninger et al. 2017, Dahan et al. 2017). The relative abundance of psyllids haplotypes may have an important implication on the spread of the pathogen because some haplotypes (e.g. Southwestern) have not been reported to carry on Lso. Also, the variation of haplotypes through the seasons may be associated with the ability of some haplotypes to colonize other hosts available during the field season.

Zebra Chip Appearance and Spread

Zebra Chip (ZC) was first reported in 1994 in Mexico, and was later discovered in the U.S. in 2000 (Munyaneza et al. 2007, Secor and Rivera-Varas 2004). In 2011, ZC reached the most important potato production regions of the United States (Greenway 2014): Idaho, Washington and Oregon (Crosslin et al. 2012a, Crosslin et al. 2012b). These states represent up to 57% of the total United States potato production (Greenway 2014). ZC can reduce potato production by 50%, and up to more than 85% in severely affected fields (Munyaneza et al. 2011). Moreover, a 93% reduction in the marketability of the infected potato tubers have been reported (Munyaneza et al. 2008). In addition to the US, ZC is also present in potato fields in Mexico, Central America and New Zealand (Secor and Rivera-Varas 2004, Liefting et al. 2009,

Munyaneza 2012). In 2017, ZC was reported for the first time in Alberta, Canada (Johnson et al. 2018).

Transmission and Development of "*Candidatus* Liberibacter solanacearum"

Psyllids are known as vectors of different species of Liberibacter, which is one of the well-studied phloem-restricted and gram-negative group of bacteria. Attempts to grow *Liberibacter* spp. *in vitro* have been unsuccessful, perhaps because phloem sap may contain some essential nutrients for their growth that have yet to be identified (Bove and Garnier 2002). Liberibacter circulates within the insect vector and is transmitted in a persistent manner (Perilla-Henao and Casteel 2016, Eigenbrode et al. 2018).

In the ZC pathosystem, after the acquisition of Lso by potato psyllids, insects are able to effectively transmit Lso after a latent period. This period is the time in which Lso is translocated from the alimentary canal to salivary glands (2-3 weeks at 20-30°C) (Cooper et al. 2014, Sengoda et al. 2013). Adults of the potato psyllids are efficient in transmitting Lso with a transmission success rate of up to 70% for a single psyllid and up to 100% for multiple psyllids, within 48 hours of inoculation access period (IAP) (Rashed et al 2012). Twenty adults can transmit Lso in 1 hour of IAP and one Lso-infected psyllid can inoculate Lso within 6 hours of IAP (Buchman et al. 2011). However, the nonuniform distribution of the pathogen inside the plant host may influence the Lso acquisition by the potato psyllid (Levy et al. 2011, Rashed et al. 2012). This may be due to the fact that Lso infects significantly more leaflets of the leaf which share the same vascular connection with the inoculated leaf (Cooper et al. 2015). Therefore, the number of psyllids acquiring Lso is significantly higher when they are exposed to the whole leaf that has a direct connection with the Lso-infected leaf (Cooper et al. 2015).

This observation may be consistent among psyllid haplotypes because the haplotype of the potato psyllid is not affecting the transmission success; Central, Northwestern and Western haplotypes are similarly efficient in transmitting Lso in potatoes (Mustafa et al. 2015).

In addition, plant mechanism(s) of defense activated by Lso may influence potato psyllid performance. Overall, plant defense response occurs by the recognition of microbial-, pathogen- or herbivore-associated molecular patterns (MAMPs or PAMPs or HAMPs); or effector molecules (Wu and Baldwin 2010, Zhao et al. 2015). Defense responses are expressed as the production of signaling molecules (e.g., salicylic acid), secondary metabolites, pathogenesis-related proteins (PRs) and gene expression of resistance genes in the host (Agrios 1997, Hove and Jander 2008). The heterogenous distribution of Lso throughout the potato plant (Levy et al. 2011) and the expression of plant defense could vary among host genotypes. Therefore, in ZC pathosystem, the efficiency of Lso transmission could also be influenced by the potato psyllid behavior, the variability among host genotype, and plant defense response.

Following successful transmission, ZC symptoms become visible in foliage and in tubers, over time. Foliar symptoms include curling of young leaves, chlorosis, yellowing/purpling of young leaves, deformation of branches, aerial tubers, and necrosis of the leaves and potato vine (Secor et al. 2009, Wen et al. 2009, and Rashed et al. 2014). Symptoms in fresh tubers are observed in fresh tissue as necrotic “flecking” and brown spots around tuber cortex tissue (Miles et al. 2010). These symptoms have been mainly linked to elevated levels of phenolic compounds (Wallis et al. 2012). However other pshyiological changes has been also observed during infection such as: changes in levels of polyphenol oxidases, and reducting sugars (Wallis et al. 2012, Rashed et al. 2013). When the fresh tuber tissue is exposed to air, polyphenol oxidase (PPO) catalyzes the oxidation of phenolics to quinones, and the outcome

is observed as brown-colored areas on the tuber tissue (Navarre et al. 2009, Wallis et al. 2012). Symptomatic tubers also possess relatively higher levels of some amino acids, reducing sugars, and enzymes related to plant-pathogen defense such as PPO, peroxidases, chitinases and β-1,3-glucanases (Wallis et al. 2012), and possibly salicylic acid (SA) (Navarre et al. 2009). Moreover, ZC symptoms are also characterized in fried chips as a brown tissue discoloration; such discoloration is more pronounced than on fresh tissue (Munyaneza et al. 2012). This pronounced severity of discoloration has been attributed to the elevated concentration of certain amino acids and reducing sugars (Wallis et al. 2012) that are known to be precursors for acrylamide production when exposed to high temperatures (Stadler et al. 2002). ZC symptom severity is known to be influenced by the infestation time, host plant response and environmental factors. The severity of ZC symptoms increases with the duration of infection. Early-season infections produce severe symptoms in tubers whereas symptoms in late-season infections may not be visible in the tubers (Rashed et al. 2013). The severity of ZC symptoms is positively correlated with levels of certain phenolic compounds, PPO, reducing sugars and defense enzymes (Rashed et al. 2013). However, these defense-associated compound also appear to vary among potato genotypes (Rashed et al. 2013; Wallis et al. 2014, Wallis et al. 2015), a variability that could also explain variations in host symptom expression (Wallis et al. 2015). Therefore, the lack of changes in tuber biochemistry due to Lso infection has been proposed as a potential mechanism of ZC resistance (Wallis et al. 2015).

Zebra Chip Control

All commercial potato cultivars in the U.S. are determined to be susceptible to ZC (Levy et al. 2015, Munyaneza et al. 2011). Currently, growers are focused on the application

of multiple systemic and broad spectrum-insecticides to manage ZC (Anderson et al. 2013, Prager et al. 2016). However, due to their high fecundity and short generation time (Goolsby et al. 2012), the potato psyllid develops resistance to these frequently-used insecticides (Liu and Trumble 2007, Tiwari el at. 2012, Prager et al. 2013). Moreover, the use of broad-spectrum insecticides may even promote population growth of the potato psyllid (Prager et al. 2016). Systemic insecticides may not prevent Lso transmission. This is because they need to be ingested by the psyllid which may result in Lso transmission during the process (Levy and Tamborindeguy 2014, Prager et al. 2016). This would be a likely scenario, especially when the efficacy of the systemic chemicals starts to fade away over time and as the plant continues to grow (dilution effect). Adult psyllids are known to be highly efficient in transmitting Lso and extended feeding time would contribute to effective transmission (Buchman et al. 2011). Finally, the negative effects of chemical applications on beneficial insects (Buttler and Trumble 2012) and costs associated with frequent insecticide application (Greenway 2014, Guenthner et al. 2012, Cooper and Bamberg 2016) are other reasons that highlight the importance of adapting more sustainable alternative approaches to manage ZC. IPM has been successfully implemented in various agroecosystems to manage various pest conditions (Trumble et al. 1994, Butler and Trumble 2012). Identifying and developing resistant or tolerant cultivars to Lso and/or the potato psyllid may effectively contribute to the success of an IPM approach, which can subsequently delay the development of resistance in potato psyllids to insecticides (Gharalari et al. 2009, Cooper and Bamberg 2016). Up until now, the strategy to reduce the spread of Lso has been the chemical control of the potato psyllid vector (Goolsby et al. 2012, Horton et al. 2016, Greenway 2014, and Prager et al. 2013). Thus the host-plant resistance approach has been proposed to reduce ZC incidence (Munyaneza 2012).

Host Plant Resistance to Zebra Chip

Host plant resistance against insect pests/pathogens can be classified as antixenosis and antibiosis. Antixenosis is the ability of the host plant to produce components or structures that reduce the presence of an intruding organism (Cooper and Bamberg 2014); for example, the production of certain secondary metabolites *in planta* reduces the preference of some species of aphids (e.g., *Brevicoryne brassicae*) for a particular host plant (Van Emden and Harrington 2007). Antibiosis, on the other hand, can result in reduced survival of the invader due to the negative impact the host plant compounds on its biology (Cooper and Bamberg 2014, Diaz-Montano et al. 2014). For example, the production of a high level of toxins by some varieties of *Vigna unguiculata* can kill *Aphid craccivora*, as this toxin appears non-detectable by the aphid (Van Emden and Harrington 2007). In addition to antixenosis and antibiosis, tolerance can be defined as the expression of heritable features in the host plant that would limit symptom expression in the presence of a pest/pathogen (Agrios, 1997, Van Emden and Harrington 2007).

For ZC, sources of resistance or tolerance can be found in the wild as well as in potato breeding clones. Potato genotypes derived from wild species such as *Solanum bulbocastanum, Solanum habrochaites* and *Solanum verrucosum* were reported to show antixenosis (Cooper and Bamberg 2014) and antibiosis to the potato psyllid (Cooper and Bamberg 2016, Levy and Tamborindeguy 2014). Butler et al. (2011) reported a negative effect of four potato genotypes on the behavior (e.g., duration of probing) of potato psyllids. Further research showed reduced fecundity of potato psyllids when they were exposed to several potato genotypes through no-choice experiments (Diaz-Montano et al. 2014). Also, evidence of resistance to the potato psyllid has been found in another wild Solanaceae species, *Solanum peruvianum* (Mill). The

R gene Mi-1.2 of *S. peruvianum* confers resistance against species from four different taxonomic categories including nematodes, aphids, whiteflies and potato psyllids (Perilla-Henao and Casteel 2016, Walling 2008). The Mi-1.2 gene has been isolated and incorporated into commercial tomato varieties. The potato psyllid had lower oviposition on the transformed tomatoes that carry the Mi-1.2 gene (Casteel et al. 2006), which can be categorized as antibiosis. While some promising results have been reported, the observed resistance traits against the vector do not provide sufficient ZC control since Lso transmission is still possible. Therefore, there is also a need to identify sources of resistance to Lso, in order to achieve an effective management of ZC.

The evaluation of some potato genotypes showed a reduced Lso transmission success (Butler et al. 2011) and antibiotic effect against Lso (Diaz-Montano et al. 2014). Furthermore, Rubio-Covarrubias et al. (2017) reported ZC tolerance in fresh tubers from selected potato genotypes. The tolerance in these genotypes was associated with reduced concentrations of phenolic compounds and lower ZC symptom severity of fresh tubers. So far, the expression of defense in response to Lso infection in symptomatic tubers is characterized as significant changes in tuber biochemistry (Wallis et al. 2012, 2015). However, the level of such physiological response varies among potato cultivars since the initial biochemical profile of tubers show differences across cultivars. Hence, the progress of the disease (e.g., symptom severity) is likely to be different among cultivars (Wallis et al. 2014).

Most recently, Rashidi et al. (2017) showed that clones derived from *Solanum chacoense* (A07781-10LB, A07781-3LB, and A07781-4LB) are relatively tolerant to ZC, with low susceptibility to Lso infection under greenhouse conditions. The development of disease however is the result of the interactions among environment, pathogen and plant host.

Therefore, field experiments will be required for evaluating sources of resistance to ZC to confirm greenhouse findings. Controlled conditions can have an influence on the expression of disease symptoms as well as the success of Lso transmission (Ni et al. 2009, Down et al. 2001). Moreover, interactions between Lso and its potato host continue post-harvest and during storage (Rashed et al. 2015, 2018). This may provide a chance for the pathogen to continue impacting tuber quality. Rashed et al. (2018) evaluated Lso and disease development in Russet Norkotah tubers, which were infected 14, 10 and 4 days before harvest, under commercial storage conditions. The increase in the proportion of Lso-infected tubers, Lso titer and phenolic compounds, over time confirmed the continued development of the disease during storage (Rashed et al. 2018).

Up until now, all screening studies have been focused on vector-plant-pathogen interactions before harvest, typically considering a single time of infection/infestation (Butler et al. 2011, Diaz-Montano et al. 2014, and Rashidi et al. 2017). However, field infestation by the potato psyllids may occur at different times in different geographical locations. In the PNW the infective psyllids usually arrive later into the growing season so that late infestation of potato fields is common. Tubers infected late in the season may be asymptomatic and have low Lso concentrations; many late-season infections cannot be detected at harvest (Rush et al. 2015, Rashed et al. 2018, and Wallis et al. 2017). Since Lso continues to interact with tuber tissue throughout storage, late-season infection can be considered an important issue for the potato industry where late-season infections are likely to occur. Therefore, screening for resistance might be expanded to stored tubers.

Effect of Lso on the Potato Psyllid

The negative impact of Lso on some of the potato psyllid fitness traits, such as fecundity and nymphal survivorship, has been reported (Nachappa et al. 2012, Yao et al. 2016, Frias et al. 2018). The reduction in fecundity has been shown to be correlated with the increase in the vector Lso titer load (Nachappa et al. 2014). Moreover, Lso haplotype B has a stronger effect on the fitness of the potato psyllid than Lso haplotype A; Lso B causes a significantly greater reduction of nymphal survival compared to Lso A in infected psyllids (Yao et al. 2016). The interaction between Lso and its insect vector has also been studied at the genomic level. A recent study has shown a significant reduction of the expression of genes involved in insect reproduction such as vitellogenin (*BcVgI-like*) in Lso B-infected psyllids (Frias et al. 2018). The reduced production of *BcVgI-like* transcript may be associated with a significant decline in the number of developing oocytes in Lso B-infected females (Frias et al. 2018). In addition to Lso effects on reproduction, transcriptomic analyses of Lso-infected and uninfected adult potato psyllids have shown that Lso also affects the expression of genes involved in metabolism and to some extend those associated with stress- and immune-related responses (Nachappa et al. 2012, Yao et al. 2016). For instance, Lso-infected psyllids show a highly significant level of expression of phenoloxidase (Yao et al. 2016), an enzyme with an important role in defense against bacteria highly pathogenic in invertebrates (Liu et al. 2007). Also, the fact that Lso induces the expression of genes involved in metabolism, can indicate an important alteration of psyllid physiology (e.g., respiration rate) due to Lso infection. In a closely related pathosystem, Las was shown to alter cellular metabolism (e.g., ATP production) of its vector, *Diaphorina citri* and induces energetic stress, shorter lifespan and altered feeding behavior (Killiny et al. 2016). Therefore, it is conceivable to propose that the presence of Lso could

induce a change in the level of expression of genes altering the metabolic process involved in survivorship of Lso-infected psyllid vector during winter.

The Development of the Potato Psyllid on Wild Solanaceous Hosts

It has long been assumed that potato psyllids migrate from breeding sites in the southern U.S. and northern Mexico to colonize potato fields in the PNW. However, this hypothesis has been reevaluated due to the recent developments showing the potato psyllid's ability to survive cold temperatures (Murphy et al. 2013, Horton et al. 2014) on wild solanaceous hosts such as bittersweet nightshade (*Solanum dulcamara*), matrimony vine (*Lycium* spp.), *Lycium barbarum*, and *L. chinense*. (Munyaneza et al. 2012, Murphy et al. 2014, Horton et al. 2016, Thinakaran et al. 2017, Wenninger et al. 2019). The physiological mechanisms involved in winter survival of potato psyllids are still unknown. Psyllids may be able to overwinter through entering a quiescent state (Horton et al. 2015); the development of immature ovaries or mature eggs from adult females in winter may be favored by warmer temperatures at the end of winter (Horton et al. 2015). Likewise, other insect species such as aphids (e.g., *Myzus persicae*) have the overwintering ability (Broadbent et al. 1949). One of the strategies used by aphids (e.g., *Pemphigus bursarius*) for overwintering is the development of a special morph called hiemalis. This morph is compounded by apterous stages of aphids that can survive without a host plant during winter; hiemalis can remain in the soil during winter (Van Emden and Harrington 2007). The survival success of the hiemalis morph is due to physiological adaptations such as accumulation of energy reserves (e.g., higher levels of triglycerides) that results in cold tolerance of the hiemalis morph (Phillips et al. 2000). Conversely, the potato psyllid appears to require a host plant for successful overwintering

(Murphy et al. 2013, Horton et al. 2015). It was proposed that after potato psyllids break their quiescent state in the autumn, they may move from wild host to potato crop (Horton et al. 2016). However no evidence hasbeen found yet to support this hypothesis. Although recent studies have suggested that the movement of potato psyllids between wild hosts and potatoes in spring may be possible (Wenninger et al. 2017, 2019, Dahan et al. 2017), more studies are needed to prove that the dispersal from wild hosts to potatoes occurs. Therefore, the association between the potato psyllid and alternative hosts would allow the potato psyllids to maintain their population until they move into the potato crop in the next field season (Horton et al. 2014, 2015, and 2016). Under laboratory conditions, the potato psyllid is tolerant to cold temperatures; nymphs can survive at -15ºC and adults show 40% survival when placed in -10ºC for 24 hours. Moreover, the survival rate of potato psyllids may be increased when they have access to suitable microclimates, which would protect them against cold temperatures (Henne et al. 2010). However, it is possible that the overwinter survival of potato psyllid varies across the haplotypes and/or is based on their overwintering host species. In the PNW, the Northwestern haplotype is the predominant population, which may overwinter on *S. dulcamara* (Swisher et al. 2013a, Horton et al. 2015), *Lycium barbarum* and *L. chinense* (Thinakaran et al. 2017). The high population of the Northwestern haplotype in the growing season is linked to its ability to overwinter (Swisher et al. 2014, Dahan et al. 2017). At the end of summer, it is possible to find a mixture of the vector haplotypes on wild hosts (e.g., *S. dulcamara)* but this diversity of psyllid populations disappears in winter (Horton et al. 2014). This suggests that other populations such as Central and Western haplotypes may not be able to overwinter or that they would move to a more suitable wild host species for overwintering.

Cold Hardiness in Insects

Insects have two strategies to survive at low temperatures; freezing tolerance or freezing avoidance (Bale 2002, Clark and Worland 2008). Those strategies involve the production of specific chemicals (e.g., polyols and sugars), antifreeze proteins, (e.g., AFPs) and ice nucleating agents or proteins (INAs and INPs) in the insect (Bale 2002). Insects can tolerate freezing temperatures by the regulated freezing of body water in extracellular spaces, and protection of membrane bilayer structure with cryoprotectants (Bale 2002, Storey and Storey 2012). The avoidance of freezing is led by previous changes in behavior and physiology such as the location of overwintering place and reduction of body water followed by a deep cooling of body fluids. This process is assisted by the synthesis of antifreeze proteins (AFPs) and accumulation of carbohydrates cryoprotectants (e.g., glycerol) (Bale 2002, Storey and Storey 2012). Under freezing conditions, the metabolic rate of insects is reduced, and they survive by slow catabolism of stored body-fuel reserves (Bale 2002). The response of insects to cold temperatures also involves variations in gene expression (Qin et al. 2005), that may be influenced by the time of exposition to cold temperatures. For example, in *Drosophila melanogaster*, the upregulating of 20 and 69 genes is observed in a time of exposition at -0.5 ºC for 2 hours and 10 hours, respectively (Zhang et al. 2011).

Plant-mediated Interactions among Pathosystems

Under natural circumstances, a host plant species can be the target of of multiple pathogens and herbivores. Consequently, multiple pathosystems can develop and interact in the same plant host. As they continue to interact with the host plant, pathogens could also influence interspecific interactions in the other existing pathosystems (e.g., pathogen- and

vector- host plant interactions) (Shapiro et al. 2013). Interspecific interactions are especially interesting in obligate parasites such as viruses and fastidious bacteria (Andret-Link and Fuchs 2005) since they are mostly transmitted by vectors and require a living host to multiply. Many viral and bacterial pathogens are known to colonize vascular tissue of host plants since they are a source of nutrients and can function as a pathway for translocation and spread among tissues (Perilla-Henao and Casteel 2016). In viruses, viral particles move intracellularly from their initial replication through the cellwall, then it moves to adjacent cells through plasmodesmata and across various cells to reach vascular tissue to initiate systemic infection (Hull 2014). In phloem-restricted bacteria, the pathogen is directly inoculated into the vascular tissue since these vectors feed exclusively on phloem (Bove and Garnier 2002). The movement of viruses through vascular tissues has a similar translocation pattern to plant photoassimilates, and it is influenced by the arrangement of leaves on the plant stem (Leiser et al. 1992). The *Tobacco rattle virus* (TRV, genus *Tobramovirus*) requires at least 4 hours to move out of primarily inoculated cells of *Nicotiana clevelandii* (Derrick et al. 1992). The *Cauliflower mosaic virus* (CaMV, genus *Caulimovirus*) needs 5 days to move from inoculated leaf and reach vascular tissue of turnip plants (Leisner et al. 1992). In addition, the transport and accumulation of viruses (e.g., *Potato virus Y* and *Potato virus X*) in potato plants depends on variety resistance, the combination of viruses and the order which they are inoculated on the host plant (Rusetsky and Blotskaya 2008). Coinfection by two or more viruses from different groups does occur. One of the most interesting interactions is synergism, which is characterized by a cooperative coexistence of non-related groups of viruses. For example, the coinfection of *Potato virus Y* (PVY, genus *Potyvirus*) and *Potato virus X* (PVX, genus *Potexvirus*) in potato results in an increase of PVX titer (Goodman et al 1964). The variation of accumulation of

PVX is in part due to the suppression of RNA gene silencing by the helper component potyviral protein (HC-Pro) encoded by PVY (Vance et al. 1991, Anandalakshmi et al. 1998). Therefore, PVY-PVX coinfection favors the development of PVX as well as its transmission since PVX titer is increased.

