PROCEEDINGS OF THE 14TH ANNUAL SCRI ZEBRA CHIP REPORTING SESSION

F. Workneh and C.M. Rush Editors

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Portland, OR

Nov. 9-12, 2014

PREFACE

Zebra chip of potato (ZC) was first documented from potato fields around Saltillo, Mexico in 1994, and in 2000 it was identified in South Texas. In the USA, the disease initially was considered a regional problem in South Texas, but by 2006 ZC had been identified from all potato production areas in Texas, and also in Arizona, California, Colorado, Kansas, Nebraska, Nevada, and New Mexico. Outside of the USA, ZC has been reported from Guatemala, Honduras, Mexico and New Zealand. Early studies of ZC were hampered by lack of knowledge concerning disease etiology, but in 2007, the potato psyllid, *Bactericera cockerelli*, was definitively associated with ZC and in 2008 two independent studies reported the association of *Candidatus* Liberibacter spp. with ZC. It now has been repeatedly demonstrated that transmission of *Candidatus* Liberibacter solanacearum by the potato psyllid results in diagnostic symptoms of ZC, while infestations by potato psyllids without *Candidatus* Liberibacter solanacearum do not cause ZC. However, questions still exist concerning the effect of pathogen and vector variability on disease severity.

Soon after ZC was first identified in South Texas, representatives from *Frito Lay*, approximately four farmers and two plant pathologists met to discuss how to deal with the new disease. Grower sponsored research projects were initiated the next year, and the same small group met again, after the 2001 harvest, and in an informal setting presented their findings and observations. This meeting constituted the first ZC reporting session. After the disease was identified in potato production regions outside of Texas, the National Potato Council and the US Potato Board recognized the potential danger of this new disease and begin to support additional research. In 2007, the Texas Legislature appropriated $2 million to support research on ZC and in 2009; a multistate, multidisciplinary group of scientists were awarded $6.9 million, from the Federal Specialty Crop Research Initiative (SCRI) Program, to study all aspects of ZC.

On November 9-12, 2014, 118 scientists, farmers, and personnel from agri-industry and potato processing companies, representing five countries, attended the 14th Annual Zebra Chip Reporting Session. Each year, the goal of the meeting is to provide a forum to facilitate collaboration and multidisciplinary research on all aspect of ZC. Those who attend present research results on a wide variety of topics including pathogen detection, vector/pathogen diversity, epidemiology, pest management, breeding for resistance, economics, and disease risk assessment and forecasting. The high quality of information presented in an informal setting to a multidisciplinary group with common interests always makes for an enjoyable, professionally rewarding experience. This volume serves as a record of information presented at our most recent meeting and represents the first published Proceedings of the ZC Reporting Session. It is hoped that the information presented in this Proceedings will be useful to all those interested in ZC.

Charlie Rush
ZC SCRI Program Director

ACKNOWLEDGEMENTS

The publication of this Proceedings and the research reported herein was made possible through a Federal grant from the United States Department of Agriculture-National Institute of Food and Agriculture-Specialty Crop Research Initiative (USDA-NIFA-SCRI) Program, Grant #2009-51181-20176 and through the Texas Department of Agriculture.

The organizers of this meeting would like to express their gratitude to Ms. Patty Garrett for facilitating local arrangements for this meeting. We also would like to acknowledge Bayer Crop Science, Frito Lay, DuPont, Syngenta, NW Potato Research Consortium, ConAgra Foods, and Dow AgroSciences for covering expenses for the Welcome Reception and Hospitality events. Finally, we appreciate the efforts of Kay Ledbetter in recording interviews and Donnie Parrack or recording presentations.

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2014 SCRI ZC ANNUAL REPORTING SESSION
NOV. 9-12, 2014

SCRI Zebra Chip

Overview of the 2013-2014 Potato Psyllid Area-wide Monitoring Program

Workneh[1], F., Henne[2], D. C., Paetzold[1], L., Silva[2], A., Warfield[2], B., Bradshaw[3], J. D., and Rush[1], C. M.

[1]Texas A&M AgriLife Research, Bushland, TX 79012; [2]Texas A&M AgriLife Research, Weslaco, TX 78596; University of Nebraska –Lincoln, Panhandle Research and Extension Center, Scottsbluff, NE 69361.

Abstract

Potato psyllids (*Bactericera cockerelli*) vector the putative bacterial pathogen of potato zebra chip (ZC), '*Candidatus* Liberibacter solanacearum' (Lso). The current project is part of the regional psyllid surveys which have been conducted for the last several years to provide growers with information on the spatial and temporal distribution of the insect for making timely decisions on management. During the 2013-2014 season, in the Lower Rio Grande Valley (LRGV), psyllid counts over time in treated commercial fields followed patterns which are similar to past seasons. In all fields, psyllid counts were initially near zero but increased gradually over time reaching maximum near harvest. As expected, unsprayed plots had much higher psyllid densities than the sprayed commercial fields. Greater percentages of (nearly 14%) of psyllids from the untreated (control) plots tested positive for Lso than those from commercial fields (<1%). Treated commercial fields in the northern regions had <1% the psyllids testing positive as well. As in the past, regional psyllid distribution followed a bimodal pattern, the southern and the northern regions having greater psyllid counts than the Texas Panhandle. Nearly 90% of the collected psyllids were of the Central haplotype. For the Lso, type B accounted for most of the identified haplotypes with nearly 45%, followed by type A, which was 26% while the A+B mix accounted for 16%. The rest of the psyllids (nearly 14%) were unclassifiable.

Introduction

Potato psyllids (*Bactericera cockerelli*) vector the putative bacterial pathogen of potato zebra chip (ZC), '*Candidatus* Liberibacter solanacearum' (Lso). Periodic monitoring of the insect is critical for initiation of timely management actions. Since the advent ZC in the US, the regional psyllid survey program has been providing growers with weekly psyllid prevalence reports to enable them make necessary management decisions. The 2013-2014 project was a continuation of the ongoing regional program which followed similar protocols of collection and identification of psyllids. Currently there are several identified haplotypes of the potato psyllid (Swisher et al., 2013) and two known haplotypes of Lso across the US (Wen et al., 2013). In addition to information on regional psyllid abundance and their Lso status, knowledge of time- and space-related distributions of the different haplotypes of both the insect and Lso is important for understanding of the ecology and epidemiology of the disease. Thus, in the 2013-2014 both psyllid and Lso haplotype tests were incorporated into the survey program in all psyllid collections across the region.