As previously shown within viruses, co-infection of pathogens from different taxonomic groups can have effects on the expression of plant defense which in turn may affect the regular pathogen development by changes in the plant host quality (Mauck et al. 2012). Overall, plant defense activation is characterized by alteration in morphology, physiology and production of signaling molecules (e.g., SA), secondary metabolites, PRs and gene expression (e.g., R genes) (Kogovsek et al. 2013). But defense system operating on a plant host may vary with the pathosystem. For instance, RNA silencing has major impacts on pathogenic viruses (Hull, 2014) while that the induction of SA can negatively impact bacteria pathogens (Casteel et al. 2012). Changes in the plant host due to the pathogen infection can also influence vector preference of tissue infected with viral, bacterial (Eigenbrode et al. 2002, Casteel et al. 2012, and Prager et al. 2015) and fungal plant pathogens (Blood et al. 2018). For example, Davis et al. (2017) showed that the infection of insect-borne *Bean leafroll virus* (BLRV, genus *Luteovirus*), transmitted by the pea aphid (*Acyrthosiphon pisum* L.), is beneficial for its vector development. Moreover, the level of pathogen infection in the host plant may also impact vector preference as shown in the maize bushy stunt phytoplasma (MBSP) pathosystem that is transmitted by the corn leafhopper, *Dalbulus maidis* (Hemiptera: Cicadellidae). If vector-borne pathogens can manipulate host plants to appear more attractive to the vectors (Eigenbrode et al. 2002, Casteel et al. 2012; Ingwell et al. 2012, Davis et al. 2017, Blood et al, 2018), then they may also be able to affect vector behavior from another pathosystem interacting with the

same plant host. For example, it has been shown that *the Tobacco mosaic virus* (TMV) infection alters the tomato physiology, which results in a reduction in the attractiveness of the tomato plants to the potato psyllid vectors of Lso. Consequently, Lso titer is relatively reduced in TMV-infected plants (Prager et al. 2015). Another example is the PVY infection in potato which was shown to have an indirect effect on other insect pests the Colorado potato beetle (*Leptinotarse decemlineata*, CPB); pre-existing PVY-infected plants on the field are more vulnerable to CPB (Petek et al. 2014). Finally, the interaction of two vector-borne pathogens on the same host species can generate plant-mediated effects on the vector(s) involved. For example: Cucumber plants were inoculated with the bacteria *Erwinia tracheiphlia* and the *Zucchini yellow mosaic virus* (ZYMV). The bacterial pathogen is transmitted by the specialist vector cucumber beetle, *Acalumma vittattum*, and ZYMV is transmitted in a non-persistent manner by several generalist aphid species. ZYMV-infection has an indirect effect on the bacterium's vector since *A. vittattum* reduced its attractiveness to the host plant when it was ZYMV-infected. This response was associated with a reduction in the incidence of the bacterial disease that was observed in plants inoculated with ZYMV and *E. tracheiphila* (Shapiro et al. 2013).

Plant-mediated Interactions between *Potato virus Y* and "*Candidatus* Liberibacter solanacearum"

PVY is a threat to potato production in the U.S. (Karasev and Gray 2013, Dupuis et al. 2017, Funke et al. 2017). This is considered the most important viral pathogen of potatoes and the top important problem in the seed potato industry in North America (Gray et al. 2010). PVY is transmitted naturally by a wide range of aphids in a non-persistent manner (Karasev

and Grey 2013, Mondal et al. 2016, Shrestha et al. 2014); virus particles are retained by the vector for only a few hours, and no latent period is required for a successful transmission (Hull 2014). This method of transmission is different for some phloem-limited bacteria, including Lso, where there is a persistent association with their vectors, and they interact in a circulative manner (Perilla-Henao and Casteel 2016) requiring a latent period for successful transmission (Sengoda et al. 2013, Cooper et al. 2014).

In potato, both PVY and Lso are translocated from plant to the progeny tubers and both pathogens can be transmitted from one stem to other stems of the same plant through vascular system of the mother tuber (Levy et al. 2011, Dupuis 2017). In PVY, the virus can accumulate in all tissues, but the highest quantities of viral RNA and viral particles are localized in symptomatic leaves and stems (Kogovsek et al. 2011). The accumulation of Lso is primarily in stems (Levy et al. 2011, Munyaneza et al. 2012). Since, PVY and Lso would interact directly in phloem tissue, the colonization of PVY may potentially affect the development of Lso by direct and indirect interaction. PVY replication can be inhibited by SA (Spoel et al. 2007), whereas Lso infection would result in a reduction in the transcription of metabolites involved in SA synthesis (Casteel et al. 2012). Consequently, Lso may be able to manipulate the host plant by suppressing its defenses to provide suitable conditions for the development of its vector and facilitate vector colonization on the plant host (Casteel et al. 2012). Thus, the inhibition of SA transcripts by Lso may have a direct effect on PVY development since both PVY and Lso colonize phloem. Conversely, biotrophic pathogens such as PVY, induce SA pathway-related defenses (Pieterse et al. 2012), and the development of Lso may be directly affected for the activation of those SA – related defenses. Further, pre-existing PVY infection

can have an indirect effect on the potato psyllid behavior since other viral infections have been shown to have a negative impact on this insect vector (Prager et al. 2015).

Phytohormones such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) regulate the response of plants against herbivore and pathogen attacks. The production of SA is associated with plant pathogen infections (Glazebrook, 2005, Casteel et al. 2012), whereas the production of JA is associated with herbivore infestations (Su et al. 2015, Stotz et al. 2000, Walling, 2009). A mechanism associated with plant-mediated interactions is the suppression of essential defense transcripts by the pathogen in host plants which favor the development of their vectors (Casteel et al. 2012, Casteel et al. 2015, Su et al. 2015, Abe et al. 2011, and Bak et al. 2017). The persistently transmistted viruses *Tomato yellow leaf curl virus* (TYLCV, vector: *Bemisia tabaci*) and *Tomato spotted wilt virus* (TSWV, vector: *Frankliniella occidentalis*) can suppress the anti-herbivore response in plants, which subsequently favors their vectors by the disruption of downstream defenses in the jasmonic acid (JA) pathway (Su et al. 2015, Abey et al. 2011). However, JA regulation by TSWV is not beneficial for the vector of TYLCV (Chen et al. 2018). For instance, the fecundity of *F. occidentalis* is significantly reduced when TYLCV is present in tomato plants. It would suggest that TSWV and TYLCV are likely to compete for host resources to manipulate their vectors (Chen et al. 2018). Therefore, pathogens from different taxonomic groups (e.g., PVY and Lso) but sharing the same host plant and transmission mode may activate mechanisms *in planta* to reduce the performance of the other vector.

Both PVY and Lso are important pathogens in potato, and coinfections can occur in fields. In Idaho, however, Lso infections are likely to occur later in the season when PVY is already present in the field (Nolte et al. 2009, Rondon 2012). A clear understanding of PVY-

Lso interaction has direct implications in the epidemiology of ZC and PVY infections. However, host/vector responses from multiple interactions are difficult to predict since they are likely to be regulated by different, interconnected, and/or unknown molecular interactions such as the ones between host plant, plant virus, and insect pest/vector (Petek et al. 2014, Prager et al. 2015).

Research Objectives

1. Evaluate the transmission success and susceptibility to "*Ca.* Liberibacter solanacearum" in selected potato genotypes

2. Evaluate Lso development and ZC symptom progress during storage

3. Evaluate the effect of cold temperatures on potato psyllid mortality in relation to their Lso status and the vector haplotype

4. Evaluate the impact of Lso and PVY coinfection on ZC pathosystem

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Chapter 2: Effect of the level of Lso infection on the development of zebra chip and identification of sources of resistance in potato genotypes

Abstract

The potato psyllid (*Bactericera cockerelli* Sulc.)-transmitted "*Candidatus* Liberibacter solanacearum" (Lso) is a pathogen negatively impacting the potato industry in the USA and several other potatoproducing countries. Liberibacter has been linked to zebra chip disease (ZC), a condition that severely affects the yield and quality of potato tubers. There have been efforts to find sources of resistance to Lso or its insect vector in wild solanaceous species and potato varieties and breeding clones through greenhouse evaluations. Although findings from greenhouse studies are valuable, results need to be validated in the field. This study was conducted to evaluate the relative resistance/tolerance to ZC at harvest in selected potato genotypes. Lso transmission success (greenhouse evaluation), Lso titer, and ZC severity (field evaluations) were determined and compared among eight potato genotypes. Evaluations of Lso titer and symptom severities were conducted on plants infested with the infective psyllids at 77, 12, or 4 days before vine-removal (DBVR). The evaluated genotypes were classified according to their relative resistance to Lso and tolerance to ZC symptoms. Results showed that Lso transmission success in A07781 siblings was below that of the susceptible control ('Russet Burbank'). A07781-4LB and A07781-3LB genotypes were categorized as relatively resistant to the pathogen and highly tolerant to ZC symptoms, and A07781-10LB was considered as still susceptible to Lso but relatively tolerant to symptom expression. Also, A07781 siblings have shown a reduced incidence of Lso in tubers at 77 and 12 DBVR.

Therefore, A07781 siblings exhibit a potential source of resistance or tolerance to ZC at harvest.

Introduction

Zebra Chip (ZC) disease represents an economic problem for the potato industry since it severely affects the yield and quality of tuber production in the United States, Mexico, Central America and New Zealand (Secor and Rivera-Varas 2004, Liefting et al. 2009, Munyaneza 2012). This disease has been linked to the pathogen "*Candidatus* Liberibacter solanacearum" (Lso), a phloem-limited bacterium, which is transmitted by the potato psyllid *Bactericera cockerelli* (Sulc) (Hansen et al. 2008, Liefting et al. 2009). Symptoms of ZC in the foliage are characterized by upward rolling of the top leaves, purplish discoloration, shortened internodes, leaf-scorch, and the formation of aerial tubers (Secor et al. 2009). The most characteristic symptom of ZC in fresh tubers, however, includes brown discoloration and necrotic flecking of internal tuber tissue (Miles et al. 2010, Munyaneza et al. 2007).

No practical integrated approach has been yet developed for ZC management, and there are currently no potato cultivars with an acceptable level of resistance to ZC. As such, the spread of Lso is managed primarily by frequent insecticide applications to control the psyllid vector. The use of calendar-based insecticide applications is costly and unsustainable (Goolsby et al. 2012, Greenway 2014, Prager et al. 2013). Integrated pest management (IPM) can be an effective strategy to manage ZC (Munyaneza et al. 2012). Since host plant resistance is an effective component of IPM in managing vector-borne plant pathogens (Butler at al. 2011, Munyaneza et al. 2012, Diaz-Montano et al. 2013), identifying sources of resistance to Lso and/or its vector becomes essential.

Resistance to ZC has been investigated in wild solanaceous species (Perilla-Henao and Casteel 2016, Cooper and Bamberg 2014, Cooper and Bamberg 2016, Levy and Tamborindeguy 2014) and potato breeding clones (Butler et al. 2011, Diaz-Montano et al. 2014; Rubio-Covarrubias et al. 2017, Rashidi et al. 2017). These studies reported genotypes with degrees of resistance against the potato psyllid (Butler et al. 2011, Diaz-Montano et al. 2014), or Lso (Butler et al. 2011, Diaz-Montano et al. 2014; Rubio-Covarrubias et al. 2017, Rashidi et al. 2017). In a greenhouse study, Rashidi et al. (2017) showed that clones derived from *Solanum chacoense* (A07781-10LB, A07781-3LB, and A07781-4LB; Table 1) expressed lower susceptibility to Lso and ZC expression at harvest. However, additional verifications are needed because the development of the bacterial pathogen is not only influenced by potato genotypes (Rashed et al. 2011, Rashidi et al. 2017) but also by the time of infection (Rashed et al. 2011) and likely abiotic environmental variables influencing plant-pathogen interactions in the field (Borges de Oliveira et al. 2006, Costello et al. 2017, Down et al. 2001). Early-season infections produce severe symptoms in tubers, whereas symptoms in late-season infections may not be visible in the tubers (Rashed et al. 2013; Rashed et al. 2015, Rush et al. 2015). Therefore, field evaluations should also consider the effect of genotype and time of infection as sources of variability which can influence insect-plant-pathogen interactions.

The present study was conducted to evaluate the transmission success and susceptibility to "*Ca*. Liberibacter solanacearum" in eight selected potato genotypes. These genotypes were first evaluated for Lso transmission success via greenhouse assays. Field evaluations were performed to determine the susceptibility of the eight genotypes to Lso infection under standard agricultural practices in PNW.

Materials and Methods

Plant and insect material

Eight potato genotypes (*Solanum tuberosum* L.) were selected for this experiment (Table 2.1). These genotypes were previously reported as tolerant/resistant to ZC and/or potato psyllids (Butler et al. 2011, Diaz-Montano et al. 2014, Rashidi et al. 2017). The variety Russet Burbank was used as a susceptible control. The genotypes evaluated were provided by the USDA-ARS, Small Grains and Potato Germplasm Research Unit, Aberdeen, Idaho.

Colonies of the potato psyllids from the Central haplotype (Swisher et al. 2012) carrying the Lso-B biotype (Wen et al. 2013), were reared on potato (var. Russet Burbank) and maintained in climate-controlled growth chambers [18-27°C; 16:8 Light(L): Dark(D)], in 60 x 60 x 60 cm bugdorm cages (BioQuip Products, Rancho Dominguez, CA). The haplotype of potato psyllids was confirmed by polymerase chain reaction (PCR) and followed a restriction analysis which is described by Swisher and Crosslin (2014). Prior to each inoculation (see below), Lso incidence in colonies was estimated using PCR (Crosslin et al. 2011) on 10 individuals from each cage. The Lso incidence ranged between 90 and 100 percent in both years of the study.

Greenhouse evaluations: Liberibacter transmission success

The transmission success study was conducted in greenhouses of the University of Idaho, Aberdeen Research and Extension Center, Aberdeen, ID, during the winter of 2016. Tubers from eight potato genotypes were planted in 7.57-liter pots containing a mixture of 70% sand, 20% peat moss (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada), 10% vermiculite (Therm-o-Rock West INC., Chandler, AZ, USA) and fertilizer (Osmocote; Scott-

Sierra Horticultural Products Co., Marysville, OH, USA). Plants were maintained in controlled greenhouse conditions with temperatures ranging between 16 (night) and 23 (day) ºC, on a 12D:12L photoperiod.

Plants were arranged in a completely randomized design in two time-blocks, planted one week apart, and each located in a different greenhouse. For each time-block, there were 12 plant-replicates per genotype, including two uninfected control plants. Control plants never tested positive for Lso by qPCR. Plants were inoculated two weeks after emergence (> 80% germination). For inoculations, one frame-less leaf clip cage (BioQuip Products, Rancho Dominguez, CA) with 2.54-cm of diameter was installed on one leaflet of a fully expanded middle leaf of each plant. Three infective psyllids were released into each leaf cage using an aspirator. For control plants infested with non-infective psyllids, three Lso-negative psyllids were released into each leaf cage. All plants were covered individually with a mesh bag for the 48 hours of inoculation access period (IAP).

After IAP, psyllids from each plant were removed and then placed into a 2-ml tube and stored at -20°C until Lso quantification by quantitative Polymerase Chain Reaction (qPCR) described below. After removing insects, plants were sprayed with 2.8 ml per gallon of Warrior II (248g/L of Lambda-cyhalothril [a.i]; 1.9 E-06 g of a.i per plant) (FMC Corporation, GA, USA) and 7.4 ml per gallon of Movento (480g/L of Spirotetramat [a.i]; 0.043g of a.i per plant) (Bayer CropScience, NC, USA) immediately, and also one week after psyllid removal, to reassure successful removal of all potato psyllids. Plants were maintained in the greenhouse for approximately 10 weeks. Tubers were harvested two weeks after vine-removal. Tubers less than 2 cm in diameter were discarded. From the remaining tubers of each plant, four were randomly selected for Lso analysis to determine transmission success. The selected tubers were

sampled at the stolon attachment end by removing 100 mg of tissue using a 6-mm Harris UNI - CORE^{TM} (GE Healthcare Life Sciences, Buckinghamshire. UK). Samples were stored in -20°C until DNA extraction and Lso analisys by qPCR.

DNA extraction and Lso quantification

Total DNA from tuber samples and psyllids (composite sample of three psyllids) were extracted using the CTAB (hexadecyltrimethyllammonium bromide) method. DNA extraction of tubers was performed following the methods of Rashidi et al. (2017) whereas DNA from psyllids was extracted as specified by Marzachi et al. (1998). DNA of all tissue samples were quantified (absolute quantification) by qPCR using SYBR Green in a CFX Real-Time PCR System (BioRad Laboratories, Hercules, CA, USA). The qPCR reaction contained 150 nM of each of the primers, HLBr and LsoF (Li et al. 2006, Li et al. 2009), 1X SsoAdvanced Universal SYBR Green Supermix (BioRad Laboratories, Hercules, CA, USA), and 1 µl of DNA template. The amplification program was as follows: one cycle at 98˚C for 2 min, followed by 40 cycles of 95˚C for 10 sec, and 62˚C for 20 sec, followed by a melt curve (65˚C to 95˚C, 0.5˚Cs^{-1} increments). A plasmid (pIDTSMART – KAN) containing a known copy number of Lso was used to build a standard curve, developed according to eight 10-fold serial dilutions, and to estimate copy numbers (Levy et al., 2011). Negative controls including DNA from healthy plants and water (no template control) were also included in all qPCR analyses.

Field Evaluations: Relative susceptibility to Lso infection and tolerance to ZC

Field experiments were conducted in 2016 and 2017 at the Kimberly Research and Extension Center, Kimberly, ID. Seed potato pieces were planted on May 4, 2016 and May 3,

2017. Seed potatoes from each of the eight genotypes were hand-planted on two 91-cm wide rows (4 plants/row), 30-cm apart (one seed piece/genotype/cage). The planted plot was covered with 1.5 x 2.4 x 1-m (W x L x H) field cages constructed from netting 4750 plastic mesh (U.S. Global Resources, Seattle, WA) over SunGUARD® II fiberglass rods (Geoteck Inc., Stewartville, MN), secured into the ground at both ends. There was a total of 40 and 32 cages in 2016 and 2017 experiments, respectively. Cages were arranged in a randomized complete block design, with 10 blocks in 2016, and 8 blocks in 2017. Each block included four cages, each infested at either 77, 12 or 4 days before vine-removal (DBVR). One cage per block was not infested with the potato psyllids and was used as a non-inoculated control.

The psyllids used in this study were obtained from the colony of Lso-infected potato psyllid of the Central haplotype. The colony was maintained in growth chambers as previously described. Inoculations were initiated three weeks after emergence (> 80% emergence for all genotypes). For inoculation, five Lso-positive psyllids were released at the base of the individual plants (40 psyllids per cage). Plants were exposed to Lso-infected psyllids for an IAP of 7 days. Cages were sprayed twice, one and two weeks after inoculation, with a mix of 8ml/gallon of Movento (480g/L of Spirotetramat [a.i]; 0.043g of a.i per plant) (Bayer CropScience LP, NC) and 6ml/gallon of Agri-Mek (22g/L of Abamectin [a.i]; 1.6 E-03g of a.i per plant) (Syngenta Crop Protection, NC). At the end of the season, the vine was removed 129 days after planting. The removal of the foliar tissue was performed mechanically by cutting vines with pruning shears. Tubers were harvested by hand two weeks after vine-removal. Tubers were immediately stored at 12.7 ℃ and evaluations were performed between the 1st and 3rd day after harvest. The temperature of 12.7 ℃ was the initial step of the cold storage procedure used for the study described in the chapter 3 of the present dissertation.

Four tubers per plant were randomly selected for evaluations of ZC symptom severity and Lso quantity at harvest. Tubers less than 2 cm in size were not included in the study. ZC symptoms were evaluated at the stem end (stolon attachment end). ZC severity was scored on a scale of '0' to '3'. Score "0" represents asymptomatic tubers whereas "3" represents severe tuber symptoms (Rashed et al. 2013). For Lso quantification, 100 mg of tuber tissue from tubers evaluated in their ZC symptoms was removed from the stem end, using a 6-mm Harris UNI - CORE^{TM} (GE Healthcare Life Sciences, Buckinghamshire, UK). Samples were stored in -20°C for DNA extraction and Lso quantification. Quality and quantity of DNA were verified by nanodrop measurement (NanoDrop Lite, Spectrophometer, Thermo Scientific, Madison, WI). DNA extraction and Lso quantification by Real-time PCR (qPCR) were performed as described above under DNA extraction and Lso quantification.

Relative resistance and tolerance to ZC in the evaluated genotypes

To categorize the relative susceptibility and tolerance of genotypes, pathogen titer (Y = Lso titer) and ZC symptom severity score (X = symptom score) were used to construct a two-dimensional graph area that was divided into four quadrants. Each quadrant was defined as a category of relative susceptibility to the pathogen and tolerance to the infection. The evaluated genotypes were placed into a scatter plot graph according to their average Lso titers and symptom severity scores. Quadrants were delimited by averages of both Lso titers and ZC symptom severity score across all genotypes in the study (Rashidi et al. 2017). Genotypes were categorized as relatively susceptible or low susceptible to Lso, and relatively tolerant or intolerant based on ZC symptom expression. High susceptibility is defined as the inability of a plant to stop or reduce the development of the pathogen (Agrios 1997). Tolerance refers to

the ability of a plant to limit symptom expression, regardless of the level of pathogen multiplication (Agrios 1997).

Statistical analysis

Transmission success of "*Ca*. Liberibacter solanacearum" was analyzed with the software IBM SPSS ver. 24.0. Generalized linear mixed model (GLMM) with binomial distribution was used to evaluate Lso infection success in tubers. Infection success was considered as the response variable and was treated as a categorical variable (0 = non Lso infected, 1 = Lso infected). The initial model included block, genotype and interaction terms (e.g., block-genotype interaction) as main effects, plant replicates as a random effect, and psyllid titer (continuous variable) as a covariate. Factors with non-significant ($P > 0.05$) effect were removed from the model, in a stepwise approach. Genotype was kept as the main factor regardless of its significance, and block factor without significant effect was kept as the random factor.

GLMM was also used to compare Lso titers. For each plant, Lso quantity and ZC symptom severity were estimated based on average titer and ZC score in the four randomly selected tubers. The effect of genotype on Lso titer (continuous variable) was analyzed using a normal distribution. To deal with zeros in the data set a fixed value of 1.5 was added to all Lso concentrations prior to log-transformation. This transformation was performed because data had not a normal distribution. The log of 1.5 is smaller positive value which might not alter the trend of the original data. Year, genotype, time of infestation, and year-by-genotype interaction were included as main factors, and block was treated as the random factor in the model. Factors with non-significant effect were removed from the model in a stepwise approach to improve the sensitivity of our model to detect differences. GLMM was also used

to compare the susceptibility to Lso and tolerance to ZC among genotypes in relation to the time of infestation (77, 12, and 4 DBVR). The initial model contained Lso titer as the response variable, potato genotype, time of infestation, year and interaction terms as fixed factors, and block as the random factor. Factors showing a non-significant effect on the response variable were removed in a stepwise approach. The effect of genotype on ZC symptom severity (ordinal variable) was evaluated using a nonparametric approach, the Kruskal-Wallis test. Scores of ZC severity (dependent variable) were compared among the time of infestations. The incidence of ZC in tubers was analyzed by GLMM with a binomial distribution. Incidence was treated as binomial variable (0 = non-infected, 1 = Lso infected).