Materials and Methods

Psyllid survey. As in the past (Henne et al., 2013), the 2013-2014 psyllid regional survey began in the fall of 2013 in the Lower Rio Grande Valley (LRGV), south Texas, and progressed northward following successions of potato planting seasons. Survey locations included Texas (Edinburg, Weslaco, Pearsall, Olton, Spring Lake, and Dalhart), Kansas (Garden City and Hugoton), Nebraska (Scottsbluff and Alliance), Colorado (Alamosa, Fort Morgan, and Wray), New Mexico, and several locations in Minnesota and North Dakota. Multiple commercial fields were surveyed in many of these locations. Some of these locations also contain unsprayed plots wherever possible. In each field, five yellow

sticky traps were installed on wooden stick at 50m interval beginning form the southern edge of the field towards the center for adult- psyllid monitoring. The sticky traps were collected and replaced on weekly basis. In addition, one-hundred compound leaves were collected from around the field edges for egg and nymph assessments. Both stick traps and leaves were shipped overnight to the Weslaco AgriLife Research Station for identification and counting.

Psyllid testing for Lso. Tests of psyllid samples for Lso, and identification of both the psyllid and Lso haplotypes were conducted in Dr. Charlie Rush's laboratory at the AgriLife Research Station at Bushland, TX.

Results and Discussion

Psyllid survey. In LRGV, counts of psyllds per trap gradually increased over time from near zero early in the season and peaking to around 5/trap in April (near harvest). However, in one of the fields, over 12 psyllids/trap were detected near the end (Fig. 1). Egg counts followed a pattern similar to that of the adults with little or no detection early and peaking to near 1.5/leaf late in the season with the exception of the field that had the highest psyllid counts, which had close to 4 eggs /leaf (Fig. 2). Nymph counts peaked around the third week of March and declined towards the end of the season (Fig. 3). As expected, untreated control plots (UTC1, UTC2, and UTC3) had substantially higher psyllid counts than treated commercial fields (Fig. 4). Psyllid counts in UTC1, which was planted earlier than UTC2 and UTC3 continued to increase until the end of the season culminating at above 30 psyllids/trap. Psyllid counts in Pearsall followed similar pattern of over time progression to that of LRGV with just around 5/trap at the highest in some of the fields towards the end of the season. The exception to this is one field, which had around 55 psyllids/trap a week before the last trap collection date (Fig. 5).
As in the past (Henne et al., 2012), the overall regional psyllid survey showed a bimodal pattern of psyllid abundance, in which greater numbers of psyllids were caught on traps in the southern (LRGV and Pearsall) and northern regions (Colorado and Nebraska) than in the Texas Panhandle (Olton and Dalhart, Fig. 6).

Psyllid haplotyping and testing for Lso. A total of 6076 psyllid samples were received during the 2013-2014 season. Of these, 4075 were tested for Lso and for identification of Lso and psyllid haplotypes (Fig. 8). Of the total psyllid numbers, 88.7% were the Central haplotype, 3.9% were Western, and 6.7% were Southwestern type, while 0.7% of the psyllids were unclassifiable. Approximately 2.1% of the Central haplotypes tested positive for Lso, while 3.2% and 0.4% of the Western and Southwestern types, respectively, tested positive for the pathogen. The 2.1% Lso for the Central haplotype is without those collected from pepper and tomato fields (but only from potato fields) from LRGV, which were about 35% positive for Lso. Overall, during the 2013-2014 season, about 2% of psyllids collected from potato fields tested positive for (Fig. 9).
Samples collected from potato fields in New Mexico exhibited dramatic shifts in proportions psyllid haplotypes over time. There were a total of 305 psyllids collected from New Mexico during the season and, initially, all psyllid samples were of the Southwestern type but the percentage declined over time and was eventually overtaken by the Western type (Fig. 10). Similar displacements in haplotype proportions were observed in psyllids collected from the natural vegetation (Li et al., 2014).

Lso haplotyping. Lso type B was the most encountered haplotype comprising 44.6% of all the Lso types followed by type B, which accounted for 25.8%. Mixed A+B types comprised 16% of the total while 13.6% were unclassifiable (Fig 11).

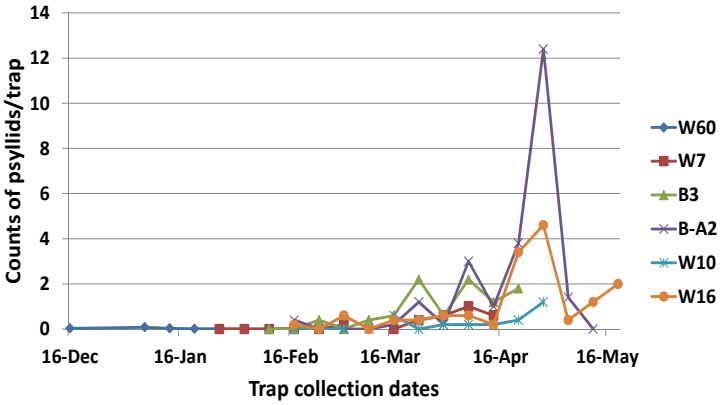

Fig. 1. Counts of psyllids/trap in different fields over time (Dec. 16/2013 to last week of May 2014) in the Lower Rio Grande Valley of Texas.