Results

Lso transmission success among genotypes

There was no significant difference in the probability of successful Lso transmission among the evaluated genotypes (GLMM, $F_{6,79} = 0.437$; $P = 0.449$). The Lso inoculation rate ranged between 33% (A08399-6LB) and 75% (A05379-211), with none of the genotypes significantly different from the susceptible control, Russet Burbank (Pairwise, $F_{7,79} = 1.210$; $P = 0.421$). Transmission success rates in A07781-4LB, A08399-6LB and PALB3016-6 were estimated below the 55% overall average. Russet Burbank and A05379-211 were the two genotypes with relatively higher rates of transmission success (Fig. 2.1).

Relative susceptibility of potato genotypes in relation to the time of infestation

Lso titer was significantly affected by the time of infection ($F_{2,411} = 467.4$, $P < 0.001$), with tubers infected early in the season having the highest Lso titers. The least Lso titer was detected in tubers which were infested 4 days before vine removal (4 DBVR) (Fig. 2.2). The

effect of genotype however was only of borderline significance ($F_{7,411}$= 2.00, P = 0.053), with the least Lso titer observed in A07781-3LB (mean copy number per 100mg of tuber tissue [±standard error] = $5.9*10^5$ [$2.1*10^5$]. No effects of year (*P* = 0.117) or year-by-genotype (*P* = 0.962) were observed.

ZC symptoms were not present in tubers that were infected four days before vine removal. Thus, symptom severity comparison among genotypes only included tubers from plants that were infected 12 and 77 DBVR. An initial Kruskal-Wallis analysis detected significant difference in the severity of symptoms between the two years of the study with greater symptom score in 2017 (0.89 [±0.08]) than 2016 (0.66 [±0.05]). Thus, among-genotype symptom comparison within each infestation time treatment was conducted separately for each year.

In both years, significant differences were observed in the severity of the symptoms expressed by the potato genotypes (2016: 77 DBVR, *H* = 51.45, *P* < 0.001; 12 DBVR, *H* = 15.77, *P* = 0.027; 2017: 77 DBVR, *H* = 18.74, *P* = 0.009; 12 DBVR, *H* = 27.65, *P* < 0.001) (Fig. 2.3, A-B). The analysis of symptoms severity at 4 DBVR was not presented because tubers were overall asymptomatic; ZC symptoms score were likely to uninfected control treatment. In the infestation conducted 77 DBVR, the A07781 genotypes expressed milder symptoms compared to the susceptible control (Fig. 2.3, A-B). However, a different pattern was observed in tubers infested 12 days before vine-removal (12 DBVR) as symptom severity was higher than for the susceptible control, especially in A07781-3LB and A07781-10LB, in both 2016 and 2017.

Relative resistance to Lso and tolerance to ZC symptoms were compared among the eight evaluated genotypes by plotting the relationships between pathogen quantity and

symptom severity in freshly cut tubers from early infestation (77 DBVR). Since the interaction between year and potato genotype was not significant for Lso quantity, and because symptom expression in 2016 and 2017 followed more-or-less a similar pattern (Fig. 2.3, A-B), 2016 and 2017 were pooled and graphed in a single plot (Fig. 2.4). Genotypes were classified based on the analysis of relative susceptibility and tolerance as described by Rashidi et al. (2017). The three genotypes belonging to the A07781 family were categorized as either resistant or tolerant in relation to other evaluated genotypes. Relatively low Lso titer and mild ZC expression placed A07781-4LB and A07781-3LB into the resistant quadrant, while relatively high Lso titer but mild symptom expression placed A07781-10LB in the tolerant genotype quadrant. Our susceptible control Russet Burbank, the commercial variety Western Russet, A05379-211, and A08399-6LB fell into the intolerant quadrant since even relatively low titer levels triggered severe expression of ZC symptoms. PALB03016-6 showed relatively higher symptom severity scores and Lso quantities. They were thus categorized as relatively intolerant and susceptible genotypes.

Lso incidence across times of infestation

The percentage of tubers that tested positive for Lso was compared among the evaluated genotypes in 2016 and 2017 at 77, 12, and 4 DBVR. The initial analysis showed that Lso incidence in tubers was significantly affected by year ($F_{1,418} = 16.08$; $P < 0.001$), with a significantly higher number of Lso-positive tubers in 2016 than 2017 ($F_{1,418} = 17.312$; $P < 0.001$). The percentage of Lso-positive tubers reached 71% in 2016 and 28% in 2017. A significant effect of time of infestation was also present ($F_{2,418} = 53.13$; $P < 0.001$). Lso incidence however was not influenced by time of infestation by year interaction ($F_{2,418} = 0.564$;

P = 0.569). Pairwise comparations revealed that the number of Lso-infected tubers varied significantly among the three times of infestation ($F_{2,418} = 392.4$; $P < 0.001$). The average of Lso incidence in tubers was 98, 42, and 13% at 77, 12, and 4 DBVR, respectively.

In the 77 DBVR treatment, ZC incidence in A07781-4LB and A07781-10L was estimated at 93.7 and 92.9%, respectively. All of remaining genotypes showed 100% infection. In the 12 DBVR treatment, ZC incidence was significantly affected by year ($F_{1,128} = 13.7$; $P < 0.001$), but not by the host plant genotype ($F_{7,128} = 1.2$; $P = 0.296$), and no effect of genotype by year interaction was detected ($F_{7,128} = 0.1$; $P = 0.997$). The percentage of infected tubers was significantly higher in 2016 than 2017 ($F_{1,135} = 16.9$; $P < 0.001$), reaching 58% in 2016 and 27% in 2017. In the 12 DBVR treatment, the lowest Lso incidences were associated with A07781-3L (27.7 %), A07781-4LB (33.3%) and A05379-211 (33.3 %).

In the 4 DBVR treatment, ZC incidence was not significantly affected by either genotype ($F_{7,135} = 16.9$; $P = 0.743$) or year ($F_{1,135} = 3.156$; $P = 0.078$), ranging between 5% (PALB03016-6) and 22.5% (A05379-211).

Discussion

The relative susceptibility of eight potato genotypes to ZC was compared based on the following criteria: the transmission success of Lso by its potato psyllid vector, Lso concentration within plant tissue, and the severity of expressed symptoms in relation to the time of infestation. Lso transmission success assays were conducted in the greenhouse, and Lso titer and symptom expression were conducted in the field. The field component of our study intended to confirm findings from a previous greenhouse study, which detected resistance and/or tolerance in three genotypes, all belonging to the A07781 family (Rashidi et

al. 2017). Overall, the A07781 siblings emerged as a promising group in our field evaluations, suggesting a way forward toward developing commercial genotypes that will lower susceptibility to the disease.

The potato psyllid vectors are highly efficient in transmitting Lso (Buchman et al. 2011, Rashed et al. 2012) from plant to plant, but the transmission rate varies among the host genotype (Rashidi et al. 2017, Butler at al. 2011). A07781-4LB, A07781-3LB, A07781-10LB, A05379-211 and Russet Burbank, the five genotypes shared between the two studies, had transmission success rates below 37% in Rashidi et al. (2017). In our present study, with the exception of A07781-4LB, the remaining four genotypes had transmission success rates exceeding 50%. The relatively higher rate of transmission success may be due to the difference in the number of Lso-infected psyllids used for inoculations. Rashidi and colleagues (2017) used two potato psyllids to conduct inoculations, whereas four potato psyllids where used in the present study. It is known that the likelihood of successful transmission increases as the number of potato psyllid vector increases (Rashed et al. 2012). Similar to previous studies (Rashed et al. 2012; Rashidi et al. 2017) transmission success was not affected by the Lso titer of the potato psyllid vectors.

The genotype A05379-211 is derived from *S. etuberosum* (Butler et al. 2011). In our study A05379-211 had a transmission rate of 75%. Previously, much lower transmission success rates of A05379-211 were reported as 44.4% (Buttler et al. 2011) and 20% (Rashidi et al. 2017). Again, the difference of our results with Rashidi et al. (2017) may be explained by the number of psyllids used in inoculations. However, our results are not directly comparable to those of Butler et al. (2011) because they evaluated transmission success in leaves and not the tuber tissue.

The potato psyllid infestations may occur at different times throughout out the season in a pattern that appears to be location-specific. For example, early-season infections are common in southern states which corresponds to the vector movement from its southern overwintering sites (e.g., Mexico). In Idaho, on the other hand, there is an increase in the number of potato psyllids captured in the field late in the season (Wenninger et al. 2017). So far, the incidence of Lso-infected psyllids collected on the field from 2012 to 2015 ranged between 2.4 to 26.4% (Dahan et al. 2017). Since it has been shown previously that host plant susceptibility can be influenced by the plant developmental stage (e.g., time of infection) (Rashed et al. 2014), our results has shown that Lso titer and ZC severity vary with the time of infection; these findings support reports from earlier studies that showed greater ZC symptom expressions are associated with infections that occur early in the season (Rashed et al. 2015). Overall, Lso titers and the severity of ZC symptoms were greater in 2017 than 2016. Environmental factors are likely to explain the observed differences across years especially since both potato psyllids and Lso are sensitive to fluctuations in temperature. While optimal conditions for potato psyllid development is around 27ºC, its development starts to be negatively affected at 32ºC (Butler and Trumble 2012). Likewise, temperatures over 32ºC can be detrimental to Lso development (Munyaneza et al. 2012). Maximum and minimum temperatures registered between May and September in the vicinity of our field sites ranged between 1.9 ºC and 36.6 ºC, in 2016, and 3.4 and 37.2 ºC, in 2017 (https://www.usbr.gov/pn/agrimet/). The overall average of Lso titer trend to be higher in 2017 than 2016 at 77 and 12 DBVR (data not shown).

Moreover, although the same potato psyllid colony was used to conduct field inoculations in 2016 and 2017, potential differences in the quality of individuals at the time of

inoculations might have also impacted the amount of the initial inoculated inoculum which could subsequently impact disease development (Rashed et al. 2012, 2014). Relative resistance and tolerance of the potato genotypes were characterized based on the relationship between pathogen titer and ZC symptom expression at 77 DBVR. Results have shown that A07781-4LB and A07781-3L are relatively resistant to the pathogen and tolerant to ZC symptoms. A07781-4LB and A07781-3L genotypes might reduce the pathogen infection and also maintain a reduced ZC symptom severity. Likewise, A07781-10LB is relatively susceptible to the pathogen but tolerant to ZC symptoms. Although the pathogen developed successfully in A07781-10LB, this genotype has still shown low expression of symptoms, a pattern that was consistent across the duration of the study. An earlier greenhouse study showed that A07781 siblings have a Lso transmission success rate ranging between 41% (A07781-4LB) and 64% (A07781-10LB); A07781-4LB had a Lso transmission success rate below the average (55%), and both A07781-3LB and-10LB had over the average Lso transmission rate. However, all of A07781 siblings expressed relative resistance and tolerance in the field study. Overall, our field results are similar to those of previous greenhouse studies on characterization of host resistance to ZC. Rashidi et al. (2017) analyzed the relationship between Lso titer and ZC symptom severity of potato genotypes including A07781-4LB, A07781-3L, and A07781-10LB genotypes. They found that the A07781 group had relatively low Lso susceptibility and high ZC tolerance. This demostrates a consistent expression of traits associated with resistance or tolerance in the family A07781. Western Russet, A05379-211, and A08399-6LB, on the other hand, were considered less susceptible to Lso but highly intolerant to ZC. Russet Burbank (susceptible control) showed low Lso susceptibility but expressed greater symptom severity. The expression of tolerance traits in tubers can be associated with a decrease in the intensity

of host physiology response following Lso infection. Some of the potato clones expressing tolerance to ZC have reduced production of phenolic compounds that are associated with ZC symptom expression in tubers (Wallis et al. 2012, Wallis et al. 2015). Therefore, low susceptibility to the pathogen and high severity of symptoms in tubers suggest a strong physiological response to pathogen infection.

A07781 genotypes are derived from *Solanum chacoense* (Rashidi et al. 2017). Germplasm derived from wild species are shown to be a source of resistance to plant pathogens (Jasky et al. 2018, Cooper et al. 2016, Casteel et al. 2006). Other potato breeding clones from *S. chacoense* have also expressed tolerance to ZC symptom severity (Wallis et al. 2015) and resistance to other diseases caused by bacteria (e.g., *Streptomyces scabies*) (Jasky et al. 2018). Resistance genes (e.g., R gene) identified in wild solanaceous species (e.g., *Solanum peruvianum)* can confer resistance against species from four different taxonomic categories (Casteel et al. 2006, Perilla-Henao and Casteel 2016). Therefore, genotypes from *S. chacoense* (e.g., A07781 siblings) may be a stable source of resistance to ZC.

In our field study, we have conducted inoculations under controlled conditions (5 psyllids per plant). Our results showed that the development of ZC at harvest varies with the time of infestation in the field and that the susceptibility of genotypes to Lso can be influenced by environmental conditions (e.g., year of season). However, genotypes from the A07781 family showed consistent expression of resistant and/or tolerance to ZC across both growing seasons, as well as in a previous greenhouse study (Rashidi et al. 2017). Overall, A07781 siblings can be considered as a potential source of resistant or tolerant genes to ZC during the field season. As the development of Lso has been reported after harvest (Rashed et al. 2018), future studies would be required to evaluate the suitability of the A07781 family after storage

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Tables

Table 2.1. Selected potato genotypes for initial greenhouse and field study

Genotype	Background	Reference
Russet Burbank	Susceptible control; US commercial cultivar	Munyaneza et al. 2011
A07781-4LB	Selected based on low ZC symptoms severity in the greenhouse experiment	Rashidi et al. 2017
A07781-3LB	Selected based on low ZC symptoms severity in the greenhouse experiment	Rashidi et al. 2017
A07781-10LB	Selected based on low ZC symptoms severity in the greenhouse experiment	Rashidi et al. 2017
A08399-6LB	Sri-Lankan cultivar HilStar in background	NIFA report, 2016
PALB3016-6	Reduced oviposition; *S. guerreroense* in pedigree	NIFA report, 2016
A05379-211	Reduced growth index, reduced ZC severity in the greenhouse experiment	Butler et al. 2011, Diaz-Montano et al. 2013
Western Russet	Reported ZC tolerance/resistance in New Zealand	NIFA report, 2016

*Poster: Identification of resistance to zebra chip diseases in species-derived germplasm and international potato cultivars for use in the development of zebra chip resistant and tolerant potato cultivars for the U.S.

Figures

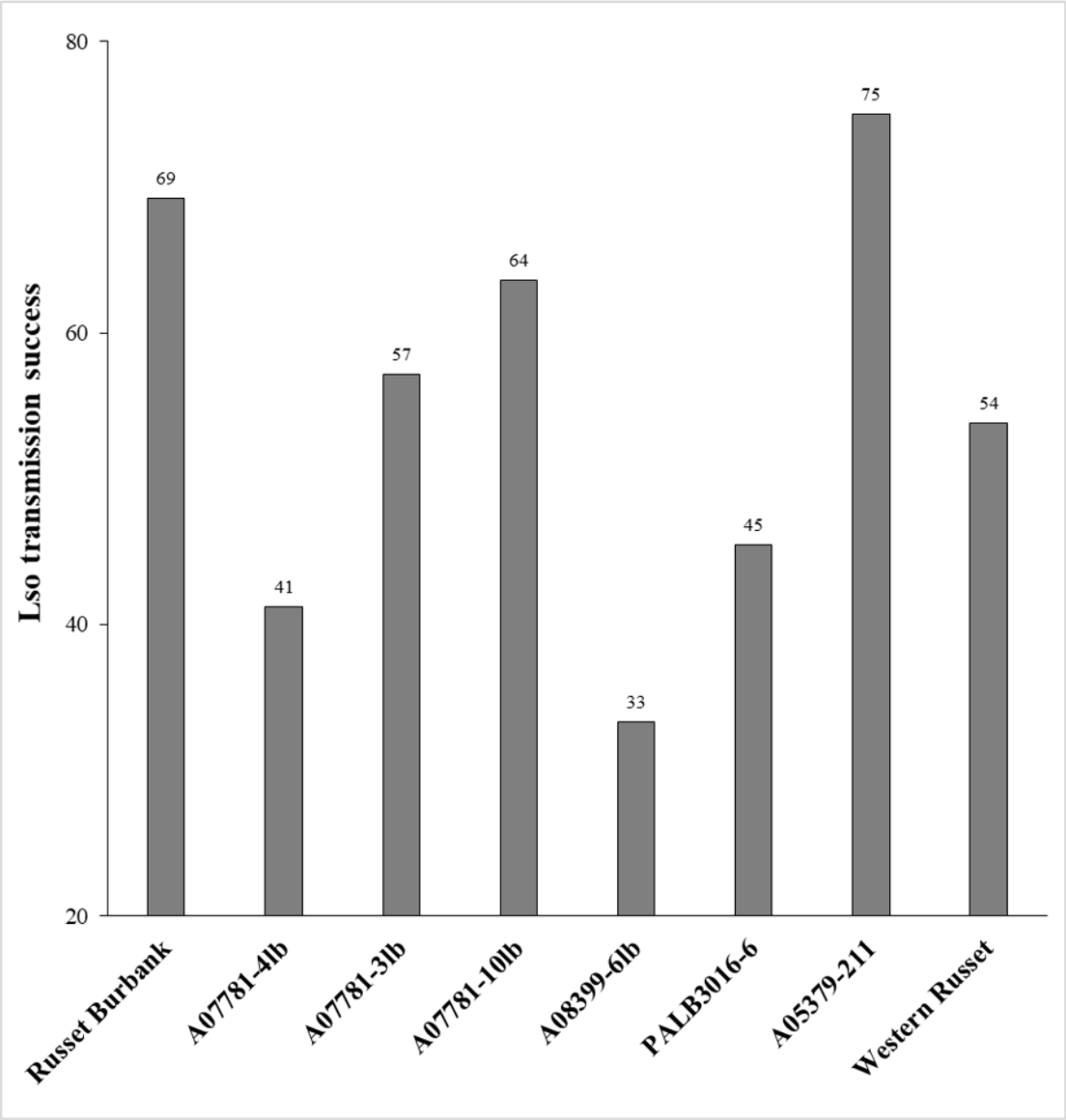

Figure 2.1. Percentage of tubers infected with "*Candidatus* Liberibacter solanacearum" by inoculation with Lso-infected potato psyllids. Lso infection was analyzed in fresh tubers of each genotype. Russet Burbank: susceptible control.

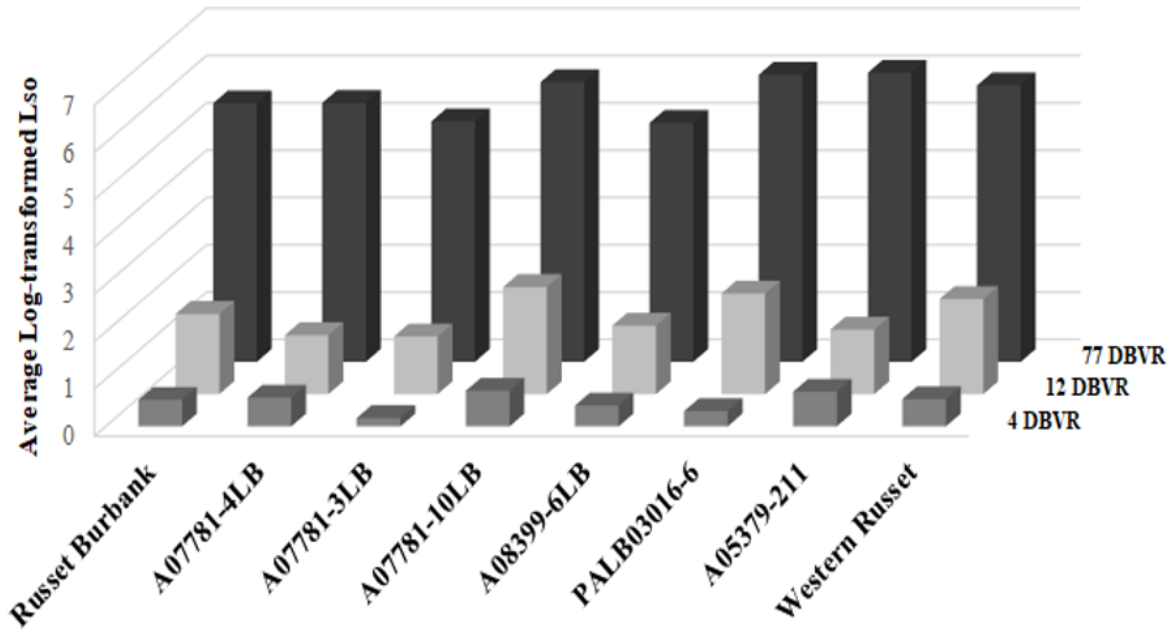

Figure 2.2. Lso quantity of selected potato genotypes from season 2016 and 2017 infested at either 77, 12 and 4 days before vine-removal (DBVR). Lso quantity was analyzed in tuber samples at harvest. Russet Burbank: susceptible control.