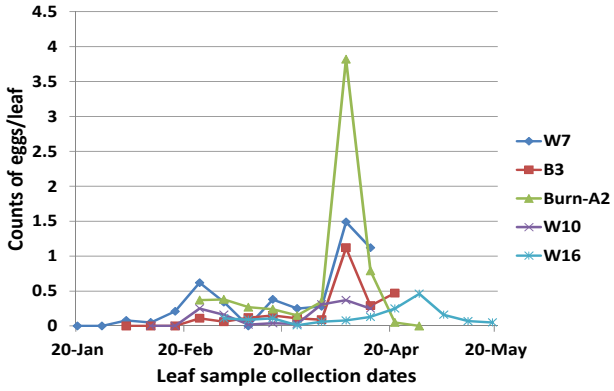

Fig 2. Counts of eggs/trap in different fields over time (Dec. 16/2013 to last week of May 2014) in the Lower Rio Grande Valley of Texas.

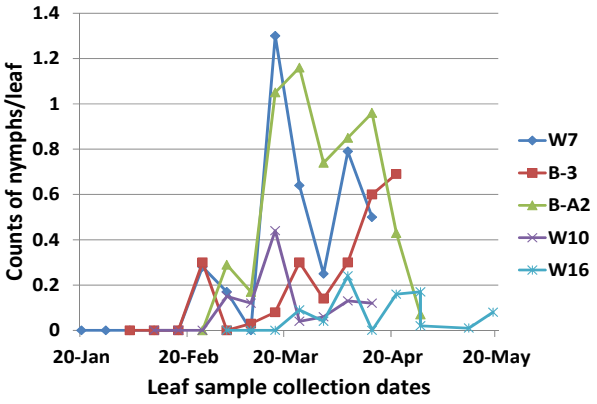

Fig. 3. Counts of nymphs/leaf in different fields over time (Dec. 16/2013 to last week of May 2014) in the Lower Rio Grande Valley of Texas.

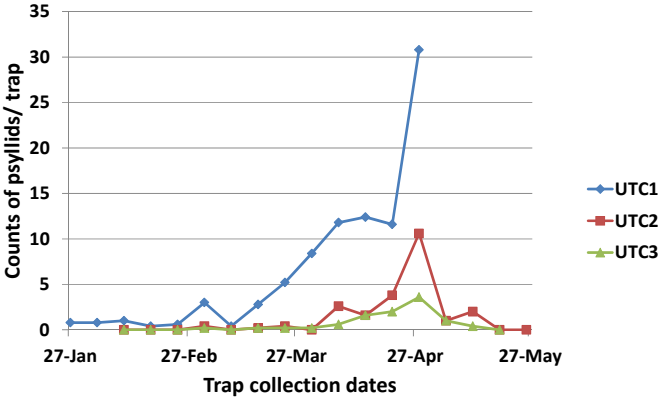

Fig. 4. Counts of psyllids over time in untreated plots in the Lower Rio Grande Valley of Texas.

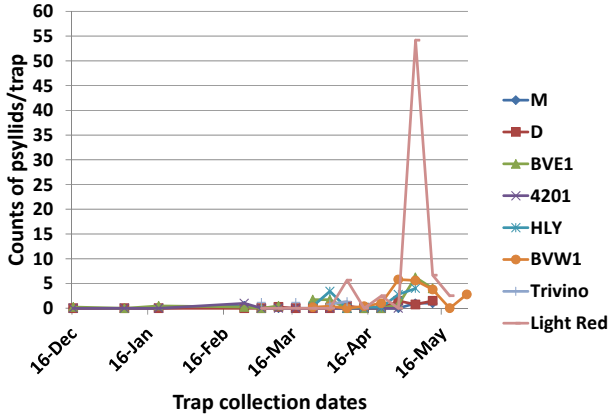

Fig. 5. Counts of psyllids/trap in different fields over time Dec. 16/2013 through Ausgust 2014 in the Pearsall Texas.

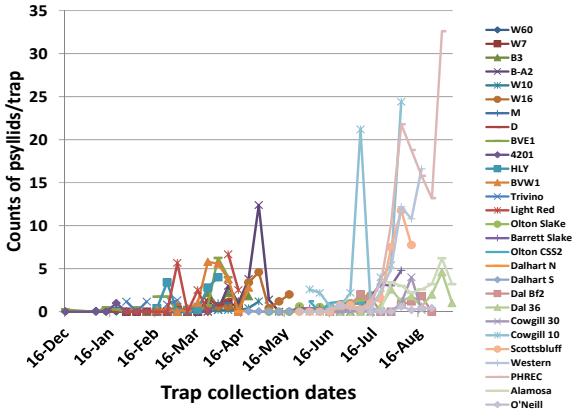

Fig. 6. Regional over time (2013 through August 2104) distribution of psyllid counts from south Texas to Nebraska.

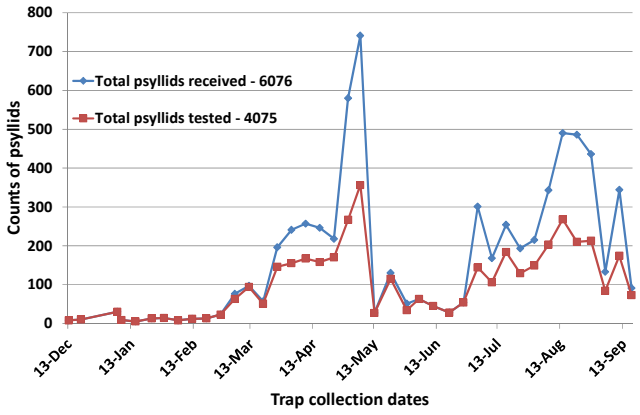

Fig. 8. Counts of psyllids received and tested for Lso during the 2013-2014 field season.

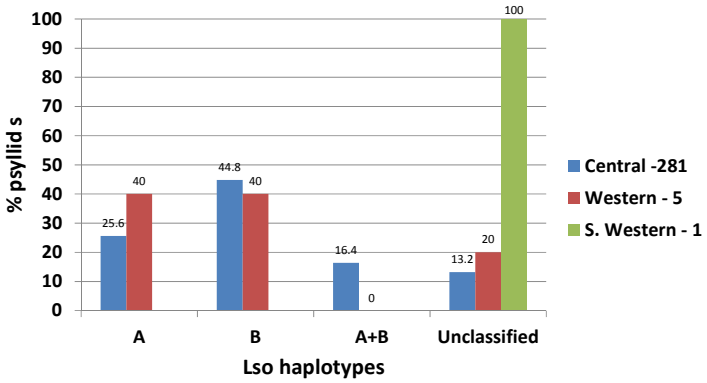

Fig. 9. Haplotypes of regionally collected psyllids and their Lso (status (minus those collected tomato & pepper plots in LRGV).