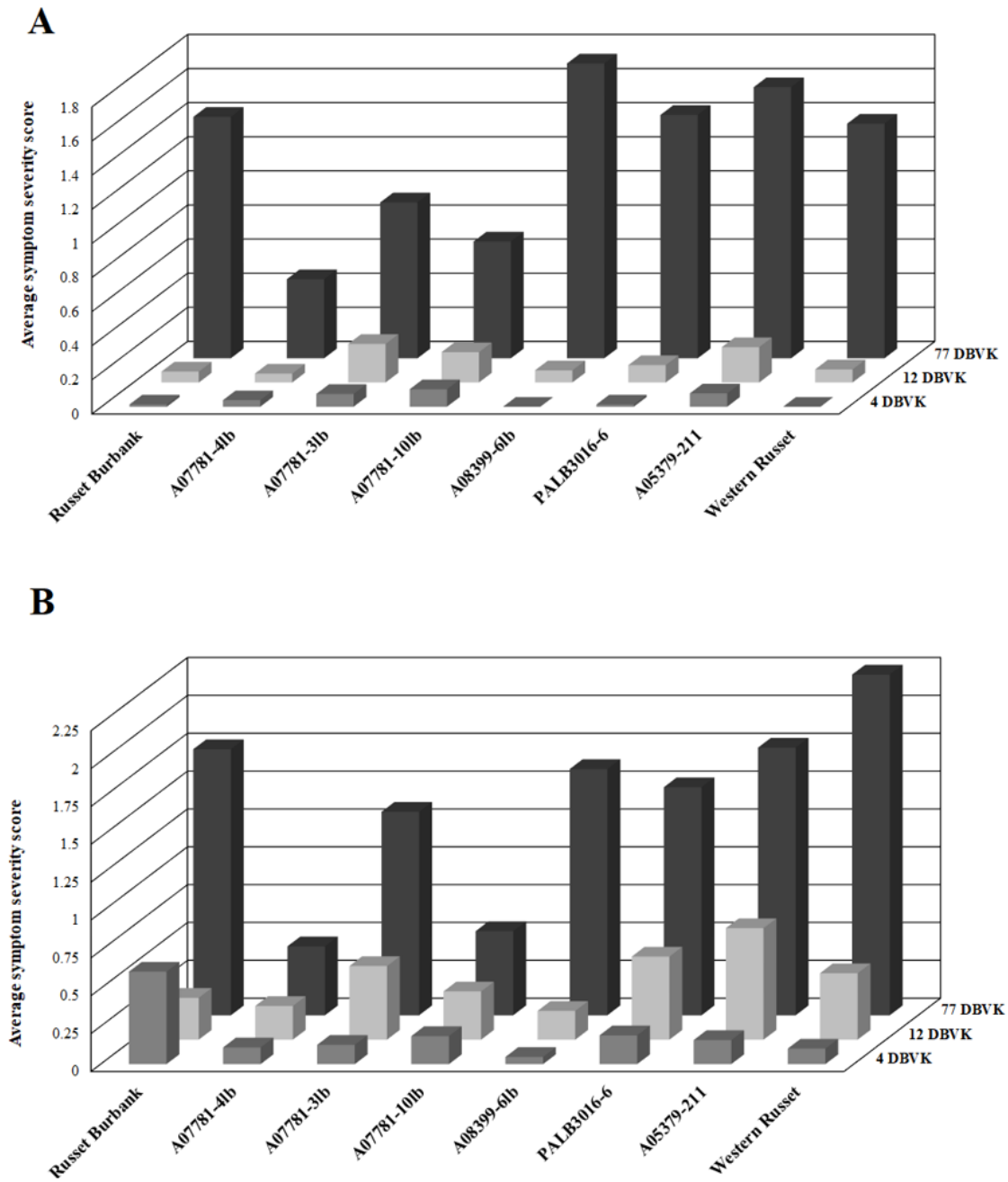

Figure 2.3. ZC symptom severity in potato genotypes infested at either 77, 12 and 4 days before vine-removal (DBVR). Severity of ZC symptoms was evaluated in tubers at harvest from season (A) 2016 and (B) 2017. Russet Burbank: susceptible control.

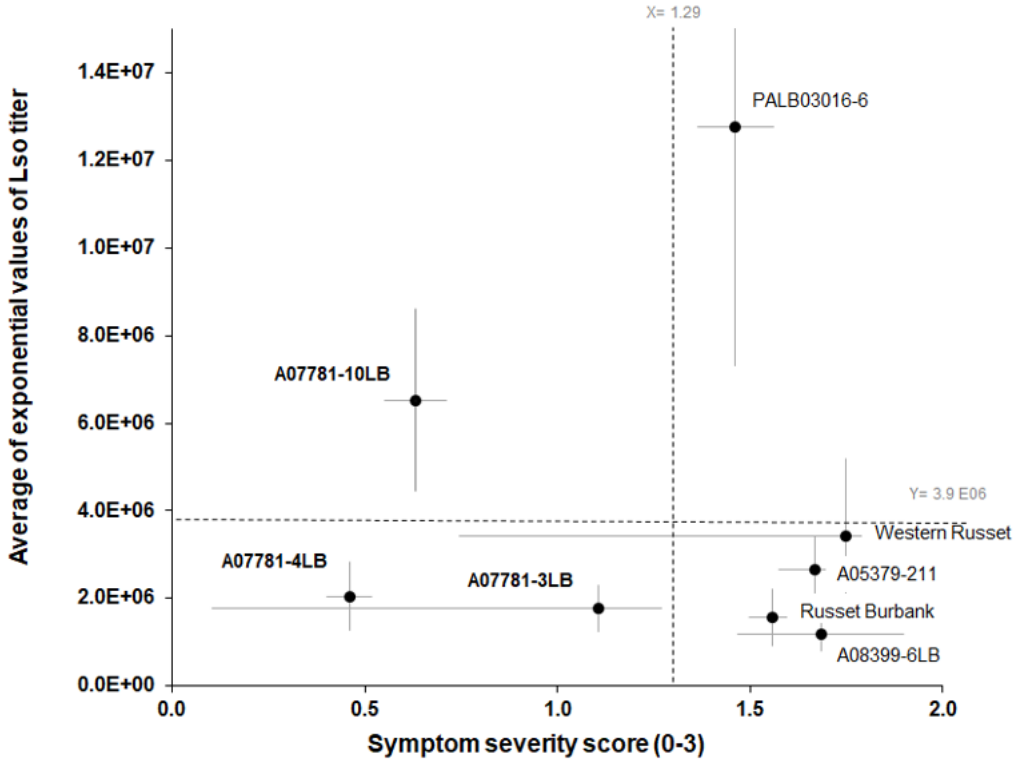

Figure 2.4. Relationship between Lso quantity and severity of ZC symptoms of selected potato genotypes from season 2016 and 2017 infested at 77 DBVR. Lso quantity and ZC symptom severity were analyzed in tuber samples at harvest. Lso titer represents averages of exponential values of Lso-transformed Lso titer. Russet Burbank: susceptible control. Dotted lines represent overall averages of symptom severity and Lso titer across the eight genotypes. Error bars represent standard error (+/- 1).

Chapter 3: Evaluation of zebra chip disease development among selected potato genotypes during cold storage

Abstract

"Candidatus Liberibacter solanacearum" (Lso) is a vector-borne plant pathogen associated with zebra chip (ZC) disease in potatoes, and is transmitted by the potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae). ZC is a disease of potato that negatively impacts commercial production because it both reduces yield and negatively affects potato tuber quality. The negative impact of ZC on potato quality is not just limited to tubers at harvest; Lso continues to develop in harvested potato tubers during storage, and such development can be influenced by the timing of infections in the field. Although some studies have reported resistance or tolerance to ZC at harvest, such responses have not been studied post-storage. The goal of this study is to evaluate the progress of Lso development and ZC symptoms during storage in potato genotypes, which were also screened during the field season. Tubers from eight potato genotypes were infected with Lso in the field at 77, 12 and 4 days before vine-removal (DBVR), then stored following typical commercial storage practices. Changes in Lso titer of each genotype were recorded between harvest and the end of the storage process. Also, relative susceptibility and Lso incidence were compared among genotypes after storage. Lso multiplication was observed in all the times of infestations with Lso-positive psyllids across genotypes, but a significant increase was only observed in tubers infected 12 DBVR. The analysis of relative susceptibility after storage has confirmed A07781 siblings as tolerant genotypes. The number of tubers infected with Lso had increased significantly at 4 DBVR. Results indicated that the development of Lso in tubers post-harvest was affected by

the time of infection in the field. Some genotypes such as those belonging to the A07781 family cannot only limit expression of ZC symptoms in freshly harvested potatoes, but also maintain their tolerance of ZC after storage.

Introduction

Since zebra chip (ZC) was first reported in the United States in 2000, the disease has caused severe economic losses to the potato industry (Munyaneza et al. 2012, Munyaneza et al. 2015), especially in fields in Oregon, Washington, and Idaho where ZC was first identified in 2011. These areas represent more than 50% of US potato production (Crosslin et al. 2012a,b). The disease affects the fresh market, table-stock, and export potato industry (Munyaneza et al. 2015). The pathogen which is associated with the bacteria "*Candidatus* Liberibacter solanacearum" (Lso) is transmitted by the potato psyllid *Bactericera cockerelli* Šulc (Hemiptera: Triozidae) (Hansen et al. 2008, Liefting et al. 2009). Typical symptoms (i.e., yellowing/purpling of young leaves) in foliage are observed within 3- or 4-weeks following infection (Secor et al. 2009, Rashed et al. 2014). Symptoms in fresh tubers are observed as necrotic "flecking" and brown spots around the tuber cortex tissue (Miles et al. 2010). The severity of symptoms in fresh tubers has been positively correlated with levels of phenolic compounds (Wallis et al. 2012, Rashed et al. 2013). When the fresh tuber tissue is exposed to air, polyphenol oxidase (PPO) catalyzes the oxidation of phenolics to quinones and the outcome is visualized as brown-colored areas on tuber tissue (Navarre et al. 2009, Wallis et al. 2012). In fried chips, ZC symptoms are characterized as a brown tissue discoloration (Munyaneza et al. 2012) which might be caused by the elevated concentration of certain amino acids and reducing sugars (Wallis et al. 2012). Potato plants are susceptible to being infected

through the entire season; early-season infection produces high severity of symptoms and reduction of yield. Consequently, programs to manage ZC have been focused on vector control to minimize early and mid-season infestations.

Studies have shown that plants infected late in the season are likely to be asymptomatic (Rashed et al. 2014, Rush et al. 2015). Thus, late-season infection is a significant threat to potato quality, especially where potatoes are stored for long periods after harvest (Rashed et at. 2018, Munyaneza et al. 2012, Wallis et al. 2017). Symptoms in tubers are visible 2 weeks after plant exposure to Lso-infected psyllids (Rashed et al. 2014), but Lso can move from the infected leaf to the tuber within only two days (Rush et al. 2015). Despite this quick translocation of Lso, plants that are infected only days before harvest can produce tubers. These tubers not only do not express any ZC symptom, but also will have very low concentrations of Lso that are not detectable by our current most sensitive molecular tests (Rush et al. 2015, Rashed et al. 2018). Detection of late-season infections is very important because evaluations of ZC at harvest may result in an underestimation of disease incidence (Rush et al. 2015). This is a major concern for the Pacific Northwest, where most of the tubers harvested go into storage and where the abundance of psyllids is usually higher later into the growing season (Wenninger et al. 2017). Moreover, the pathogen continues impacting tuber quality following harvest because interactions between Lso and its potato host continue post-harvest and during storage (Rashed et al. 2018, Wallis et al. 2017). An increase of ZC symptom severity, Lso titer, and phenolic compound levels has been detected during storage and those changes might vary with holding temperature, potato variety, and time of infection (Rashed et al. 2018, Wallis et al. 2017).

Since the interactions between Lso and tubers continue during storage, it is important to extend tolerance and resistance evaluations of potato genotypes into the cold storage stage. In the present study, Lso development and ZC symptom progress was determined in eight potato genotypes that were previously screened for resistance to Lso and tolerance to ZC in the field (Chapter 3).

Materials and Methods

This study was conducted in the University of Idaho, Potato Storage Research Facility at Kimberly R&E Center, Kimberly and the Eastern Idaho Entomology Laboratory, Aberdeen Research and Extension Center, Aberdeen, ID. Tuber evaluations were conducted between September and July of 2016 and 2017.

Tuber material

Tubers used in this study were from field studies, which were conducted at the University of Idaho, Kimberly R & E Center, Kimberly, ID, in 2016 and 2017. In both years, seed pieces from eight potato genotypes were provided by the USDA-ARS, Small Grains and Potato Germplasm Research Unit, Aberdeen, ID. Seed potatoes were hand-planted early in May. Tubers were planted on two 91 cm-wide rows. The rows were covered by field cages prior to potato emergence. Each cage contained one seed from each of the 8 evaluated genotypes, planted in a random order within the cage. Cages of 1.5 x 2.4 x 1-m (W x L x H) were constructed by stretching mesh (netting 4750) over SunGUARD® II fiberglass rods, secured into the ground at both ends. Three weeks after emergence (> 80% germination), plants were inoculated with Lso. To inoculate plants, five Lso-positive potato psyllids (Central haplotype) carrying the Lso-B biotype (Wen et al. 2013), were released at the base of individual

plants. The Lso-positive colony was maintained in 61 x 61 x 61-cm bugdorm (BioQuip Products, Rancho Dominguez, CA) and reared on Russet Burbank potato plants placed in a growth chamber, with temperatures ranging between 21 and 26°C. The conditions of temperature were the same on a 16:8 hrs photoperiod (Light: Dark). Overall, cages were arranged in a randomized complete block design. Each block included four cages; three were infested at either 77, 12 or 4 days before vine-removal (DBVR), and one was left non-infested as control. At the end of the season, the vines were mechanically removed. The field study is detailed in chapter 2 (page 45). The tubers were harvested two weeks after vine-removal. Two groups of four tubers per plant were selected for storage; one group was sampled and evaluated at harvest, and the other group was kept intact for post-storage quantification of symptoms and Lso. Tubers that were less than 2 cm in diameter were not included in the study.

Storage process and evaluations

After evaluations at harvest, potato tubers were stored at the University of Idaho, Potato Storage Research Facility at Kimberly R&E Center, Kimberly, ID. Tubers were stored inside plastic mesh bags, and bags were placed inside of plastic crates. Crates were stored under the following conditions: 1) 12.7°C for two weeks (healing period), 2) temperature ramp-down to 8.8°C at 0.3°C per day within approximately 2 weeks, and 3) holding temperature of 8.8°C for approximately 21 weeks (Fig. 3.3). The sprouting of tubers in storage was prevented by applying 22 ppm of chlorpropham (CIPC; Decco, Elf Atochem North America, Monrovia, CA) 67 days after harvest. At the end of the storage period, potato tubers were evaluated for their ZC symptom severity and Lso titer. Following storage, the intact stored tubers, and the tubers that were already sampled at harvest, were evaluated for ZC symptom severity and Lso titer.

The tubers that were evaluated at harvest were re-sliced at the stem end (also see below). ZC severity was scored on a scale of 0 to 3; score "0" represents asymptomatic tubers, whereas "3" represents severe tuber symptoms (Rashed et al. 2013). For Lso quantification, 100 mg of tuber tissue was removed at the stem end, from the close proximity of the previous sampling, using a 6-mm Harris UNI - CORE^{TM} (GE Healthcare Life Sciences, Buckinghamshire, UK). Samples were stored in -20°C for DNA extraction and Lso quantification. DNA extraction and quantification of Lso was performed as described in chapter 2.

The subset of tubers that was not sampled or sliced at harvest (intact) was used for categorizing the potato genotype with respect to their Lso titer and ZC severity. This was done because post-storage symptom severities may be somewhat difficult to distinguish due to the increase in phenolic contents and PPO activity on exposed tissue.

Statistical analysis

The data was analyzed with IBM SPSS Statistics ver. 24.0. A generalized linear mixed model (GLMM) with normal distribution was used to compare Lso titer among tubers samples at harvest and after storage. The sampling time was considered as the repeated measure, since some tubers were sampled twice. Prior to the analysis, titer values were log-transformed. The initial analysis included infestation time, year, genotype, time of sampling, and interaction terms, with block as a random factor.

Lso change during storage was also analyzed in tubers that were sampled both at harvest and after storage. First, a value of 1.5 was added to all Lso titers to make all titer values greater than zero. Then the change of Lso was calculated in each plant following the calculation

suggested by Rashed et al. (2018). The resulting value was used to analyze the change of Lso by GLMM. The analysis included infestation time, year, genotype, and interactions terms, with block as a random factor.

Relative susceptibility was evaluated in the tubers that were kept intact at the end of storage in the treatment 77 DBVR. The genotype A05379-211 was not considered in the analysis of intact tubers because it had an insufficient number of repetitions in 2017 (N=2). The model included Lso titer as the response variable and genotype, time of infestation, year and interaction terms as fixed factors and block as a random factor. The effect of ZC symptom severity was analyzed using the nonparametric Kruskal-Wallis test. Scores of ZC severity were compared among the time of infections and genotypes.

The number of ZC-affected tubers was compared between harvest and after storage by GLMM with a binomial distribution and a logit link function. Incidence was treated as binomial variable (0 = non-infected, 1 = Lso infected) was considered as the response variable. The time of evaluation (harvest and after storage) was considered as the repeated measure. The model included infestation time, year, genotype, time of sampling, and interactions terms, and block as a random factor.

Results

Change in "*Ca.* Liberibacter solanacearum" titer during storage

Lso titers were affected by sampling time and were significantly higher after storage when compared to pre-storage (harvest) (GLMM, $F_{1,831} = 18.270$; $P < 0.001$). Also, significant effects of infection time ($F_{2,831} = 736.7$; $P < 0.001$) and genotype ($F_{7,831} = 2.16$; $P = 0.035$) were detected. The titer of Lso after storage was significantly higher in tubers from plants infested

at 77 and 12 DBVR (*Ps* < 0.001) compared to the treatment 4 DBVR. However there is not significant difference in Lso titer between 77 and 12 DBVR infections treatments. Althrough the effect of genotype was significant in the model, non-genotype has shown a significant higher or lower Lso titer after storage. The shift in Lso titer was significantly higher in the treatment 12 DBVR ($F_{1,832}$ = 14.46; *P* < 0.001) and 4 DBVR ($F_{1,832}$ = 5.24 ; *P* = 0.022),), further analysis was focused on the effect of genotype on the change in Lso titer at 12 DBVR. This was because in this infestation time treatment significant differences in Lso titers were detected between before and after storage (*P* < 0.001). In the 12 DBVR treatment the change in Lso during storage was not affected by genotype ($F_{7,128}$ = 1.012; *P* = 0.426), year ($F_{1,128}$ = 3.850; *P* = 0.052), or year-genotype interaction ($F_{7,128}$ = 0.935; *P* = 0.482). Although the change of Lso was not influenced by genotype, changes in Lso titers during storage in A07781-4LB, A07781-10LB, A05379-211, and A08399-6LB were all less that that of the susceptible control, Russet Burbank (Table 3.2).

Change in the number of Lso-infected tubers during storage

Incidence of Lso in tubers changed during storage. The number of Lso-affected tubers was significantly higher after storage than that of harvest (GLMM, $F_{1, 837}$ = 9.165; *P* = 0.003) (Fig. 3.1-A). Pairwise analysis has shown that the number of Lso-infected tubers after storage compared to harvest was significantly higher in the treatment 4 DBVR (*P* < 0.001) (Fig. 3.1-B). Also, the incidence of Lso-affected tubers after harvest significantly affected by year ($F_{1, 837}$ = 37.798; *P* < 0.001) and the time of infestation ($F_{2, 837}$ = 109.464; *P* < 0.001) However, it was not affected by the potato genotype ($F_{7, 830}$ = 1.763; *P* = 0.091) and year-time of infestation

interaction ($P = 0.328$). Since year-time of infestation interaction was not significant, data form 2016 and 2017 were analyzed together.

Relative susceptibility after storage

The relative susceptibility after storage was also analyzed in intact tubers that were stored directly from harvest. Lso titer was only significantly affected by the time of infestation ($F_{2,388}= 228.037$, $P < 0.001$) and year ($F_{1,388}= 18.857$, $P < 0.001$). There were significant effects of genotype on ZC symptom expression (Kruskal-Wallis: Chi square = 51.336, $P < 0.001$, df =6) with the lowest ZC symptom expression being associated with the A07781 genotypes. The interaction between year and potato genotype was not significant for either Lso quantity or the expression of ZC symptoms. Thus, 2016 and 2017 were pooled and graphed in a single plot (Fig. 3.2). The A07781-3LB and A07781-4LB genotypes were categorized as relative resistant to Lso and tolerant to ZC symptoms whereas A07781-10LB was categorized as relative tolerant to ZC symptoms but susceptible to Lso. Russet Burbank, Western Russet, and A08399-6LB fell into the intolerant quadrant since even relatively low titer levels triggered severe expression of ZC symptoms. PALB03016-6 showed relatively higher symptom severity scores and Lso quantities. They were thus categorized as relatively intolerant and susceptible genotype.

Discussion

The results presented here showed that the titer of "*Ca*. L. solanacearum" in potato genotypes was higher in tubers after storage in both early- and late-season infected tubers. This effect suggested Lso development during storage. The change of Lso during storage was

determined to be the highest in tubers from plants which were infected two weeks before vine removal. The development of Lso post harvest was also reflected in the significantly higher number of Lso-infected tubers after storage compared to the ones at harvest. However, what exactly led to this observed variation in Lso changes among genotypes, and across time of infestation treatments, remained unclear. This study also categorized relative susceptibility to Lso and ZC symptom severities of the evalaued potato genotypes after storage, confirming that genotypes from the A07781 family remain tolerant to the expression of ZC symptoms and exhibit relativly low levels of susceptibility to Lso after storage.

The present study supports previous findings of Rashed et al. (2018) that determined Lso development continues during storage, and that it varies depending on the time of infestation in the field. In our study, the change in Lso titer varied among the three times of infestation treatments (77, 12, and 4 DBVR). The highest change in Lso titer was found in the 12 DBVR treatment. It may be possible that tubers infested at 12 DBVR were placed into storage with a low or in some cases undetectable level of the infection. Infection was present at harvest and it might developed during storage. Lso development showed considerable variability among genotypes. It is important to note that Lso titer in potato can vary with host genotype, the holding temperature, and the stage of storage (Wallis et al. 2017). Our study only quantified the change of Lso between harvest and the end of the storage. We are unable to specify whether the Lso increase is associated with a particular stage during the storage, e.g., curing versus cold storage (Fig. 3.3). Nonetheless, our results confirm that Lso and ZC can continue influencing harvested tubers during harvest. The change in Lso during the storage process was not significant in the 4 DBVR and 77 DBVR treatments. The load of Lso at 77 DBVR was the highest titer at harvest. Thus, the development of Lso during storage was very

low because the pathogen might have been reached already its maximum development at harvest. Conversely, the concentration of Lso in the 4DBVR tubers was mostly undetectable at harvest (low titer) with a low initial pathogen load thus the growth of Lso was likely to be slower.

The development of Lso during storage was also demonstrated by the increase in the incidence of Lso-infected tubers after storage. Although, the change of Lso in tubers was significant at 12 DBVR, the number of Lso-positive tubers was observed only significant in the tubers from 4 DBVR treatment. Potato plants are susceptible to Lso infection throughout the growing season. Although tubers from late-infection can test negative for Lso at harvest, Lso can continue its multiplication during storage (Rush et al. 2015). In plants exposed to infected psyllids late in the season, Lso can reach tubers in four days (Rashed et al. 2018).