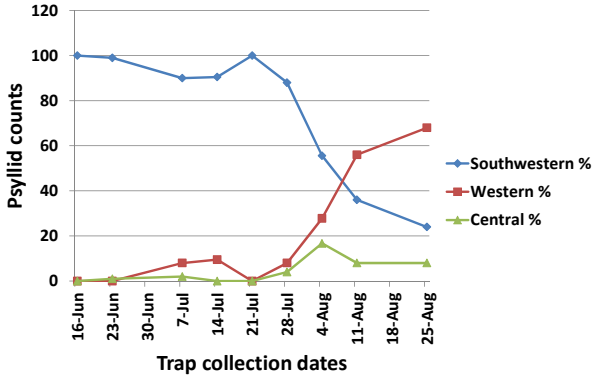

Fig.10. Trends in incidence of psyllids haplotypes collected from New Mexico potato fields.

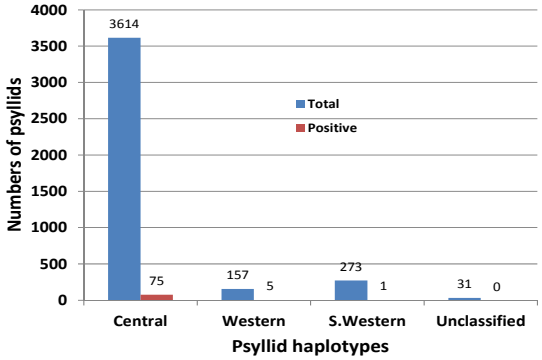

Fig.11. Haplotypes of Lso in regionally collected psyllids.

Acknowledgements

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References

Henne, D. C., Aniciso, J., Bradshaw, J. D., Whipple, S. Carpio, L. Schuster, G., Richmond, J. Walker, S., Porter, P., Barrett, S., Willet, j., Mathews, J. M., Deroo, K., Seger, P. Crosslin, J., Hamlin, L., and Goolsby, J. 2012. Overview of the 2011-2012 potato psyllid area wide monitoring program. Pages 1-4. In: Proceedings of the 12th Annual SCRI Zebra Chip Reporting Session. F. Workneh, A. Rashed, and C. M. Rush eds. Oct. 30 - Nov. 2, San Antonio TX.

Paetzold, L. Rashed, A., Workneh, F., and Rush, C. M. 2014. Temporal and spatial variations of psyllid haplotypes occurring in indigenous vegetation of Texas. Pages… In: Proceedings of the 14th Annual ScRI Zebra Chip Reporting Session. F. Workneh and C. M. Rush Eds. Nov…- …2014. Portland, OR.

Swisher, K. D., Munyaneza, J. E., Crosslin, J. M. 2013. Temporal and spatial analysis of the potato psyllid haplotypes in the United States. Environ. Entomol. 42:381-393.

Wen, A., Johnson, C., and Gudmestad, N. C. 2013. Development of multiplex and conventional real-time PCR for detection and genotyping of '*Candidatus* Liberibacter solanacearum'. Pages 68-72. In: Proceedings of the 13th Annual SCRI Zebra Chip Reporting Session. F. Workneh and C. M. Rush eds. Nov. 3-6, 2013. San Antonio, TX.

Three Years of Monitoring Potato Psyllids, '*Candidatus* Liberibacter solanacearum', and Zebra Chip in Idaho

Wenninger[1], E. J., Olsen[1], N., Thornton[2], M., Nolte[3], P., Miller[4], J., Dahan[5], J., and Karasev[5], A.
[1]University of Idaho, Kimberly Research & Extension Center, Kimberly, ID, USA
[2]University of Idaho, Parma Research & Extension Center, Parma, ID, USA
[3]University of Idaho, Idaho Falls Research & Extension Center, Idaho Falls, ID, USA
[4]Miller Research, LLC, Rupert, ID, USA
[5]University of Idaho, Moscow, ID, USA

Abstract
We monitored potato psyllids and '*Candidatus* Liberibacter solanacearum' in commercial potato fields across Idaho over three growing seasons. We sampled a total of 15, 108, and 88 fields during 2012, 2013, and 2014, respectively. During 2013 and 2014, each field was monitored either using a "Light" approach (weekly yellow sticky card trapping with four cards per field) or an "Intense" approach (10 yellow sticky traps per field, one 5-minute vacuum sample per field, and 100 leaf samples per field); only the "Intense" sampling approach was used during 2012. Sampling began during May and continued on each field until vine kill. During each year, psyllid abundance was very low at first, and gradually increased over the season. Increased sampling effort during 2013-2014 permitted assessment of psyllid phenology over more of a landscape scale. Psyllid distribution and abundance patterns over the season generally reflected the temperature/elevation gradient across the state. The first incidence of psyllids at each site generally occurred earlier for western, lower elevation sites; similarly, these sites generally exhibited higher psyllid abundance. Potato psyllid numbers have been declining during each of the last two years, and the incidence of Lso also has been relatively low. This is in contrast to 2012 in which psyllid numbers, Lso incidence, and zebra chip (ZC) incidence all were higher. ZC incidence in potato fields has been nil during the last two years, which—coupled with the low incidence of psyllids and Lso found—suggests that our monitoring program is effective at identifying the level of risk of ZC to Idaho potato growers.

Introduction
Caused by the bacterium ('*Candidatus* Liberibacter solanacearum' [Lso]) and vectored by the potato psyllid *(Bactericera cockerelli*), zebra chip (ZC) is an emerging disease of potatoes that causes millions of dollars in losses annually to growers in the southwestern United States. Despite occurrence of the potato psyllid in the Pacific Northwest over many decades, ZC was thought to not occur in this region. However, during September 2011, ZC was widely reported in Idaho, Washington, and Oregon. Because of the potential threat of this disease to Idaho's potato industry, we initiated a monitoring program in Idaho during 2012 to clarify the distribution and abundance of potato psyllids, ZC, and Lso, throughout the potato production areas in the state. During 2013-2014, we greatly expanded upon this monitoring program in order to more fully clarify the distribution and phenology of this pathosystem across the state. Knowledge of the distribution and abundance of potato psyllids in Idaho is critical to developing effective management strategies to mitigate the threat of ZC in Idaho.

Materials and Methods
Potato psyllids and Lso were monitored in commercial potato fields across Idaho during 2012, 2013, and 2014 (Table 1). Fields were monitored using one of two sample efforts: "Intense" or "Light." The Intense program was comprised of 10 yellow sticky traps per field, one 5-minute vacuum sample per

field, and 100 leaf samples per field, with samples taken at weekly intervals from mid-May through vine kill. The Light program featured 4 yellow sticky traps per field with the same timing and duration of sampling. All fields were under standard commercial production practices, which typically featured an at-plant neonicotinoid insecticide followed by several foliar insecticide sprays over the season. Monitoring began on most fields during May, but the initiation date varied by region (due to the elevation and associated temperature gradient across the state, monitoring of sites in eastern Idaho typically started later).

Sticky traps were deployed within each field around the perimeter (about 9 feet from the edge); traps were replaced weekly and adult potato psyllids on each trap were counted. For the Intense fields, within the vicinity of each of the 10 sticky trap locations, 10 leaf samples were collected each week (for a total of 100 leaf samples from each field each week); leaf samples were returned to the lab and eggs and nymphs of potato psyllids found on leaves were counted. In addition, each week a leaf blower with a vacuum attachment was used to sample insects from potato foliage of Intense fields. The windward side of each field was sampled for 5 minutes at each site each week, and samples were returned to the lab to count adult potato psyllids collected. Adult potato psyllids were tested for the presence of Lso by PCR, using the methodology of Crosslin et al. (2011).

Results and Discussion

Potato psyllid numbers have been declining during each of the last two years, and the incidence of Lso (liberibacter; the bacterium that causes zebra chip) also has been relatively low. This is in contrast to 2012 in which psyllid numbers, Lso incidence, and ZC incidence all were higher.

Psyllid numbers were relatively low this year compared to last year, which were low compared to the previous year (Fig. 1). Numbers were lower despite a relatively early detection of psyllids this year. Similar to the previous two years, psyllid numbers increased gradually over the season, which a sharp increase just at the end of the season and a sharp decline as most fields were being harvested (Figs. 1-2). Also similar to last year, psyllids were more abundant in the Treasure Valley than in the Magic Valley, and very few psyllids were observed in the Upper Snake region (Figs. 1-2).

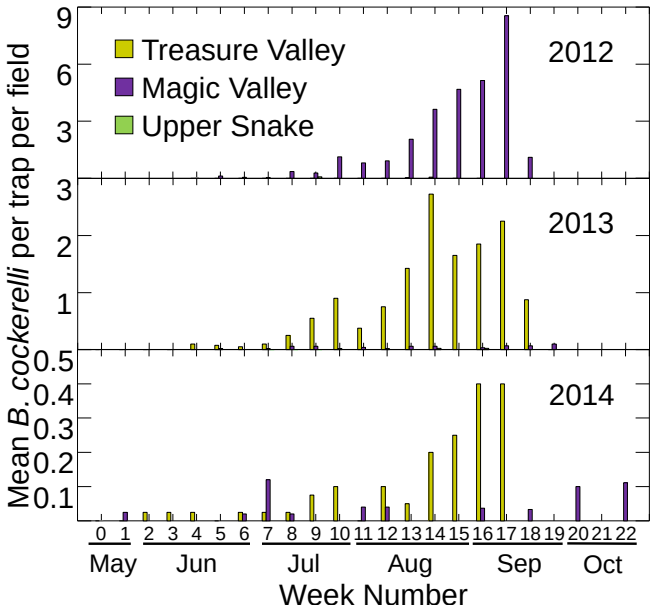


Fig. 1. Captures of adult potato psyllids on yellow sticky traps each week compared among three years and three regions in Idaho [Intense sites].

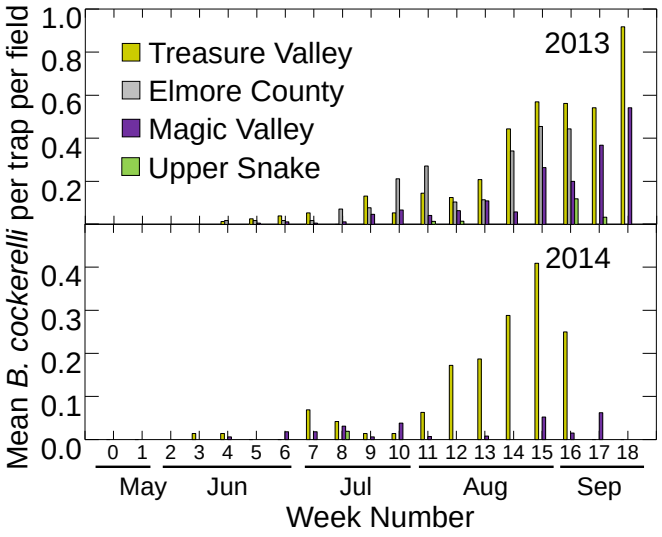


Fig. 2. Captures of adult potato psyllids on yellow sticky traps each week compared among three regions in Idaho [Light sites].

Similar to previous years, fewer psyllids were collected in vacuum samples compared to sticky trap samples. In addition, scarcely any psyllid nymphs or eggs were found during 2013-2014 in leaf samples (data not shown).

In addition to seeing a decline in the number of psyllids compared to 2012, few psyllids were found that tested positive for Lso. Indeed, only four positive psyllids were found in our monitoring network during 2014 (Table 1). The incidence of Lso during 2013 and 2014 was more in line with the rates typically seen in other parts of the country, however. The incidence of Lso in psyllids during 2012 was about 8.6 times higher than the incidence observed during the following two years. The decline in psyllid numbers observed during the last two years is even more compelling when considering that the sampling effort was much higher during these years compared to 2012 (Table 1). The number of psyllids collected per card has declined approximately five-fold each year (Table 1).