After storage, reltive susceptibility and ZC symptom expression of the three genotypes belonging to the A07781 family followed a similar pattern as that observed at harvest. In both harvest and after storage: A07781-10LB was categorized as tolerant, whereas A07781-4LB and A07781-3LB were categorized as relatively resistant genotypes at harvest in both 2016 and 2017. The level of expression of ZC symptoms in response to Lso infection in symptomatic tubers is known to be associated with changes in tuber biochemistry (Wallis et al. 2012), and such responses vary among potato genotypes (Wallis et al. 2014) and with different cold storage temperatures (Wallis et al. 2017). Lso may continue to develop and affect infected tubers post-harvest, and perhaps tuber physiology was not altered in the same direction because ZC symptoms in A07781 siblings remained lower than other the other genotypes in this study. The accumulation of phenolics is part of the tuber response to Lso infection and the levels of phenolics increases with the severity of ZC symptom (Wallis et al. 2012, Rashed et al. 2013).

Moreover, tolerance to ZC symptoms may be the result of minor changes in the physiology of Lso-infected tubers (Wallis et al. 2015), thus the mechanism driving tolerance in A07781 genotypes may be associated with a restriction of increasing of phenolics in fresh tubers, especially in A07781-4LB (resistant genotype) which even had a few tubers from the 77 DBVR treatment that remained asymptomatic at harvest and after storage as well.

In conclusion, these results suggest that Lso continues to multiply and affect tubers during storage, regardless of the host genotype. Most of the observed changes were associated with infestations that occured 12 days before vine-removal and not those of 77 or 4 days before vine-removal. The analysis of relative susceptibility after storage confirmed that the three examined genotypes from the A07781 family are relatively more tolerant, or resistant, to ZC compared to other examined genotypes. This conclusion held for greenhouse, field and storage evaluations.

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Tables

Table 3.1. Change in "*Ca*. Liberibacter solanacearum" titer between harvest and after storage in relation to time of infestation

	Average of "*Ca*. Liberibacter solanacearum" titer change		
Year	**77 DBVR**	**12 DBVR**	**4 DBVR**
2016	24.9 ± 12.7	7691.2 ± 7484.7 *	375.4± 282.0
2017	3.1 ± 2.9	44525.0± 39339.1*	45.5± 41.7

DBVR = days before vine-removal
* $P < 0.05$. Data reported are average ± SE

Table 3.2. Change in "*Ca*. Liberibacter solanacearum" titer between harvest and after storage at 12 DBVR

Genotype	**Average of "*Ca*. Liberibacter solanacearum" titer change**
Russet Burbank	15.3 ± 14.0
A07781-4LB	7.0 ± 6.3
A07781-3LB	54.0 ± 36.0
A07781-10LB	9.0 ± 15851
A08399-6LB	2.3 ± 2.0
PALB03016-6	54.1 ± 36.0
A05379-211	0.1 ± 0.06
Western Russet	51.3 ± 51

DBVR = days before vine-removal
* $P < 0.05$. Data reported are average ± SE; the values represented on the table are divided by 1000 for better observation

Figures

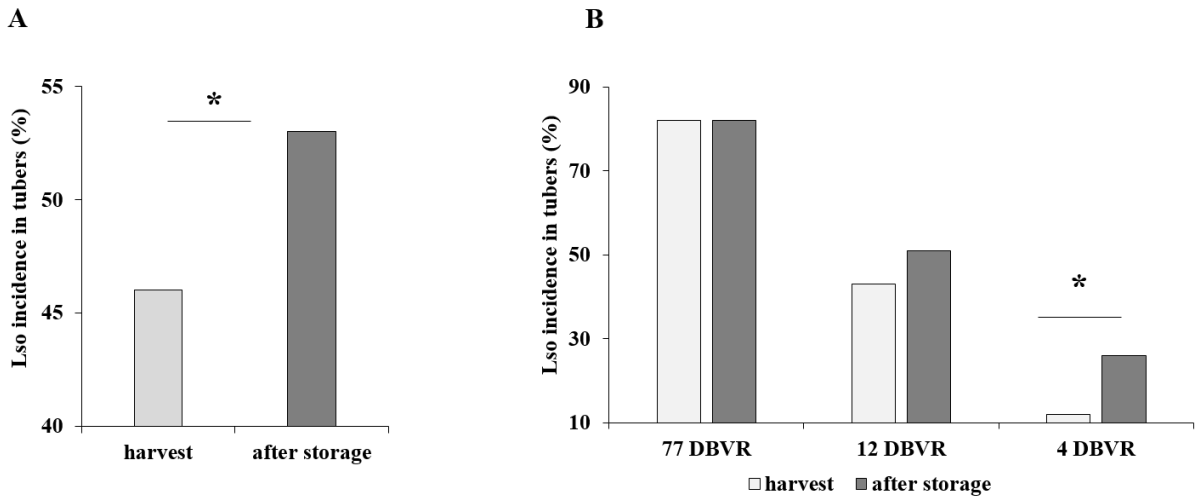

Figure 3.1. Lso incidence of tubers infected with "*Ca*. Liberibacter solanacearum". Plants were infested at 77, 12 and 4 days before vine-removal (DBVR) from season 2016 and 2017. (A) Percentage of tubers at harvest and after storage. (B) Percentage of tubers at harvest and after storage by time of infection. Russet Burbank: susceptible control. Significant values are indicated by asterisk ($P < 0.0$

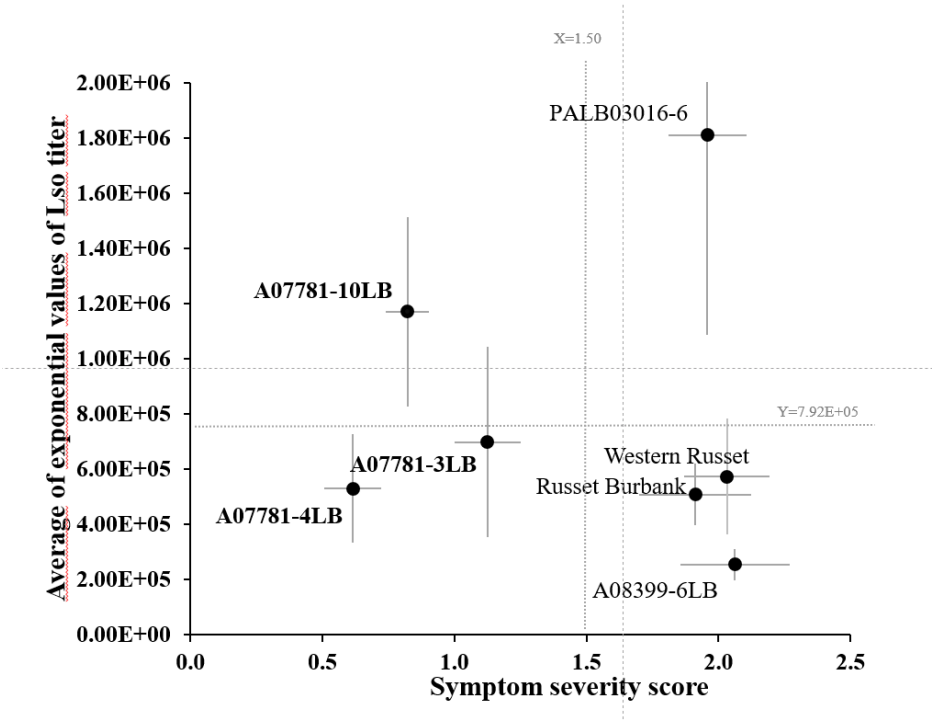

Figure 3.2. Relative susceptibility in potato genotypes after storage. Tubers infected 77 days before vine-removal (DBVR) from season 2016 and 2017. Lso quantity and ZC symptom severity were analyzed after storage in intact-evaluated tubers. Russet Burbank: susceptible control. Error bars represent standard error (+/- 1).

Chapter 4: Effect of cold temperatures on the mortality of *Bactericera cockerelli*

Abstract

Some vector-borne plant pathogens are known to affect the fitness of their vectors. Pathogens may also alter the vector physiology and their response to high or low temperatures. This can affect overwintering survival and thus vector ability to spread pathogens across seasons. *Bactericera cockerelli* Šulc (Hemiptera: Triozidae) is the vector of the bacterium "*Candidatus* Liberibacter solanacearum" (Lso), the pathogen that causes zebra chip (ZC) disease in potato. Lso has a negative effect on its psyllid vector, reducing its fecundity and survival of nymphs. Four populations of *B. cockerelli* have been described in the United States: Central, Western, Northwestern and Southwestern haplotypes. It is known that the potato psyllid can survive cold winters on native wild solanaceous species. Among the four psyllid genotypes, the Northwestern haplotype is the dominant haplotype reported to overwinter on *Solanum dulcamara* and *Lycium* spp. It is possible that the presence of Lso affects the metabolic processes required for overwintering of Lso-infected psyllids, which also may vary among haplotypes. The present study was conducted to examine survival of different potato psyllid genotypes, in the presence and absence of Lso, when exposed to subfreezing temperatures. Lso significantly increased the survival of the potato psyllid in cold temperatures. The Northwestern haplotype had relatively lower mortality rate in cold temperatures compared to other haplotypes. Thus, cold temperatures may favor overwintering Northwestern psyllids that are Lso-positive. Our results demonstrated that Lso may promote its vector's ability to acclimate to cold temperatures. Respiration analysis indicated that Lso

presence significantly increased the respiration rate of the potato psyllid vector; however, this effect does not seem to impact their overwintering ability negatively.

Introduction

Insects have two known strategies to survive at low temperatures: freeze tolerance or freezing avoidance (Bale 2002, Clark and Worland 2008). Both processes are assisted mainly by the synthesis of antifreeze proteins (AFPs) and the accumulation of carbohydrate cryoprotectants (e.g., glycerol), which are triggered by low temperatures (Bale 2002, Storey and Storey 2012). Freezing conditions stimulate a strong metabolic rate repression in insects (Storey and Storey 2012), and consequently a slow catabolism of stored body-fuel reserves (Bale 2002). The response of insects to cold temperatures has been associated with variations in gene expression (Qin et al. 2005) and the duration of exposure to cold temperatures (Zhang et al. 2011). Most cold-hardiness studies to date have been focused on Lepidoptera, Diptera and to lesser extent to Hemiptera and Hymenoptera (Andrew et al. 2013). For insect vectors, successful adaptation to local temperatures regimes may increase the transmission and persistance of plant pathogens in the environment (Kreb et al. 2017, Sternberg & Thomas 2014). Thus, vector cold hardiness has direct epidemiological implications (Phillips et al. 2000, Van Emden and Harrington 2007).

Although zebra chip disease has been impacting US potato production since 2000, there is still several questions about the ability of the potato psyllid *Bactericera cockerelli* (Hem., Triozidae) to tolerate cold temperatures. The interest to study in deep the tolerance of psyllids to cold temperatures has received more attention, until recently. This was because the research community was under the assumption that the infective psyllids simply migrate from

warmer regions of Mexico and southern US on annual basis (Munyaneza et al. 2009, Horton et al. 2015). The discovery of overwintering populations of the potato psyllids in the PNW (Murphy et al. 2013, Swisher et al. 2013b, Horton et al. 2016 and Thinakaran et al. 2017, Wenninger et al. 2019) and description of four genotypes of the vector, with two overwintering in the region triggered researchers' interest to revisit some of the theories on the potato psyllid movement and migration (Horton et al. 2016, Kaur et al. 2018, Cooper et al. 2019).

To date, four haplotypes of the potato psyllid, *B. cockerelli* (Hansen et al. 2008, Liefting et al. 2009) have been described in the United States: Central, Western, Northwestern and Southwestern (Swisher et al. 2012, 2013a, 2014). The Northwestern and Western haplotypes (mostly the northwestern haplotype) have been reported to overwinter on *S. dulcamara* and *Lycium* spp. in the region (Swisher et al. 2013b, Thinakaran et al. 2017). Recent studies have reported that psyllids from the western and northwestern haplotypes can also develop on other wild species (Cooper et al. 2019). In the absence of potato host, the adult potato psyllids are reported to survive and overwinter on native solanaceous species such as bittersweet nightshade (*Solanum dulcamara*), matrimony vine (*Lycium* spp., *Lycium barbarum*, and *L. chinense*) (Munyaneza et al. 2012, Murphy et al. 2014, Horton et. al 2016, Thinakaran et al. 2017) in the region (PNW). The majority of the surveys indicated that most field infections by Lso are associated with the western haplotype of the potato psyllids (Swisher et al. 2014, Dahan et al. 2017).

Previous studies have also shown the potato psyllids are able to survive cold winters (Murphy et al. 2013, Horton et al. 2014), with reports of nymphs and adults surviving in -15 ºC and -10 ºC, respectively, for 24 hours under laboratory conditions (Henne et al. 2010). The

physiological mechanisms involved in winter survival of potato psyllids are still unclear. Some studies have proposed that the potato psyllid may be able to survive through winter by entering a quiescence state (Horton et al. 2015). In the PNW, the Northwestern haplotype is the predominant psyllid population, along with the Western haplotype overwinters on *S. dulcamara* (Swisher et al. 2013a, Horton et al. 2015), *L. barbarum* and *L. chinense* (Thinakaran et al. 2017). This suggests that the Northwestern and Western populations are able to develop on native wild solanaceous species for overwintering.

The interaction between a pathogen and its vector can alter an insect's physiology in many different ways, including disrupting cellular metabolism, changing gene expression, and/or decreasing of fecundity of the vector (Nachappa et al. 2012, Yao et al. 2016, Frias et al. 2018, Nachappa et al. 2014, Killiny et al. 2016, Tu et al. 2013, Purcell et al. 1982, Stumpf & Kennedy 2007). The potato psyllid-Lso interaction is known to be negative for the potato psyllid: Lso infection causes reduction of fecundity in the potato psyllid (Nachappa et al. 2012, Nachappa et al. 2014). Moreover, Lso induces transcriptomic changes in the potato psyllid; most of those changes are observed in genes related to metabolism and to some extent those with stress- and immune-related genes (Nachappa et al. 2012, Yao et al. 2016). Lso induces the expression of the high level of genes in psyllids, indicates that Lso may impact psyllid physiology in important ways. All these changes could potentially impact the overwintering success of the potato psyllids. The survivorship of vectors carrying their pathogens under extreme temperatures has not yet been widely studied. Some studies in viral pathosystems have shown that mortality of vectors in extreme temperatures can be influenced by infection status (Pusag et al. 2012, Xu et al. 2016). For instance, *Bemisia tabaci*, vector of *Tomato yellow leaf curl virus* (TYLCV), suffers relatively higher mortality at low (4 ºC) and at high

(35 ºC) temperatures when it is TYLCV-infected (Pusag et al. 2013). Conversely, the *Southern rice black-streaked draft virus* (SRBSDV) when present in its vector, the white-backed planthopper *Sogatella furcifera,* confers an increase of survivorship under high temperatures (36 ºC) but lower survivorship in cold temperatures (5 ºC) (Xu et al. 2016). These studies demonstrated that pathogens can alter vector physiology to acclimate to hot or cold temperatures. In addition, the response of the potato psyllid to cold temperatures may involve expression of genes. The gene expression in insect vectors may vary with the temperature (Qin et al. 2005) and the time of exposure to those temperatures (Zhang et al. 2011). For example, in *Drosophila melanogaster*, the upregulating of 20 and 69 genes is observed in a time of exposure to -0.5 ºC, for 2 hours and 10 hours, respectively (Zhang et al. 2011). In insect vectors, it has been proposed that the pathogen can induce up or down regulation of expression of certain genes that may be associated with tolerance of thermal stress (Pusag et al. 2012, Xu et al. 2016).

The association between the potato psyllid and its alternative hosts during winter allow them to survive until they move into the potato crop in the next field season (Horton et al. 2014, 2015, and 2016). A better understanding of how Lso infection impacts potato psyllid survivorship in cold temperatures will provide further insight into the ecological effects of Lso on its vector, including how Lso is introduced at the beginning of field seasons. The objective of the present study is to evaluate the effect of cold temperatures on potato psyllid mortality in relation to their Lso status, host plant and the insect haplotype, and examine if Lso infection alters potato psyllid respiration rates.

Materials and Methods

This study was conducted in Eastern Idaho Entomology Laboratory at the University of Idaho, Aberdeen Research and Extension Center, Aberdeen, and Kimberly Research and Extension Center, Kimberly, ID from January 2018 to April 2019. Two experiments were implemented, one of them to examinate the effect of "*Ca.* Liberibacter solanacearum" on the respiration rate of *B. cockerelli,* and a second experiment evaluated the effect of cold temperatures on the mortality of *B. cockerelli.*

Plant and insect material

Bittersweet nightshade (*Solanum dulcamara*) or potato plants (*Solanum tuberosum* L., variety "Russet Burbank") were used to rear *B. cockerelli*. Bittersweet nightshade was collected from Aberdeen Research and Extension, Aberdeen, ID (N 42°58.395 W 112°49.070). Plants were propagated via cuttings and seeds; seeds were grown in seedling trays. Cuttings and plants emerged from seeds were planted in 4-inch plastic pots containing a mixture of 70% sand, 20% peatmoss (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada), 10% vermiculite (Therm-o-Rock West INC., Chandler, AZ, USA) and fertilizer (Osmocote; Scott-Sierra Horticultural Products Co., Marysville, OH, USA). Bittersweet nightshade and potato plants were maintained in a greenhouse with temperatures ranging between 16 (night) and 23 (day) ºC, on a 12:12h photoperiod (L: D). *B. cockerelli* used in these experiments came from Lso-infected and healthy colonies from each of the Central, Western and Northwestern haplotypes and reared on potato or bittersweet nightshade plants (Table 4.1). Colonies were maintained inside 60 x 60 x 60-cm tent-shaped Bugdorm cages

(BioQuip Products, Rancho Dominguez, CA) in the greenhouse. Due to space limitations, colonies from bittersweet nightshade plants were maintained in 41 x 16 x 17 cm rearing plexiglass cages. Potato and bittersweet nightshade colonies were maintained in temperatures ranging between 18 and 27 °C, and a 16:8 photoperiod (L: D). The infection status of psyllids was verified by PCR, according to Crosslin et al. (2011). The haplotype of potato psyllids was confirmed according to Swisher and Crosslin (2014). Lso-infected psyllids used for these studies were from colonies infected with Lso haplotype B (Wen at al. 2013).

Measuring respiration rate of *Bactericera cockerelli* infected and non-infected with *Ca*. Liberibacter solanacearum and comparisons among haplotypes

Experimental design

The experiment was conducted in the Eastern Idaho Entomology Laboratory at the University of Idaho, Aberdeen Research and Extension Center, Aberdeen, ID. The experiment was performed to determinate, first, if *Ca*. Liberibacter solanacearum affects the respiration rate of *B. cockerelli,* and second, if the potential effect of Lso on psyllid respiration varies across genotypes. The experiment was performed in the laboratory and was replicated at least 8 times for each treatment. The respiration rate was evaluated in Lso-infected and healthy colonies of *B. cockerelli* from each of the Central, Western, and Northwestern haplotypes which were reared on potato plants (var. Russet Burbank).

Respiration rate was measured as volume of CO_2 produced in 60 minutes at 27°C in Lso-infected and healthy potato psyllids across haplotypes. Individuals were collected inside of a 50 ml tube and were immobilized by placing them on ice for at least 10 minutes. Five

male, or female, inscets from each colony were placed inside of one previously prepared "respirometer." Respirometers were constructed by hand following the procedure presented in Yatsenko et al. (2014). A 1000 µl pipette tip was cut to fit the insertion of 20 µl capillary micropipette (Kimble^{TM} Disposable microcapillary pipets; Fisher Scientific, catalog number 13-678-18B) through the tip. Then silicon glue was applied around the area of tube insertion to seal the connection. Hereafter, this constructed unit will be refered to as a "chamber". After the glue dried overnight, a small piece of foam was placed inside the chamber. Then, 0.17 g soda lime (LI-COR, NE) (CO_2 absorbent) was added. A second piece of foam was placed inside the chamber and pressed on top of the soda lime to secure the stones. Soon after, ten live potato psyllids were placed inside the chamber, which was then sealed with plasticine putty. One respirometer was sealed without psyllids added (test control) to account for the room CO_2, in later analyses. The test control and sealed respirometers containing live psyllids were placed veritically inside a Pyrex glass rectangular chromatography tank (27.0 cm × 26.5 cm × 7.0 cm; Sigma-Aldrich) using a plastic rack. The tip of the microcapillary tubes for each chamber was immersed into a colored manometric solution, which was a mixture of water and food dye in a ratio that results in visible colorization (~1:1). The dye density was similar to the water density, which facilitated fluid movement in the capillary tube. The tank was then covered by a glass lid and sealed with petroleum jelly product to provide isolation of temperature and pressure. The volume of CO_2 produced (V_{CO2}) was measured based on the displacement of the colored solution in the glass capillary attached to each respirometer. The respiration activity of insects produced a change in CO_2 volume between the initial time and the final time of the test.

Data collection

A photograph of the tank with respirometer chambers inside was taken at the initial and the final stages of the test, using a Nikon D-5300 camera (Nikon Inc., Melville, NY), facing at a fixed 60-cm of distance of the tank (Fig. 4.1). The camera was mounted on a tripod at the fixed height of 95-cm. The start of the assay was 15 minutes after the lid of chamber was closed ('time 0'). This 15 minute interval allowed the system to equalize prior to the study. The duration of each assay was 1 hour, after which a second photo was taken ('time 1'). The tank was marked with two measuring scales in centimeters placed on each side of the tank facing the camera. Photos taken at 'time 0' and 'time 1' were analyzed in Image J software (version 1.52a. National Institutes of Health, USA). Pixel scaling in the software was set by using the scale from each picture. The displacement of the colored solution (h) in the glass capillary attached to each respirometer was measured and used for calculation of the volume of CO_2 (V_{CO2}) produced in each respirometer was used as final data. The respiration activity of insects produced a change in V_{CO2} between the initial time and the final time of the test. The V_{CO2} was calculated by the following formula: $\pi r^2 h$; where r = radius of capillary micropipette, h = distance the colored solution has moved up in the capillary micropipette, and $\pi = 3.1416$ (constant value). The V_{CO2} produced by psyllids respiration was calculated by the difference of the V_{CO2} observed between the initial time and the final time of the test. Respiration rate was calculated by the following formula: $(V_{CO2})/(N)x(T)$; where, V_{CO2} = the volume of CO_2 produced by 10 psyllids less the volume produced by the test control (non-psyllids added), N = number of individuals used (10 individuals) and T = time of the assay (60 minutes). Each picture was analyzed in Image J software two times and the average of the two measurements was reported as the final measurement.