Levels of ZC in Idaho potato fields have been nil for the last two years. Thus, the incidence of ZC during each of the past three years has been more or less consistent with the psyllid numbers and Lso incidence detected in our monitoring program. This suggests that our monitoring program has been effective at identifying the level of risk of ZC to Idaho potato growers. Updates on the monitoring program were posted at least weekly through various means in order to help growers and crop consultants use the information to make psyllid management decisions.

Table 1. Comparison of potato psyllid monitoring in Idaho among three years

	2012	2013	2014
		14 Light + 94 Intense =	13 Light + 75 Intense =
Total sites	15	108	88
Traps per week (approx.)	150	516	430
Weeks of trapping	19	19	19
Total psyllids on sticky cards	1,603	1,093	170
Total psyllids tested for Lso	1,073	1,093	170
Lso positive psyllids	250	33	4
% Lso positive	23.3%	2.8%	2.4%
Total cards read (approx.)	2,850	9,804	8,560
Psyllids per card (approx.)	0.56	0.11	0.02

References

Crosslin, J.M., Lin, H., and Munyaneza, J.E. 2011. Detection of '*Candidatus* Liberibacter solanacearum' in the potato psyllid, *Bactericera cockerelli* (Sulc), by conventional and real-time PCR. Southwest. Entomol. 36:125-135.

Progress in Regional Zebra Chip Assessment and Associated Factors

[1]Workneh, F., [2]Henne, D. C., [3] Bradshaw, J. D., [1]Paetzold, L., and [1]Rush, C. M.

[1]Texas A&M AgriLife Research, Bushland, TX 79012; [2]Texas A&M AgriLife Research and Extension Center, Weslaco, TX 78596; [3]University of Nebraska, Panhandle Research and Extension Center, Scottsbluff, NE 69361

Abstract

In 2014 regional assessments of potato zebra chip caused by '*Candidatus* Liberibacter solanacearum' (Lso) was conducted at six locations (8 fields) from south Texas to Nebraska, which included the Lower Rio Grande Valley (2 fields) and Pearsall (2 fields) both in south Texas, Olton and Dalhart (both in the Texas Panhandle), Garden City (Kansas), and Alliance (Nebraska). Compared to the previous years, disease incidence was much lower across the region although it was relatively higher in the southern region than in the northern region as usual with significant south-north declining gradient. Since the southern locations had greater zebra chip incidence with substantial year-to-year variations, we investigated the impact of winter temperatures during the last 5 years. The results showed that winter temperatures significantly impact counts of psyllids testing positive for Lso and zebra chip incidence. In the Lower Rio Grande Valley, higher minimum temperatures during the winter were significantly associated with higher counts of psyllids positive for Lso and grater zebra chip incidence. Similar results were obtained in Pearsall as well except that the relationship between minimum temperature and zebra chip incidence was not as strong as that in the Lower Rio Grande Valley.

Introduction

Potato zebra chip (ZC) caused by the putative bacterium '*Candidatus* Liberibacter solancearum', has been causing major constraints in in US potato production since its discovery in south Texas in 2000. In order to better understand the disease epidemiology, we have been conducting yearly regional ZC assessments since 2010 (Workneh et al., 2010, 2011, 2012, 2013a, 2013b). The current project is a continuation of the regional investigation where we assessed ZC incidence in selected fields across the central US from south Texas to Nebraska. Assessments during the previous four seasons provided results that are consistent across the years that the southern regions had greater ZC incidence than the northern regions and there were south-north declining trends in each of the seasons. In this report, in addition to presenting the overall ZC regional picture for the 2104 season, we will describe the impact of temperature on psyllid abundance and ZC incidence in south Texas during the past five years.

Materials and Methods

Zebra chip incidence was assessed at eight locations from mid-April through August as described previously (Workneh et al., 2013b). The locations included 2 fields each in the Lower Rio Grande Valley (LRGV) and Pearsall in south Texas, Olton and Dalhart in Texas the Panhandle, Garden City in Kansas, and Alliance in Nebraska. At each location, ZC was assessed in 20m × 30m plots around the field edges and in the center of field under the center pivot irrigation system. Number of plots per field ranged from 16 to 32. Tubers of plants suspected of having aerial ZC symptoms were dug and sliced to determine whether they have internal browning characteristic of ZC diagnostic features. If the tuber showed diagnostic symptom, the plant was considered positive for ZC. Since southern Texas has been the hot spot for ZC incidence, and the potatoes are cultivated largely during the winter season, we

investigated the impact of winter temperature on variability of psyllid abundance and zebra chip incidence over the past 5 years.

Results and Discussion

ZC was detected in seven of the eight fields investigated. As in the past, the intensity of the disease was greater in the southern regions than in the northern regions and there was a significant (R^2 = 0.68; P = 0.0075) south-north declining gradient (Fig. 1). Overall, ZC incidence across the region was the lowest this year than at any time since we started the regional assessment in 2010. In 2014, fields in south Texas where we used to see as high as50 infected plants/plot in the past, had an average of just over one infected plant/plot. To understand factors contributing to the variations, mean maximum, mean minimum, and mean average temperatures for January through March were compared for their impact on counts of psyllids positive for Lso and zebra chip incidence in Pearsall and the LRGV.