Effect of temperature on mortality of *Bactericera cockerelli* populations

Experimental design

This experiment was conducted at the University of Idaho, Kimberly Research and Extension Center, Kimberly, ID. The experiment was repeated four times (time-blocks). The *B. cockerelli* used for this experiment came from Lso-infected and healthy colonies from each of the Central, Western and Northwestern haplotype and reared on potato or bittersweet nightshade hosts. The experiment was set up with 5 individuals (male or female) per treatment. One treatment comprised each combination of the four factors: host (potato or bittersweet nightshade), psyllid haplotype (Central, Northwestern, or Western), Lso status (Lso-positive or Lso-negative), and incubation temperature (-4, 0, 4 and 20 C). Psyllids were placed in petri dishes modified to provide insects with adequate ventilation and humidity. The plastic petri dishes (VWR^{TM} International, disposable petri dishes) were 60 x 15 mm in size. Ventilation was provided through a 2.5-cm (in diameter) aperture on the lid which was sealed with a piece of mesh to prevent insects from escaping. Humidity was provided by a small piece of wet foam placed in each petri dish by an aperture of 8 mm, in diameter; the piece of foam, and was kept wet during the experiment. Petri dishes were placed inside growth chambers on dark conditions which were previously set at constant temperatures of -4, 0, 4 or 20 ºC.

There were three petri dishes per treatment (15 psyllids) and insect mortality was evaluated and recorded in each petri dish at 3, 7 or 10 days (a nondestructive approach). To record mortality, petri dishes containing psyllids were removed from the growth chamber and placed at room temperature for 10 minutes before evaluations.

Statistical analysis

The respiration rate of the potato psyllid was analyzed by a generalized linear mixed model (GLMM), with normal distribution; non-significant factors and interaction terms were removed from the analysis. Lso status and haplotype were maintained in the model as fixed factors and respiration rate as a response variable. Time-block was included as the random factor.

The effect of temperature on potato psyllid mortality was analyzed independently at 3, 7 or 10 days of exposure to different temperatures. Mortality (%) was included as the response variable; temperature, host plant, insect haplotype and Lso status were considered as fixed factors. Time-block was considered a random factor. All data were analyzed in IBM SPSS Statistics ver. 24.0.

Results

The Lso presence increases the respiration rate of its potato psyllid vector

Respiration rate (measured as the volume of CO_2 produced) of the potato psyllid vectors was not influenced by either psyllid haplotype (GLMM, $F_{2,96}$= 1.441; P = 0.242) or haplotype-by-Lso status interaction (GLMM, $F_{2,96}$ = 0.440; P = 0.646) (Fig. 4.2). but it was significantly affected by the presence of Lso (GLMM, $F_{1,96}$ = 6.092; P = 0.015), with increased respiration rates being associated with the Lso-positive potato psyllids (Fig. 4.3).

Effect of temperature on mortality of *Bactericera cockerelli* populations

The mortality of psyllids was only significantly influenced by temperature (GLMM, $F_{3,164}$= 112.58; P < 0.001) only within the first 3 days of exposure (Fig. 4.4).

In the 7th-day evaluation, the mortality of the potato psyllid was affected by the rearing host plant ($F_{1,164}$= 5.21; P = 0.024), with lower rates of mortality recorded from psyllids which were reared on bittersweet nightshade (30%) compared to those which were maintained on the potato host (42%) (P = 0.02). Psyllid mortality was also affected by its Lso status ($F_{1,164}$ = 16.541; P < 0.001), temperature ($F_{3,164}$= 77.10; P = 0.001), haplotype-by-Lso status interaction ($F_{2,164}$= 3.38; P = 0.036) and temperature-by-Lso status interaction ($F_{3,164}$= 4.86; P = 0.003). A significant reduction of mortality was detected in Lso-infected psyllids exposed to -4 º C ($F_{1,176}$= 25.387; P < 0.001) and 0 º C ($F_{1,176}$= 8.045; P = 0.005) compared to non-infected psyllids (Fig. 4.5).

Similar to evaluation conducted on day 7, on day 10, mortalilty was also significantly affected by the Lso status of the psyllids ($F_{1,163}$= 7.149; P = 0.008), temperature ($F_{3,163}$= 49.802; P < 0.001), and host-by-haplotype interaction ($F_{2,163}$= 3.235; P = 0.042) (Fig. 4.6). Psyllid mortality was observed to be significantly lower in psyllids from the Northwestern haplotype reared on bittersweet nighshade ($F_{1,163}$= 8.650; P = 0.004) compared to the ones reared on potato. The analysis of the Lso-by-temperature interaction across haplotypes at the 10th day indicated that Lso-infected potato psyllids from the Northwestern haplotype had a significantly lower mortality when they were exposed at 0 º C ($F_{1,157}$= 6.387; P = 0.012) (Fig. 4.8).

Discussion

Our results showed that Lso infection significantly increases the respiration rate of the potato psyllid, but the mortality of the potato psyllid in cold temperatures was significantly reduced by Lso presence in the insect vector. Lso-infected psyllids showed reduced mortality when they were exposed to freezing and subfreezing temperatures. Reduced mortality was

also reported for the potato psyllids reared on bittersweet nightshade. The overall, overwintering survival of the potato psyllids did not appear to be influenced by the vector haplotype. It was however, the Lso-infected Northwestern haplotype which showed superior survival after 7 and 10 days of exposure to freezing temperatures. Indicating the possibility that the Lso-positive psyllids from the Northwestern haplotype may have an overwintering advantage compared to other genotypes. However most of psyllids collected from potato fields between 2012 to 2015 in the PNW belonged to the Western haplotype (Swisher at el. 2014, Dahan et al. 2017). It is posible that the Northwestern haplotype is more adapted to wild species host and micro environments than crop conditions relative to the other haplotypes (e.g., Western). Although the Northwestern haplotype is not abundant in potato fields, this population can have importance as a source of Lso inoculum because psyllids carrying Lso can survive through the winter or they can spread Lso to several wild host species. The Northwestern and Western haplotypes are able to develop on a wide range of wild species aside from solanaceous plants (Cooper et al. 2019). Following winter, psyllids populations may acquire Lso from wild species hosts before arriving in potato fields. In addition, the previous acquisition of Lso would change the preference of potato psyllids for healthy plants (Mas et al. 2014) which is beneficial for the pathogen spread and also for the vector since healthy plants would provide psyllids with higher quality of nutrients.

The acquisition of the pathogen by its vector can result in a shortened lifespan (Killiny et al. 2017), an increased mortality under thermal stress conditions (Pusag et al. 2012), or reduction in fecundity (e.g., Lso) of the vector (Nachappa et al. 2012, Nachappa et al. 2014, Yao et al. 2016, Frias et al. 2018). Our results showed that Lso also increases the respiration of the potato psyllid. The alteration of metabolic functions of vectors by pathogen has been

reported in bacterium and phytoplasmas as well (e.g., "*Candidatus* Phytoplasma asteris") (Galetto et al. 2011, Killiny et al. 2017).

Metabolics functions take place within each cell to provide energy for physiological processes of a living organism (e.g., respiration). Killiny et al. (2017) studied the alteration of cellular metabolism of *Diaphorina citri,* vector of "*Candidatus* Liberibacter asiaticus" (CLas). CLas would induce the overproduction of energy-producing molecules (e.g., ATP production) but repress the consumption of those molecules (e.g., reduction of ATPase activity) by the vector. CLas can supply its need for these molecules by induction of changes in the metabolism of its vector (Killiny et al. 2017).

CLas is a closely related to Lso; their genomes possess several similarities at the molecular level. For instance, both CLsa and Lso encode molecules needed for synthetizing ATP and also facilitating its uptake from extracellular sources (Lin et al. 2011). If Lso, like CLsa, can induce the production of ATP in the potato psyllid to supply energy demand of Lso, the potato psyllid would be forced to obtain energy (e.g., ATP) from degradation of fuel molecules [Glucose + O_2 → CO_2 + H_2O]. Consequently, an increase of degradation of fuel molecules might result in an increase of the respiration rate. Moreover, bacterial pathogens may induce the upregulation of citric acid cycle and glycolysis enzymes in their insect vectors; the upregulation of those enzymes would accelerate the degradation of reserves and induce the consumption of more O_2 by the vector (Ramsey et al. 2015).

As the temperature decreases, the metabolic rate of insects is reduced and mechanisms involved on thermal tolerance are activated (Bale 2002, Qin et al. 2005). Under cold temperatures, insects supress their metabolim which produces saving of energy because digestion is ceased and there is reduction of demand for the ATP produced in mitochondria

(Bale 2002, Storey and Storey 2012). Thus, it would be possible that the production of oxygen-based ATP production is reduced in the potato psyllid.

In insects, glucose reserves (e.g., glycogen) are reduced significatly under freezing conditions (Stoney et al. 1993) and anti-freezing proteins (AFPs) are synthetized (Bale 2002). Consequently, energy stress would be reduced in freezing and subfreezing temperatures since the demand of ATP is reduced and reserves might be allocated to synthesis of AFPs (Storey and Storey 2012). The reduced mortality of Lso-infected potato psyllids in comparison to non-infected psyllids would suggest that Lso can indeed induce some additional changes in the physiology of its vector to enhance its survivorship in winter. More studies are required to elucitate the mechanisms behind the Lso-regulated vector survivability under cold temperature stress.

Insect vector haplotypes may have different thermal susceptibility. Mahaday et al. (2009) had found that the percentage of mortality of *B. tabacci* from Q biotype was twice mortality rate of *B. tabacci* from B biotype when they were exposed to at 37 ºC. Although we did not find significant differences in mortality due to the psyllid's haplotype, we found that Lso-infected psyllids from some haplotypes (e.g., Northwestern) were likely to have reduced mortality in cold temperatures. Thus, the susceptibility of populations of the potato psyllid freezing and subfreezing temperatures can vary. Consequently, haplotypes with low susceptibility to cold temperatures may survive longer in winter.

In our study, we found that Lso-infected psyllids reared on *S. dulcamara* had lower mortality after 7 days of exposure, and to some extent after 10 days of exposure, to freezing and subfreezing temperatures. We also found that the Northwestern haplotype of the Lso-positive potato psyllids had higher rates of survival compared to other genotypes, after 10

days of exposure to 0 ºC. Conversely, most of psyllids overwintering in the PNW in wild solanaceous species have not tested positive for Lso (Swisher et al. 2013). Thus, more studies are need to analyze the overwintering of potato psyllids on other wild species. A recent study conducted under controlled conditions has shown that the potato psyllids from Northwestern and Western haplotype are able to develop succesfully on a wide range of wild species and also they can acquire Lso from some of those species (Cooper et al. 2019). Therefore, it is important to investigate whether potato psyllids can overwinter on other wild species of host plants.

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Figures

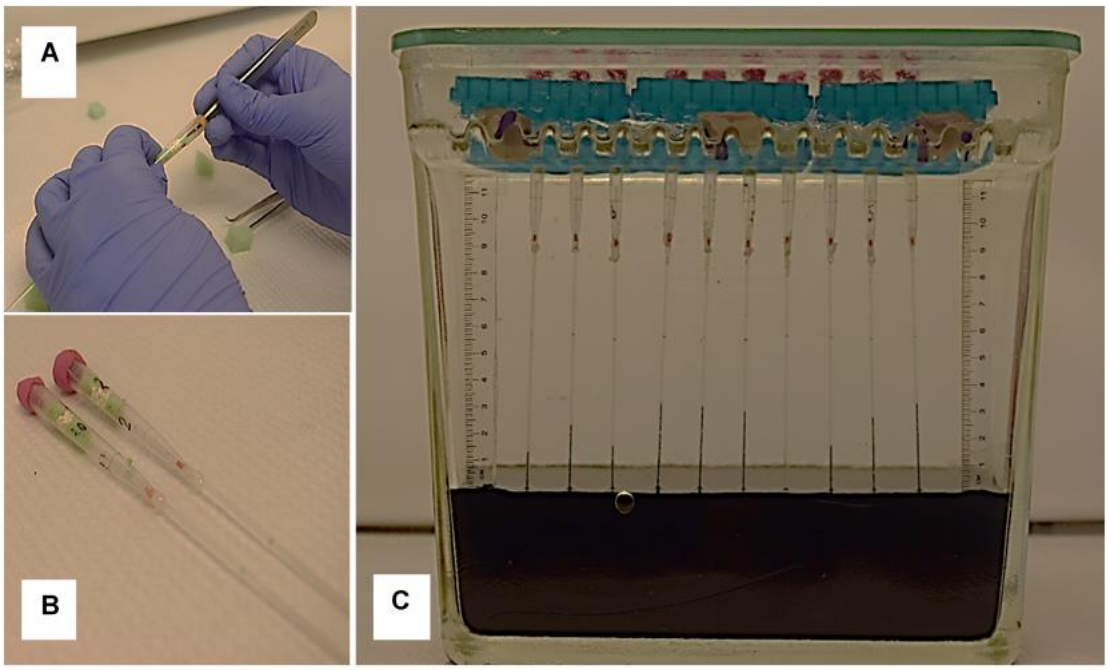

Figure 4.1. Measurement of Respiration rate of the potato psyllid. Preparation of respirometers (A); respirometers containing live psyllids (B) are placed in the chamber to run the experiment (C).

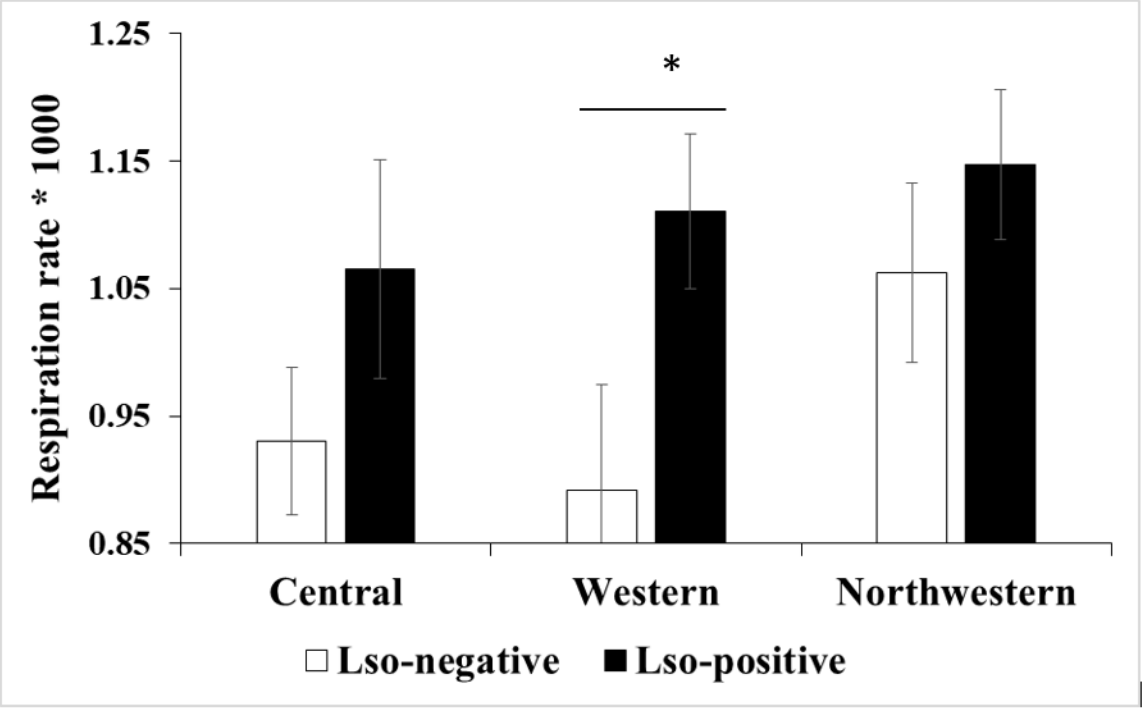

Figure 4.2. Respiration rates measured in potato psyllids of Central, Western and Northwestern haplotypes. Significant respiration rate is indicated by asterisk ($P < 0.05$). Error bars represent standard error (S.E).

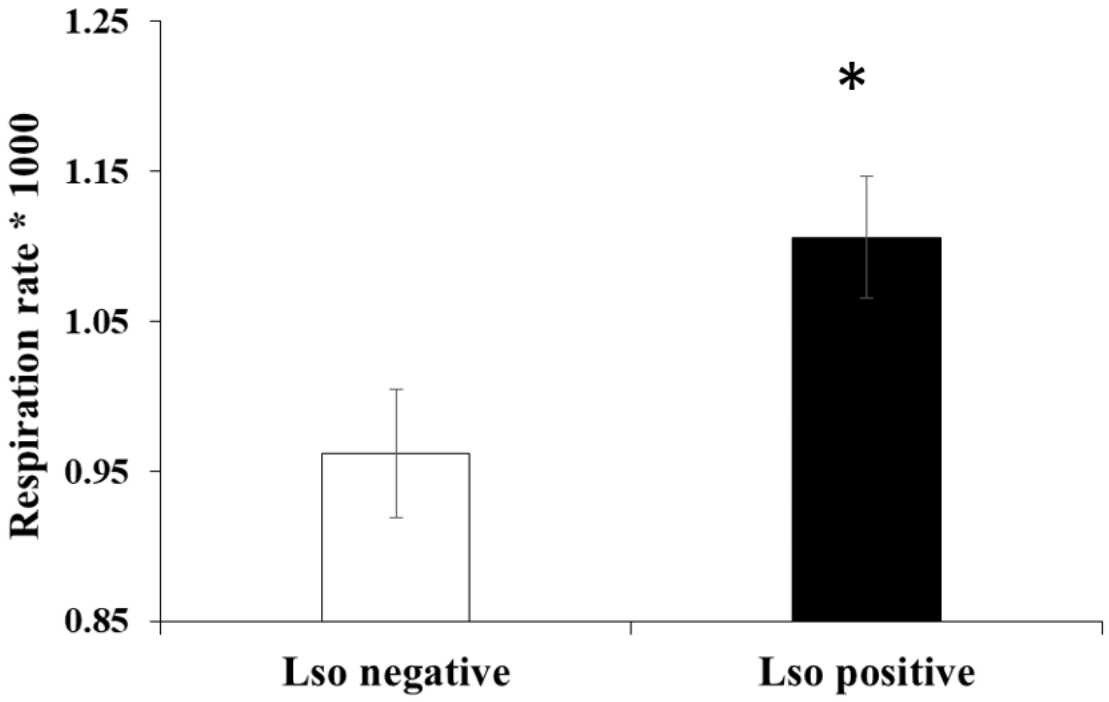

Figure 4.3. Respiration rate was significantly affected by Lso status (Lso negative/positive). Significant respiration rate is indicated by asterisk (P<0.05). Error bars represent standard error (S.E).

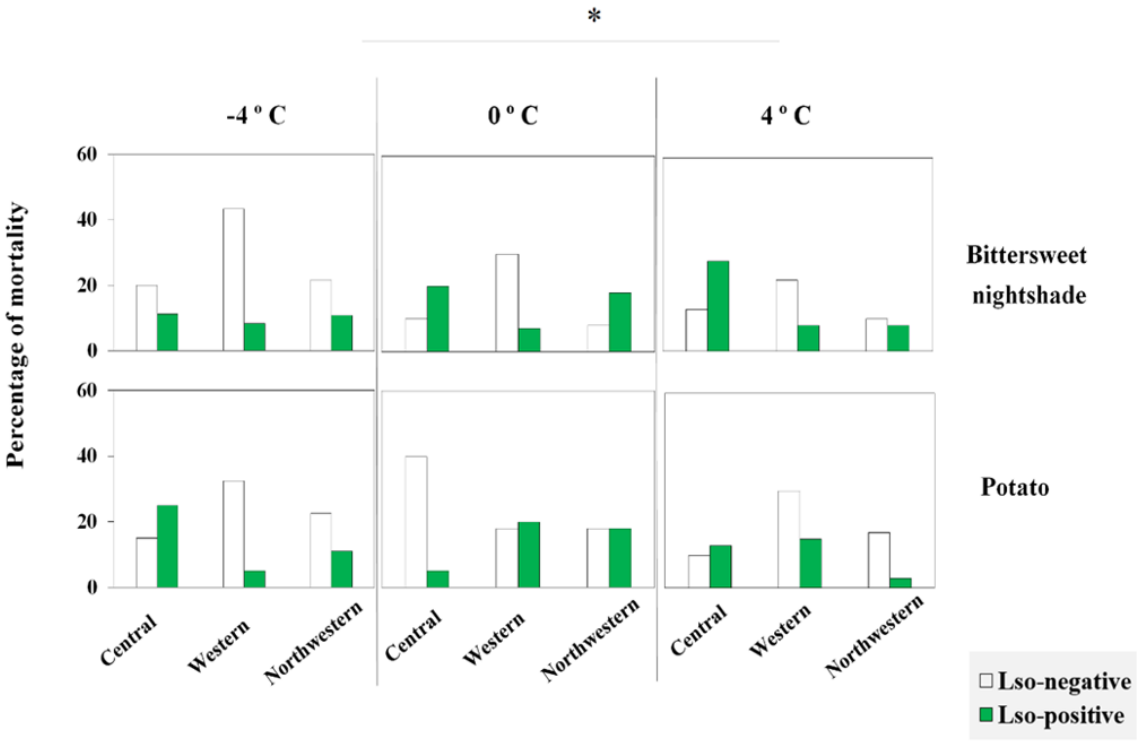

Figure 4.4. Mortality of *B. cockerelli* at 3rd days of exposition to -4, 0 and 4 ºC. Lso-negative and positive psyllids from Central, Western and Northwestern haplotypes were reared on bittersweet nightshade or potato. Significant percentage of mortality is indicated by asterisk ($P < 0.05$).

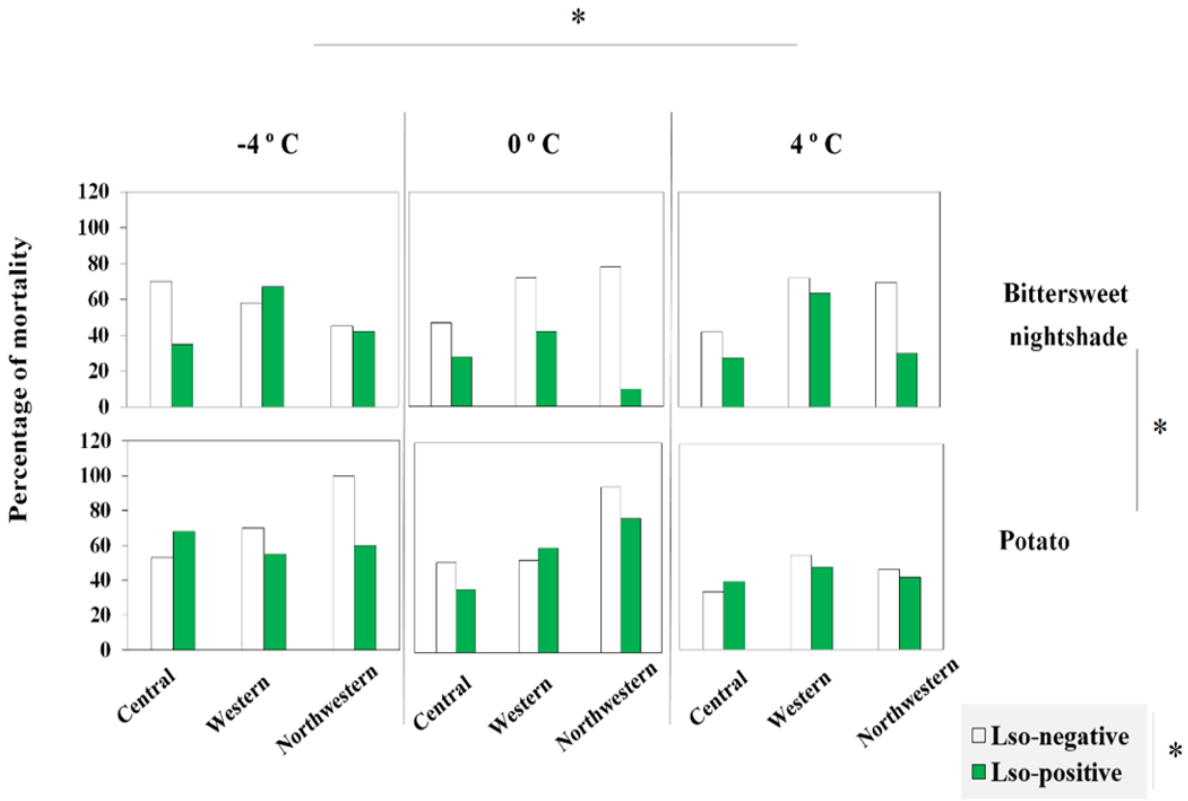

Figure 4.5. Mortality of *B. cockerelli* at 7th days of exposure to -4, 0 and 4 ºC. Lso-negative and positive psyllids from Central, Western and Northwestern haplotypes were reared on bittersweet nightshade or potato. Significant percentage of mortality is indicated by asterisk ($P < 0.05$).

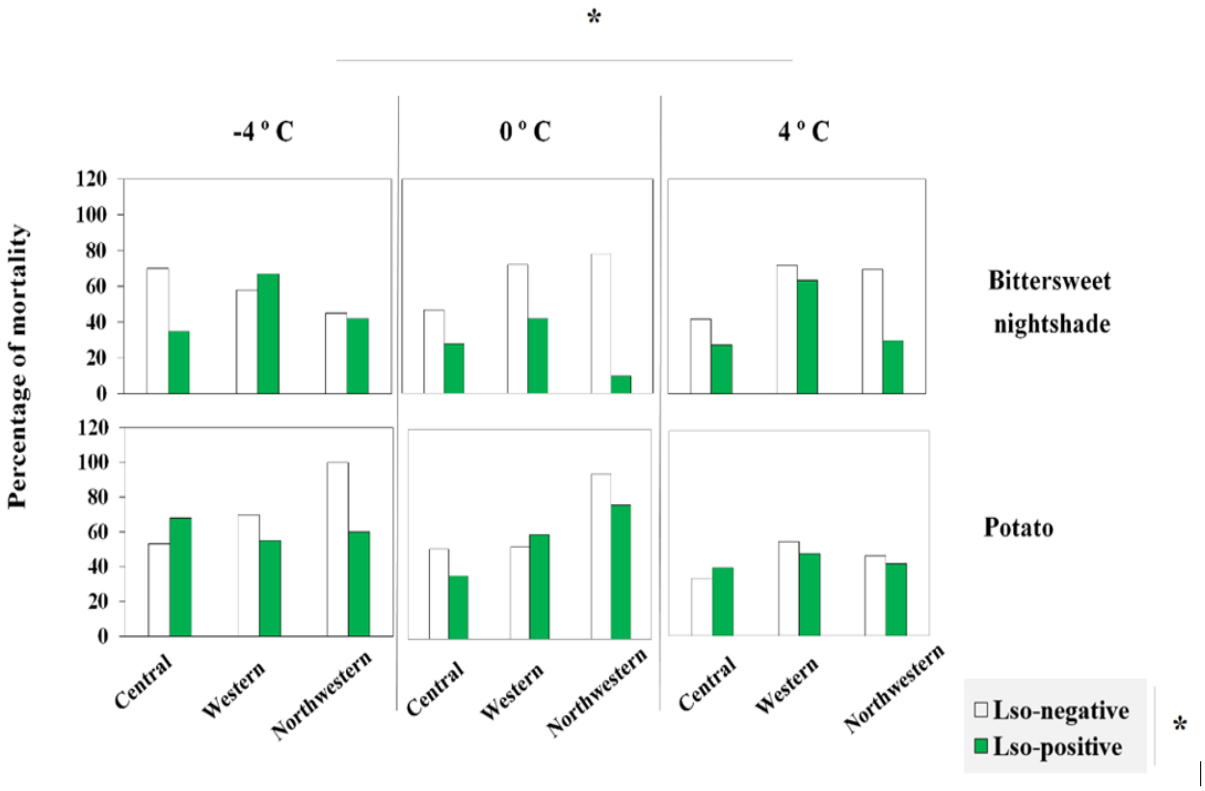

Figure 4.6. Mortality of *B. cockerelli* at 10th days of exposure to -4, 0 and 4 ºC. Lso-negative and positive psyllids from Central, Western and Northwestern haplotypes were reared on bittersweet nightshade or potato. Significant percentage of mortality is indicated by asterisk ($P < 0.05$).

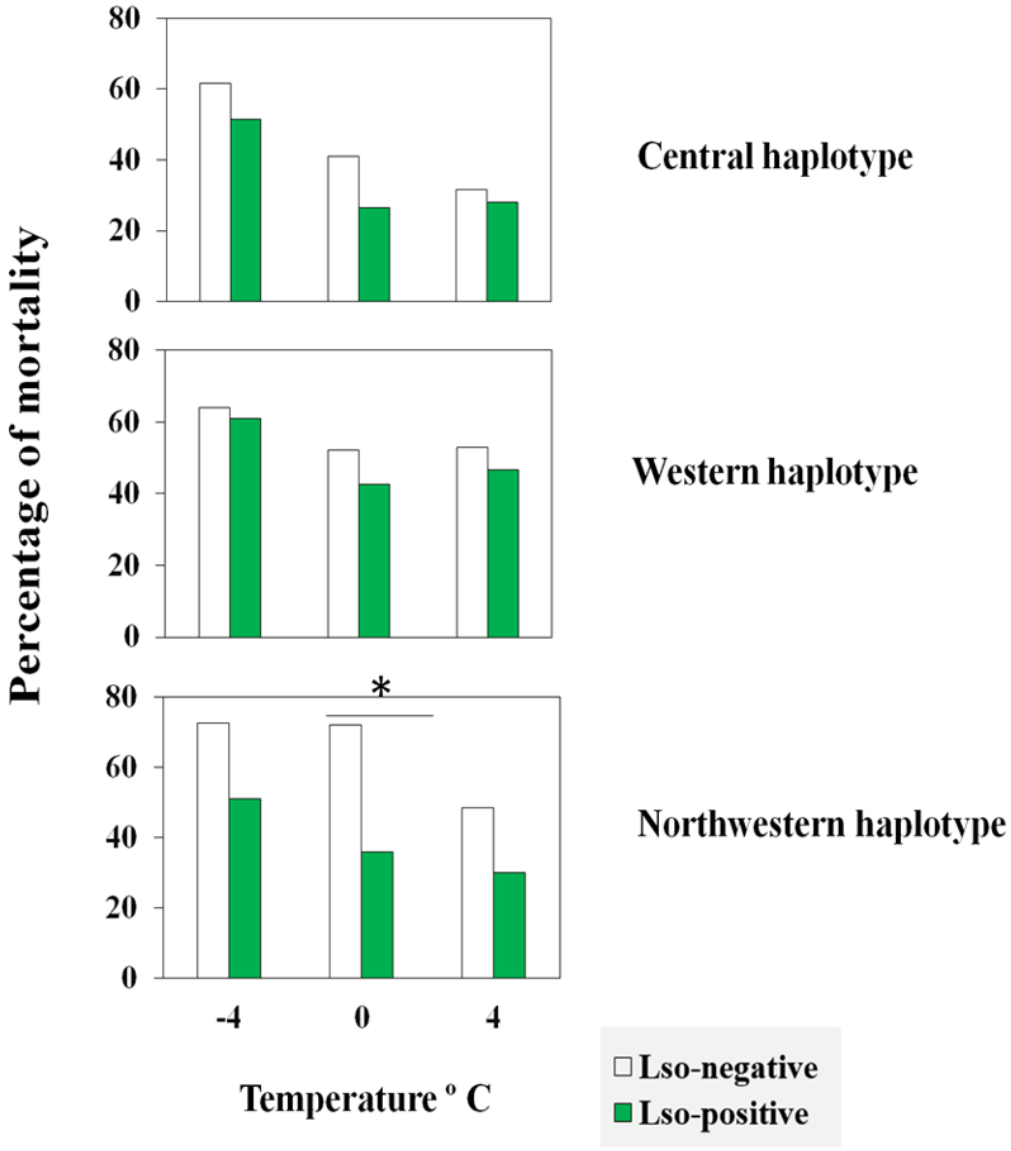

Figure 4.7. Mortality of Lso-negative and positive *B. cockerelli* from Central, Western and Northwestern haplotypes at 10th days of exposure to -4, 0 and 4 ºC. Significant percentage of mortality is indicated by asterisk ($P < 0.05$)

Chapter 5: Plant-mediated effect of *Potato virus Y* on zebra chip pathosystem

Abstract

The interaction between a viral pathogen and its host can induce changes in the host, which could affect the development of a subsequent pathogen or its vector. *Potato virus Y* (PVY) and zebra chip (ZC) pathosystems can coexist within fields. In the Pacific Northwest, they both represent threats to the potato industry productivity. PVY is currently the most important issue in seed potato production, which can be spread via both seed pieces and aphid vectors. ZC is associated with the bacterium "*Candidatus* Liberibacter solanacearum" (Lso) that is transmitted by the potato psyllid, *Bactericera cockerelli* Šulc (Hemiptera: Triozidae). ZC reduces potato yield and makes tubers unmarketable. In Idaho, PVY and ZC are likely to establish in potato fields. We evaluated the impact of PVY infection on Lso inoculation success, the development of Lso, and on the development of Lso-infected and healthy potato psyllids on tomato plants. Our results showed that Lso transmission success and Lso titer were not significantly affected by the presence of PVY. The oviposition of the Lso-positive psyllids was significantly reduced on PVY-infected plants whereas the oviposition of non-infected psyllids was not significantly influenced by the presence of PVY. The hatch rates of Lso-positive and Lso-negative psyllids was not influenced by the PVY status (positive or negative) of the host plant. Our study demonstrated that pre-existing PVY infection negatively impacts Lso vectors by reducing their oviposition. This suggests that the presence of PVY may result in a decrease in the number of infected psyllids, and thus, subsequent Lso spread. Alternatively, the

movement of infected females psyllids onto non-PVY infected plants may increase the Lso dispersal; behavior studies and also field studies are needed to confirm these possibilities.

Introduction

Under natural circumstances, more than one pathosystem can impact the same crop host, raising the possibility of interations between components of the co-occurring pathosystems. Vector-borne pathogens can manipulate their vectors directly upon acquisition (Ingwell et al. 2012, Stafford el at. 2011, Rajabaskar et al. 2014) or indirectly by changing the host plant phenotype (Eigenbrode et al. 2002, Jiménez-Martínez et al. 2004). One of the mechanisms associated with those plant-mediated effects is the induction of volatiles compounds (VOCs) blends by the host plant that attract the vectors (Eigenbrode et al. 2002, Jiménez-Martínez et al. 2004, Mauck et al. 2010). Another mechanism observed is the suppression of essential defense transcripts in host plants, which favors the development of the pathogen vector (Casteel et al. 2012, Casteel et al. 2015, Su et al. 2015, and Bak et al. 2017). Both mechanisms increase the likelihood of pathogen acquisition by the vector and facilitate its spread (Bosque-Pérez and Eigenbrode 2011). If pathogens induce changes in host plants to manipulate vector biology and/or behavior, they may also impact interspecific interactions with other existing pathosystem (e.g., pathogen- and vector- host plant interactions). For example, the first-arriving pathogen can reduce the development of a co-infecting pathogen by manipulating (e.g., virus-virus interaction) (Anandalakshmi et al. 1998, Cuellar el at. 2008, Tollenaere et al. 2017, Karyeija et al. 2000, Murphy and Bowen 2006) or evading (e.g., virus-bacteria interaction) plant defenses. Shapiro et al. (2013), has shown that infection of cucumber with a viral and bacterial pathogen resulted in a significant increase of Salicylic acid (SA) production compared to the single

bacterial infection. Likewise, pathosystems interaction can negatively impact the development and behavior of vectors from other pathogens (Shapiro et al. 2013, Prager et al. 2015, and Chen et al. 2018). Plant-mediated changes induced by a first arriving pathogen can affect another vector by reducing host plant attratciveness (Prager et al. 2015, Shapiro et al. 2013), vector oviposition rate, and/or pathogen acquisition success (Chen et al. 2018). Therefore, pathogens can not only affect the development of other pathogens, but they also might restrict the transmission of those pathogens by impacting their vectors negatively. In this chapter, we examined the effect of *Potato virus Y* (PVY, genus Potyvirus) infection on the bacterium "*Candidatus* Liberibacter solanacearum" development and the potato psyllid, *Bactericera cockerelli* oviposition. In the Pacific Northwest, both PVY and Zebra Chip (ZC) impact tuber yield and quality of potato. PVY is considered the most important viral pathogen of potatoes because it significantly affects seed potato industry (Gray et al. 2010). PVY is spread by both infected-seed pieces and aphid vectors. Several species of aphids can transmit PVY in a non-persistent manner (Shrestha et al. 2014). ZC is also considered a threat to potato production because ZC-infected tubers are unmarketable due to the characteristic tissue discoloration of fresh and fried products (Miles et al. 2010, Munyaneza et al. 2012).

Lso infection is shown to reduce transcription of metabolites involved in SA synthesis (Casteel et al. 2012). Lso may manipulate the host plant by suppressing its defenses and supporting the development and colonization of the potato psyllid vector on its host plant (Casteel et al. 2012). However, the presence of PVY may interfere with and disrupt such plant-vector interactions, as it has been observed in other pathosystems (Prager et al. 2015, Shapiro et al. 2013, and Chen et al. 2018). For example, cucumber plants previously inoculated with the *Zucchini yellow mosaic virus* (ZYMV) showed reduced attractiveness to *Acalumma vittattum*,

vector of the pathogenic bacteria *Erwinia tracheiphlia*. This response resulted in a reduction in the incidence of the bacterial disease in plants inoculated with ZYMV (Shapiro et al. 2012, Shapiro et al. 2013). In PVY-Lso interaction, pre-existing PVY infection may not only affect the development of a subsequent Lso infection but also may have an indirect effect on the potato psyllid behavior since other viral infections have been shown to have a negative impact on this insect vector. However, little is known about the potential effects of this virus on Lso and/or its potato psyllid vector. The present study aims to quantify the impact of PVY infection on Lso transmission success and evaluate the impact of PVY on the oviposition and development of the potato psyllid. A clear understanding of PVY-Lso interaction has direct implications in the epidemiology of ZC and PVY.

Materials and Methods

The present study was conducted in the Eastern Idaho Entomology Laboratory at the University of Idaho, Aberdeen Research and Extension Center, Aberdeen, ID from January 2018- April 2019.

Experimental model system

All of the experiments were conducted on tomato plant var. "Yellow Pear" (*Solanum lycopersicum esculentum* L.; W. Atlee Burpee & Co. Warminster, PA). *Potato virus Y*, strain 'O' (PVY^{o}) was used for virus inoculations. Haplotype B of Lso was used as the bacterial pathogen, and the central haplotype of the potato psyllid (*Bactericera cockerelli* Sulc), vector of Lso-haplotype B. Tomato was selected over other hosts (e.g., potato) because tomato is easier

to manage under greenhouse conditions. Tomato is also a suitable host for the potato psyllid and susceptible to the infection by Lso and PVY.

The haplotype of potato psyllids was confirmed by PCR and followed of a restriction analysis which is described by Swisher and Crosslin (2014). The PVY strain was confirmed by a multiplex PCR analysis specified by Chikh-Ali et al. (2010, 2013) and Lorenzen et al. (2006).

Plant and insect material

Tomato plants were grown from seed in seedling trays, which were later transplanted into 4-inch plastic pots. Plants were grown in a soil mix, containing 70% sand, 20% peat moss (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada), 10% vermiculite (Therm-o-Rock West INC., Chandler, AZ, USA) and fertilizer (Osmocote; Scott-Sierra Horticultural Products Co., Marysville, OH, USA). *Bactericera cockerelli* used for these experiments came from colonies maintained in the Aberdeen Research and Extension Center, Aberdeen, ID. Lso-infected and healthy potato psyllids were reared on potato (*Solanum tuberosum* L., variety "Russet Burbank") plants. Colonies were maintained in growth chambers inside 60 x 60 x 60-cm tent-shaped Bugdorm cages (BioQuip Products, Rancho Dominguez, CA) with temperatures ranging between 18 and 27 °C, and a 16:8 photoperiod (L:D). The infection status of psyllids was verified by PCR, according to Crosslin et al. (2011). The potato psyllids were confirmed to be of the central haplotype (Swisher and Crosslin 2014). Lso-infected psyllids used for these studies were from colonies infected with Lso haplotype B (Wen at al. 2013). The PVY^{o} used in these experiments was obtained from an infected tobacco (*Nicotiana tabacum* L.) source plant maintained in the greenhouse at Aberdeen Research and Extension Center, Aberdeen, ID. The tobacco plants were maintained on inside 75 x 75 x 115-cm tent shaped

BugDorm 2400F insect rearing tent with a 160 μm mesh size (BugDorm Products, Taiwan) under controlled greenhouse conditions; temperatures between 16 ºC at night and 23 ºC during the day with a photoperiod of 12:8; L:D. Tomato plants used as virus source were tested for their PVY status using ImmunoStrip® (Agdia. Elkhart, IN) before each inoculation.

PVY inoculation

PVY inoculations were performed in plants of 12 cm height. The inoculum was prepared by grinding the source plant (tobacco) leaf tissue in 0.1M phosphate buffer (2:1), pH 7.5. Carborundum 400 grit (Beta Diamond Products, INC., CA, US) was applied on at least five fully expanded leaflets of each of the experimental tomato plants. One ml of sap was rubbed on the surface of each of five leaflets of each plant using a cotton ball (Mondal et al. 2016). Mock-inoculated plants were treated by mechanical inoculation with buffer solution and the abrasive product but no virus. Following inoculation, tomato plants were maintained in the greenhouse, inside large zip-front mesh cages (BioQuip 36" jumbo cages, Rancho Dominguez, CA). Three weeks after PVY inoculation, plants were used for experiments. PVY- and mock-inoculated plants were maintained separately during the whole study under controlled conditions mentioned above. PVY status was confirmed by PVY ImmunoStrip® (Agdia. Elkhart, IN) at the end of each experiment. Only PVY- and mock-inoculated plants tested as positive and negative for PVY, respectively were considered for the final data analysis. PVY-inoculated plants tested as positive for PVY are referred to as PVY-positive, whereas mock-inoculated plants tested negative for PVY are referred as PVY-negative.

Effect of PVY on transmission success of *Ca.* Liberibacter solanacearum

An experiment was set up to determinate whether infection with PVY affects the transmission of Lso, or Lso titers. Lso transmission success and Lso titer were compared between PVY-positive (*n* = 39) and PVY-negative tomato plants (*n* = 29), following exposure to four *B. cockerelli* from a Lso-positive colony. Transmission assays were conducted on tomato plants three weeks after mechanical inoculation with PVY (and uninfected controls). Two frame-less clip cages (BioQuip Products, Rancho Dominguez, CA) with inside dimension of 1-inch were installed on two fully developed leaflets. Two infective psyllids were placed inside each leaf cage. Plants were exposed to the infective psyllids for 48 hours of inoculation access period (IAP). After IAP, psyllids and eggs were removed without damaging the leaflet, and plants were maintained for three weeks in the greenhouse. The collected psyllids were stored in -20ºC for later Lso quantification. Following the three-week period, 100 mg of petiole tissue was collected from a fully developed young leaf (~ upper third leaf), and Lso titers in both plants and psyllids were evaluated by real-time PCR (qPCR). At that time, tomato plants were tested for PVY to confirm their PVY status. Lso titer and Lso incidence were compared between PVY-infected and uninfected control plants. The experiment was conducted in three time-blocks, with a minimum of 8 replicates per treatment in each time-block.

The effect of PVY on *Bactericera cockerelli* oviposition and hatch rate

A no-choice experimental assays were set up to evaluate the impact of PVY infection on the oviposition and the hatch rate of *B. cockerelli*. The experiment was conducted in two time-blocks. A minimum of 10 replicates per treatment was included in each time-block. PVY-infected tomato plants were used 3 weeks after inoculation with the virus. PVY-positive (*n* =

43) and PVY-negative plants (*n* = 30) were exposed to four Lso-infected psyllids (two males, two females) for 48 hours of IAP/oviposition period by placing two infective psyllids inside each of the two leaf cages/plant (1 male and 1 female per leaf cage). Plants has reached were around 40-cm after IAP. These experiments were conducted similarly to the transmission assays described above. However, following the 48-hrs exposure period, psyllids were collected, clip cage was removed and eggs were counted. Plants were maintained in 27 °C, with a 16:8 photoperiod (L:D) and inspected one and two weeks after exposure to the psyllids to count the number of nymphs hatched from the eggs. Hatch rate was calculated by dividing number of nymphs per plant divided by the total number of eggs per plant, multiplied by 100. Three weeks after exposure to the potato psyllids, 100 mg of petiole tissue was collected from a fully developed young leaf (~ upper third leaf) and Lso status was verified by qPCR. At that time, the plant PVY status was also confirmed. The number of eggs and hatch rate were compared between PVY-positive and PVY-negative plants. Using a similar approach, a no choice experiment with PVY-positive (*n* = 24) and PVY-negative plants (*n* = 19) exposed to Lso-negative potato psyllids was also conducted in two time-blocks.