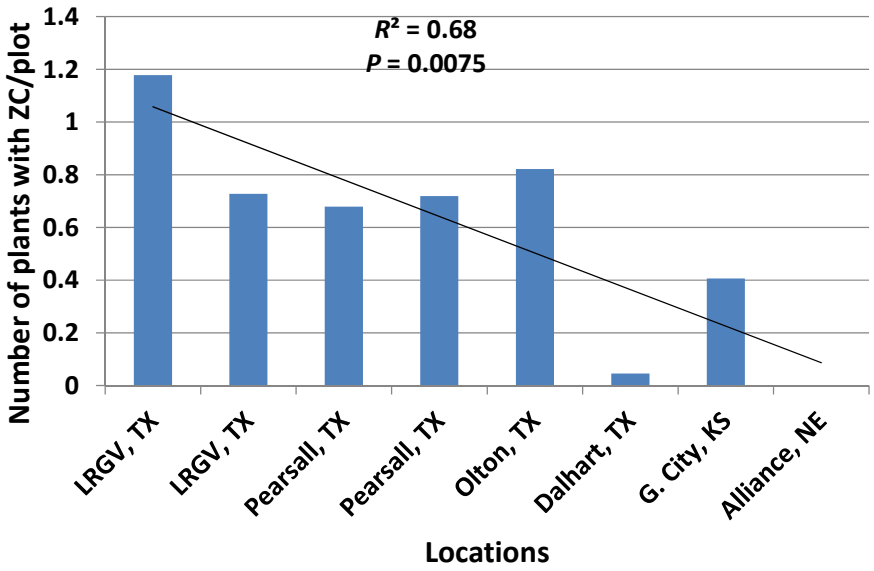


Fig. 1. Incidence of potato zebra chip at different from south Texas to Nebraska.

At both locations mean minimum temperatures during the three-month period strongly correlated with counts of Lso positive psyllids (R^2 = 0.8553, P = 0.02045, R^2 = 0.9066, P = 0.0125, for Pearsall and LRGV, respectively; Figs. 2A and 2B). The three-month temperature also was a major influencing factor in the level of disease in the spring. In the LRGV mean minimum temperature for January through March strongly correlated with the level of diseases over the past 5 years (R^2 = 0.81, P = 0.0236, Fig 3A). However, the impact of minimum temperature on ZC incidence in Pearsall was not as strong as that of the LRGV although there was a similar trend (R^2 =

0.4428, *P* = 0.1335, Fig. B).

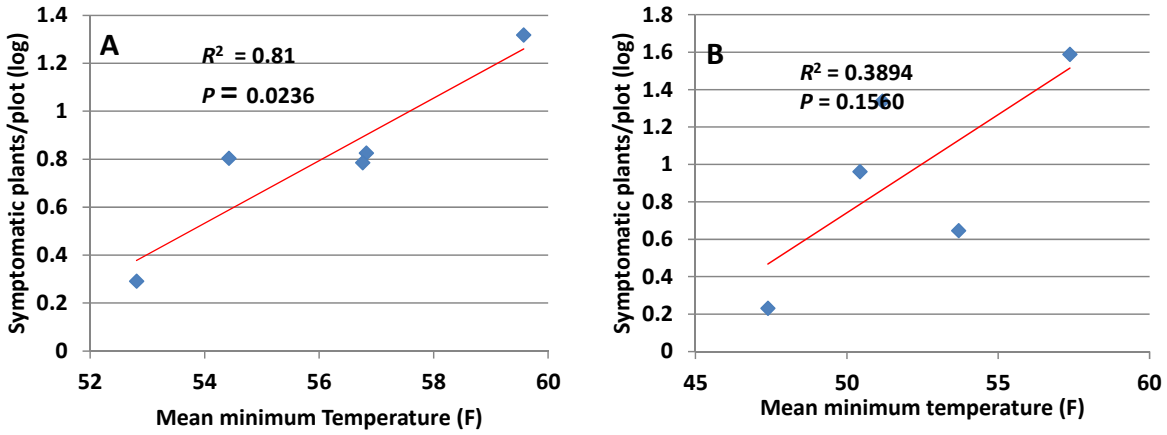


Fig. 2. Relationship between mean minimum temperature and number of symptomatic plants per plot in LRGV (A) and Pearsall (B).

In summary, major findings worth noting here are in the last five years, winter temperatures have significantly contributed to variations in counts of Lso-positive psyllids and ZC incidence. Higher minimum temperatures during the winter were associated with higher numbers of Lso-positive psyllids and higher ZC incidence.

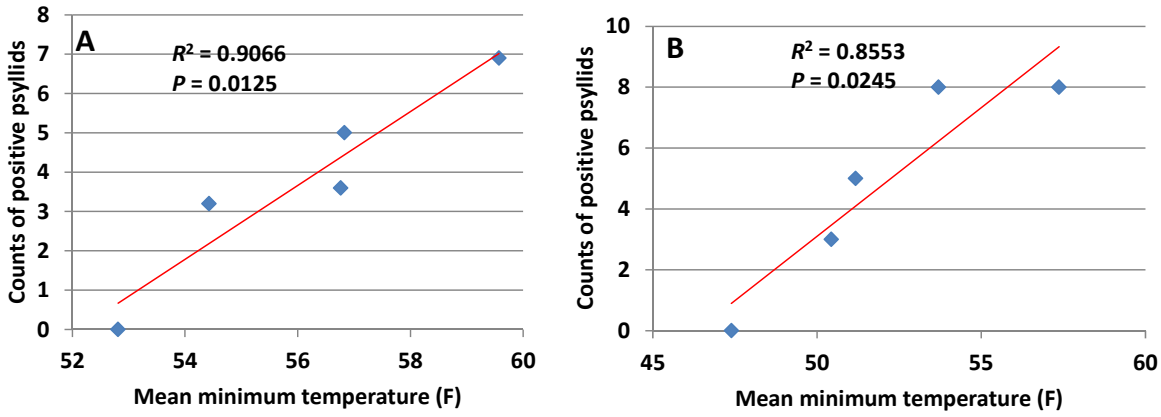


Fig. 3. Relationship between mean minimum temperature and counts of so-positive in LRGV (A) and Pearsall (B).

Acknowledgements
We thank J. Arthur, A. Dubric, and J. Gray for technical assistance. The project was partially funded by the Texas Department of Agriculture Zebra Chip Initiative and the UDA-SCRI Grant (# 2009-51181-20176).

References
Workneh, F., Henne, D. C., Paetzold, L., Harveson, R. M., Bradshaw, J. D., and Rush. C. M. 2010. Status of regional zebra chip incidence in 2010 and temperature effect under controlled conditions. Pages 10-14. In: Proceedings of the 10th Annual Zebra Chip Reporting Session. F. Workneh and C. M. Rush eds. Nov. 7-10, Dallas, TX.