Lso quantification

Plant tissue samples and the psyllids were stored in -20°C for DNA extraction and Lso quantification. Total DNA from petiole and psyllids samples was extracted using the CTAB method, following Buchman et al. (2011) and Marzachi et al. (1998), respectively. Quality and quantity of DNA were verified by nanodrop measurement (NanoDrop Lite, Spectrophotometer, Thermo Scientific, Madison, WI). Lso in both plant tissue and the psyllid was quantified by qPCR SYBRgreen using a CFX Real-Time PCR System (BIORAD). The qPCR reaction (10

ul) contained primers 150 nM or 100 nM of HLBr and LsoF (Li et al. 2006, Li et al. 2009) for psyllids and petiole tissue, respectively; 1X SsoAdvanced Universal SYBR Green Supermix (BIORAD), and 1 ul of DNA template. The amplification program was set as follows: one cycle at 98˚C for 2 minutes, 40 cycles of 95˚C for 10 seconds, and 62˚C for 20 seconds, followed by a melt curve (65˚C to 95˚C, increment $0.5^{\circ}Cs^{-1}$). Every qPCR reaction included a negative control (DNA from healthy plants) and water control (no template control). Lso copy number was calculated according to Levy et al. (2011).

Statistical analysis

Data were analyzed with IBM SPSS Statistics ver. 24.0. To evaluate the effect of PVY on Lso transmission, the Lso status (0 = negative, 1 = positive) was considered as the response variable. Data were analyzed by a generalized linear mixed model (GLMM) with a binomial distribution and a logit link function. Initially, all factors and interaction terms were included as fixed factors and block as random factor. Non-significant terms were removed, and Lso status was compared between PVY-positive and PVY-negative plants. The Lso titer was not normally distributed and it was transformed prior to analysis. Lso titer was compared between PVY-positive and -negative plants using a GLMM with a log-normal distribution.

The number of eggs was compared between PVY-positive and -negative plants using the non-parametric Mann-Whitney U test. Hatch rate (%) was normally distributed, and data were analyzed using a GLMM with a normal distribution and an identity link function. To compare hatch rate (%) between PVY-positive and negative plants, a statistical model with PVY status as a fixed factor and time-block as a random was used.

Results

Effect of PVY on transmission success of *Ca.* Liberibacter solanacearum

Lso transmission success by the potato psyllids was not influenced by PVY status of the tomato plants (GLMM, $F_{1,65} = 0.961$; $P = 0.331$; Fig. 5.1). Similarly, Lso titer (GLMM, $F_{1,66} = 0.052$; $P = 0.821$) trended to be higher in PVY-infected plants (Fig. 5.2). The Lso titer in PVY-positive plants was 1.3 times higher than that of the PVY-negative plants. The Lso transmission success was 52% in PVY-negative plants and 44% in PVY-positive plants. Although there was no significant effect of PVY on transmission success data showed the same trend in all the three blocks. The incidence of Lso in each group of PVY-negative and PVY-positive plants were 60% and 37% (block 1), 55% and 52% (block 2), and 36% and 9% (block 3), respectively.

The effect of PVY on the oviposition and the hatch rate of *Bactericera cockerelli*

Oviposition of Lso-infected psyllids was significantly reduced on the PVY-infected host (Mann–Whitney, $U = 418.5$, $n_1 = 30$, $n_2 = 43$, $P = 0.011$) (Fig. 5.3). However, the hatch rate over one week (GLMM, $F_{1,63} = 0.022$; $P = 0.883$) was not influenced by the PVY presence (Fig. 5.4). For Los-negative potato psyllids, oviposition (Mann–Whitney, $U = 245.5$, $n_1 = 24$, $n_2 = 19$, $P = 0.668$) and the hatch rate over one week (GLMM, $F_{1,34} = 0.000$; $P = 0.987$) were not influenced by the host plant's PVY status (Fig. 5.6, 5.7).

Discussion

Plants serve as hosts for multiple pathogens and herbivores. Thus, where multiple pathosystems are present, there may be interactions among systems—especially those sharing a component of the two disease triangles, such as a host plant. Our results have demonstrated that PVY can impact the ZC pathosystem by affecting the oviposition of the potato psyllid vector of Lso. However, PVY infection did not appear to have a detectable effect on Liberibacter development.

The inoculation success of Lso was not significantly affected by PVY presence, which suggests that plant-mediated defense mechanisms activated by PVY (the first arriving pathogen) may not interfere with the inoculation of Lso. Although there was not a significant effect of PVY on Lso transmission, it is still possible that the Lso incidence tends to be higher in PVY-negative plants. This possibility was reflected in the observed consistent pattern across time-blocks.. Additional replicates may help to detect statistical difference between treatments.

After successful inoculation of a pathogen into the host plant tissue by its vector, the pathogen multiplies within the host. Plant defense responses triggered by the pathogen invasion can potentially influence the development of a subsequent arriving pathogen (Karyeija et al. 2000, Tollenaere et al. 2016, Vance 1991, Voinnet 2005). For example, it has been shown that viral infections may reduce Lso titers (Prager et al. 2015). Since PVY and Lso would interact directly in the phloem tissue, the colonization of PVY may potentially affect the development of Lso through both direct and indirect interactions. However, in contrast, we observed that the multiplication of Lso in tomato plants was not significantly affected with the presence of PVY. It is known that the accumulation of PVY is hindered by the expression of SA at the early stages of infection (Nie, 2005), but once the virus reaches the plant vascular system, it can spread

systemically regardless of levels of SA in the plant (Baebler et al 2014). PVY may have a mechanism to evade SA-mediated suppression of its replication and movement (Nie, 2005). The SA induced by PVY may affects Lso at the initial stages of infection or inoculation. However after a suscessful inoculation of Lso, SA would be not an issue for Lso development since it has been shown that Lso may induce reduction in the transcription of metabolites involved in SA synthesis (Casteel et. 2012). Thus, over time, Lso may be able to suppress plant defenses related to SA and develop successfully.

Albeit non-significant, Lso titers tended to be consistently higher in plants that were infected with PVY relative to those that were PVY-free. Although it would be somewhat speculative to discuss this observed trend, I feel that it is important to consider this observation in future studies. This is because Lso titers show high spatiotemporal variablity within host plants (Levy et al. 2011, Cooper et al. 2015), which usually results in large standard deviations. This extreme variability would mask any potential diferences among treatments. Relatively high Lso titer in PVY-positive plants could result from the bacterial pathogen competing with the viral pathogen on a molecular level; thus with the multiplication of PVY would indirectly increase Lso titer, similar to what has been observed in another viral-bacterial pathosystem that shares the same host species. In the interaction between *Rice yellow mottle virus* (RYMV) and *Xanthomonas oryzae* pathovar *oryzicola* (*Xoc*) in rice, the RYMV-*Xoc* coinfection leads to a decrease in viral titer and an increase of bacterial titer. It was proposed that *Xoc* potentially switch down key enzymes involved in RYMV-mediated silencing suppression which may cause a reactivation of the defense system against the viral pathogen and consequently declining of RYMV multiplication (Tollenaere et al. 2017).

We have investigated the impact of PVY infection on oviposition and the hatch rate of Lso-infected and healthy potato psyllids. The potato psyllid haplotype (Prager et al. 2014a, Mustafa et al. 2015), host genotype (Prager et al. 2014a, Liu and Trumble 2004), Lso status of the host plant (Davis et al. 2012), and possibily Lso status of the psyllid (Mas et al. 2014) are some of the known variables that can influence the number of eggs laid by the potato psyllid vectors. These changes may be linked to the response of potato psyllids to visual and/or olfactory cues (Diaz-Montano and Trumble 2013), such as described for other species of psyllids (Demirel and Cranshaw 2006, Wenninger et al. 2009). The interaction between the ZC and a viral pathosystem can also alter the performance of the vector of Lso by induction of plant-mediated effects. Our results showed that the presence of PVY significantly reduced oviposition of the infective psyllids, but it did not affect the insect hatch rate. Conversely, PVY had no significant effect on oviposition of healthy psyllids in tomato plants. The reduction of the number of eggs laid by Lso-infected psyllids on PVY-positive plants in our study does support findings from previous studies in terms of oviposition. Prager et al. (2015) found that although the number of eggs laid by infective potato psyllids did not differ significantly between TMV-negative plants and TMV-positive plants, oviposition of the infected potato psyllids was twice as high on healthy plants when compared to infected plants. This suggests that viral infections (e.g., TMV) may reduce the oviposition of Lso-infected psyllids. Our results also demonstrated that the indirect effect of a viral infection such as PVY can result in different responses from Lso-positive and Lso-negative psyllids.

Further investigation is needed to evaluate the effect of Lso presence on PVY development. It has been reported that Lso can suppress transcripts involved in SA synthesis

(Casteel el at. 2012) and that the initial replication of PVY can be inhibited by SA (Baebler et al. 2011). Thus, Lso may facilitate the infection and spread of PVY in the host plant.

The observed adverse effect of PVY on the potato psyllid oviposition suggests that the virus presence could result in reduced secondary spread of Lso by reducing vector numbers. Another possibility would be that infected females' psyllids moves to non-PVY infected plants for oviposition. This would increase the population of Lso-infected psyllids and the Lso dispersal. However, this has yet to be confirmed in a field setup. We also need to expand the current study to evaluate whether the presence of PVY infection can influence the acquisition of Lso by psyllids (as a component of the transmission process). It is also essential to investigate whether the behavior of Lso-negative and –positive potato psyllids are differentially affected in the presence of PVY infection. For example whether the preference of healthy psyllids for Lso-infected plants (Mas et al. 2014) changes in PVY-Lso coinfected plants. Finally, various strains of PVY might affect vector behavior and Lso differently. Future studies are needed to test this possibility. Such information is required to provide a thorough understanding of the impact of PVY on ZC epidemiology.

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Figures

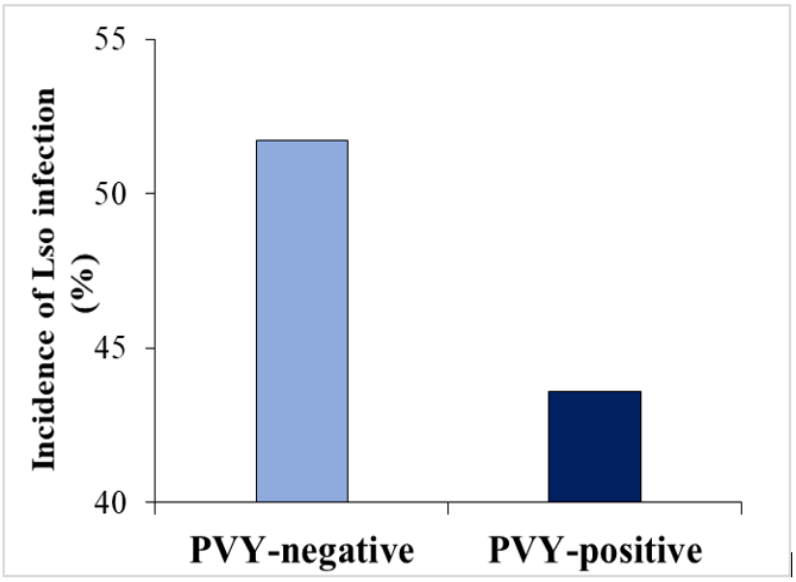

Figure 5.1. "*Ca.* Liberibacter solanacearum" transmission success measured as incidence in PVY-negative and PVY-positive tomato plants infected with "*Ca.* Liberibacter solanacearum".

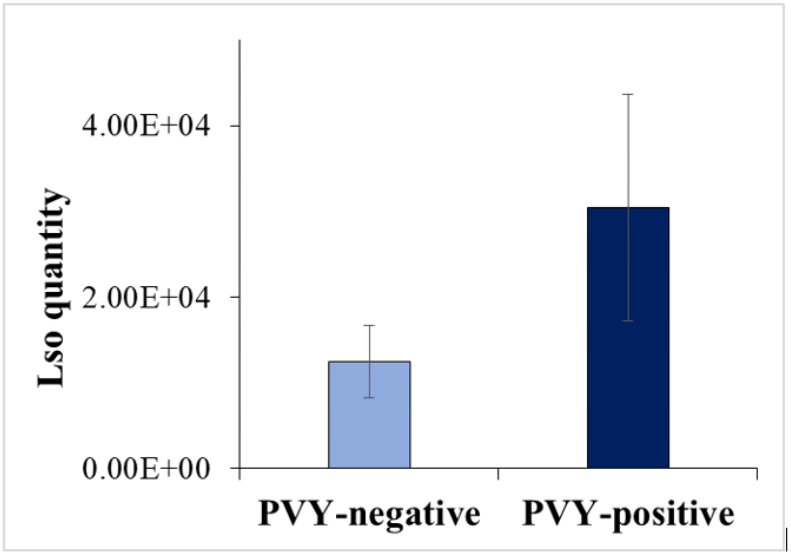

Figure 5.2. Titer of "*Ca.* Liberibacter solanacearum" in PVY-negative and PVY-positive tomato plants infected with "*Ca.* Liberibacter solanacearum". Error bars represent standard error (S.E).

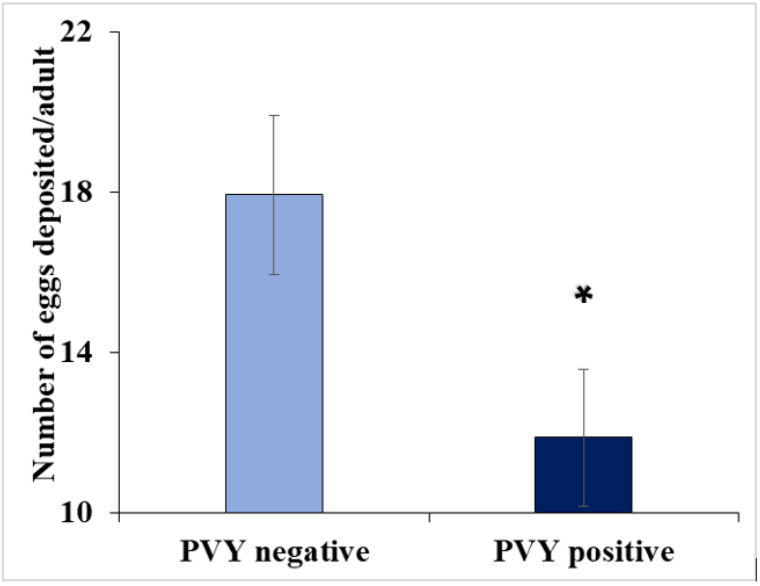

Figure 5.3. Oviposition of Lso-infected potato psyllids on PVY-negative and PVY-positive tomato plants. Significant number of eggs laid is indicated by asterisk (P<0.05). Error bars represent standard error (S.E).

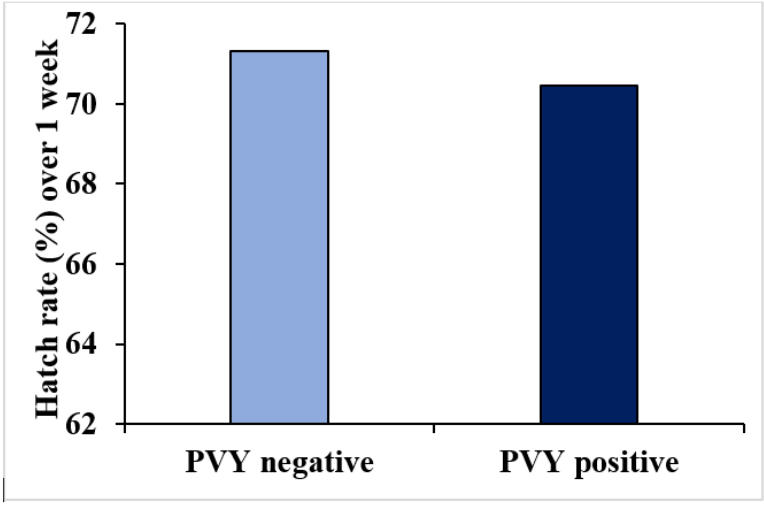

Figure 5.4. Hatching success as percentage of eggs hatched by Lso-infected psyllids on PVY-negative and PVY-positive tomato plants; over 1 week.

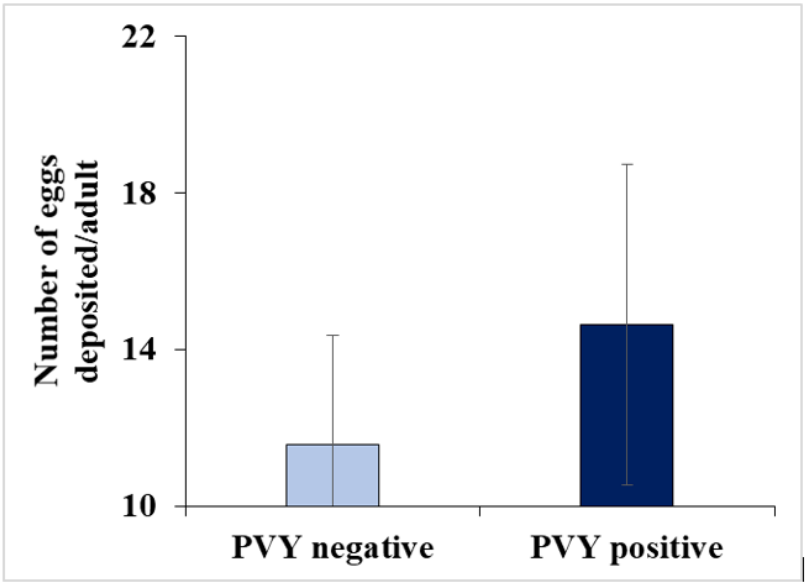

Figure 5.5. Oviposition of healthy potato psyllids on PVY-negative and PVY-positive tomato plants. Error bars represent standard error (S.E).

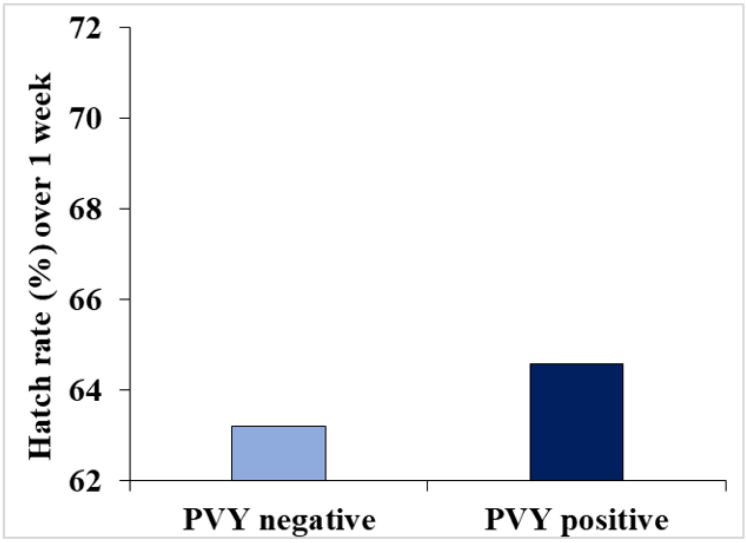

Figure 5.6. Hatching success as percentage of eggs hatched by healthy psyllids on PVY-negative and PVY-positive tomato plants; over 1 week.

Chapter 6: Summary

The research presented in this dissertation study is focused on interspecific interactions within the ZC pathosystem and also understanding potential implications of the interaction between ZC and PVY pathosystems on the epidemiology of ZC. The second and third chapters were focused on screening selected potato genotypes to identify sources of resistance/tolerance to ZC with the aim to provide information for future breeding efforts. Since, Lso-tuber interaction is not limited to the field and the pathogen continues to develop in cold temperature, evaluations were conducted at harvest and post-harvest (cold storage). The experimental design included the time of infection on the field to determinate how the time of arriving of the vector of Lso would impact on the quality of ZC-affected tubers. Potato genotypes from the A07781 family exhibited low susceptibility to Lso and tolerance to ZC symptoms both at harvest and after storage, when compared to other potato genotypes and the susceptible control. Thus genotypes belonging to the A07781 group offer potential sources of resistance or tolerance to ZC. Studies are currently ongoing to see this resistance and/or tolerance can be incorporated into the single hill generation (A07781 as a parent). The reduced expression of ZC symptoms in the A07781 genotypes from early infections or the infections occurring 12 days befor vine-kill have practical importance because infections occurring at these developmental stages can result in high losses of production at harvest and/or post-harvest. The fourth chapter aimed to evaluate the potato psyllid mortality in winter temperatures, in relation to its Lso status, haplotype, and host plant. These components are critical in understanding the overwintering capability of the potato psyllid, and are relevant for decoupling factors tshat may influeces ZC epidemiology. Findings demonstrated that Lso may enhance the ability of the potato psyllid to acclimate to winter temperatures because psyllid had a reduced mortality when they carried

Lso. In general, the haplotype and the host plant had no influence on the rate of mortality in the cold temperatures evaluated. However, the effect of the interaction between temperature-host and haplotype-temperature on mortality of the potato psyllid was present in some evaluations. Although Lso significantly influenced the respiration of potato psyllids in 20 ºC temperature, this effect was not present in freezing and subfreezing temperatures. In vector-borne pathogens, it has been observed that the susceptibility of vectors to heat and cold temperatures may be associated with the pathosystem in which pathogen and vector interact; vectors from pathosystems that develop in tropical enviroments are likely to have heat tolerance (Pusag et al. 2012, Xu et al. 2016). Thus, we would expect that the potato psyllids may have developed physiological mechanisms to be tolerant to cold temperatures. Overwintering ability of the potato psyllid is important to survival of the insect and also the Lso. Lso has been suggested to manipulate plant defenses of the plant host (Casteel et al. 2012) and behavior (Mas et al. 2014) of the potato psyllid to increase its spread in regular conditions of development. Therefore it is possible that Lso can continue to influence on potato psyllids during winter by increasing their survival period. A longer time of survival may imply more time to lay eggs or higher spread of Lso on wild host species. It would be interesting to investigate the potential ability of Lso-positive psyllids to acclimate to winter in other wild host species as well as to study the variation in the expression of genes between Lso-infected and healthy psyllids under cold temperatures. The fifth chapter evaluated effects of PVY on Lso development and the potato psyllid oviposition, and demonstrated that while the virus has no effect on Lso quantity, it may reduce the psyllid population and contribute to reduced Lso incidence in Idaho. This study improves our current knowledge of ZC pathosystem, in particular, its epidemiology.

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