Workneh, F., Goolsby, J. A., Henne, D. C., Rashed, A., Paetzold, L., Hamm, P. B., Rondon, S., Rush., C. M. 2011. Exploratory assessments of weather variables in relation to psyllid/ZC prevalence. Pages 7-11. In: Proceedings of the 11th Annual Zebra Chip Reporting Session. F. Workneh, A. Rashed, and C. M. Rush eds. Nov. 6-9, San Antonio, TX.

Workneh, F., Henne, D. C., Goolsby, J. A., Crosslin, J. M., Whipple, S., Bradshaw, J. D., Rashed, A., Paetzold, L., Harveson, R. M., and Rush, C. M. 2012. Assessing the association of management and environmental factors with regional psyllid abundance and zebra chip intensity. 2012. Pages 66-69. In: Proceedings of the 12th Annual Zebra Chip Reporting Session. F. Workneh, A. Rashed, and C. M. Rush eds. Oct. 30-Nov. 2, San Antonio, TX.

Workneh, F., Goolsby, J. A., Henne, D. C., Crosslin, J. M., Whipple, S., Bradshaw, J. D., Rashed, A., Paetzold, L., Harveson, R. M., Rush., C. M. 2013a. Potato zebra chip epidemiology: What we learned and probable future directions. Pages 6-10. In: Proceedings of the 12th Annual Zebra Chip Reporting Session. F. Workneh and C. M. Rush eds. Nov. 3-6, San Antonio, TX.

Workneh, F., Henne, D. C., Goolsby, J. A., Crosslin, J. M., Whipple, S. D., Bradshaw, J. D., Rashed, A., Paetzold, L., Harveson, R. M., and Rush, C. M. 2013b. Characterization of management and environmental factors associated with regional variations in potato zebra chip occurrence. Phytopathology 103:1235-1242.

'Candidatus Liberibacter solanacearum' (Lso) Development in Russet Norkotah under Commercial Storage Conditions

Rashed, A.[1], Wallis, C.M.[2], Paetzold, L.[3], Woodell, L.[4], Olsen, N.[4], Workneh, F.[3], Rashidi, M.[1], Wenninger, E.J.[4], and Rush, C.M.[3] 1) University of Idaho, Aberdeen R&E Center, Aberdeen, ID 83201 2) USDA-ARS Parlier, CA, 3) Texas A&M AgriLife Research, Amarillo, TX 79106, 4) University of Idaho, Kimberly R&E Center, Kimberly, ID 83341.

Abstract

Following the recent appearance of zebra chip (ZC) in the Pacific Northwest region of the US, where waves of the vector arrive towards late-summer and early fall, and tubers are stored for future marketing, recently emphasis has been placed on late-season infections. This study was set to evaluate effects of late infections under Texas field conditions on post-harvest zc development. Using infective potato psyllids, potato plants were inoculated in field cages at different dates before harvest. Tubers were sampled at harvest and throughout different stages of the storage process to evaluate changes in Lso over time. In all treatments Lso continued to develop within the infected tubers after harvest. When placed at 55°F following cold storage, 41% of the tubers collected from plants infested 4 days before harvest tested positive for Lso. Results indicated that Lso may arrive into the tuber tissue as early as 4 days after infestation by the bacteriliferous vectors, and highlights the importance of vector management until harvest.

Introduction

The identification of the putative etiological agent of zebra chip (ZC), '*Candidatus* Liberibacter solanacearum' (Lso) and its potato psyllid *Bactericera cockerelli* Sulc (Hemiptera, Triozidae) have led to new avenues of research focusing on decoupling the interspecific interactions among the bacterium, the vector, and host plants (see Munyaneza 2012, for a review). To date, visual and molecular detection of infected tubers have been used to screen for ZC-affected tubers at harvest. However, recent studies suggest that reliable detection would be a challenge if plants were infested late in the season, as Lso remains undetectable in tubers infected less than one week prior to harvest. Those tubers infected later in the season would be asymptomatic at harvest and perhaps of an acceptable frying quality. Although a preliminary study suggests that Lso may continue to interact with tubers during post-harvest (Rashed et al. 2014), development appeared to be delayed in storage at temperatures ranging 40-45°F. However, a considerable percentage of those tubers infected one week before harvest tested positive for Lso when moved into room temperature conditions. This suggests practices which prolong tuber exposure to relatively higher temperatures (i.e. temperatures > 50°F) might be conducive to development of Lso and ZC symptoms. For example, harvested tubers would go through curing process, where they are maintained at about 55°F for extended periods, to facilitate healing of damaged tubers prior to the temperature ramp-down which is performed at 0.1 to 0.5°F/day until reaching holding temperatures. Although such practices are essential to minimize quality and yield loss, they may also provide a window of several weeks in which Lso could continue to develop.

Here we present first-year results of a study, which was set to quantify the Lso development at different stages of storage and identify stages of the storage process, which may promote ZC development.

Material and Methods

The potato variety Russet Norkotah was planted in Texas A&M Agrilife Research Experimental Farm in Bushland, TX, in the April 2013. Tent-shaped mesh cages, approximately 1-m^2, made of Sungaurd II fiberglass rods and fine netting were used to cover plants (6/cage) prior to emergence. Six bacteriliferous psyllids released at the base of individual plants in cages were used for inoculations. There were 3

different infestation treatments conducted at 14, 10, and 4 days (plus a non-infected control cage/infestation) before harvest, with 3 cage-replicates per infestation (54 total infested plants). Potato tubers were harvested in August, sub-sampled for Lso quantification (qPCR, at harvest), and then shipped overnight to Kimberly, ID, and stored in the University of Idaho potato storage research facility. Using a core sampler, every individual tuber was periodically sampled at different stages of the storing process. The process consisted of: 1) curing at 55°F for 3 weeks, 2), temperature ramp-down at 0.5°F per day, 3), 8-9 weeks of 45°F holding temperature, and 4) 4 weeks of post-storage at 55°F. All storage conditions were at 95% RH.

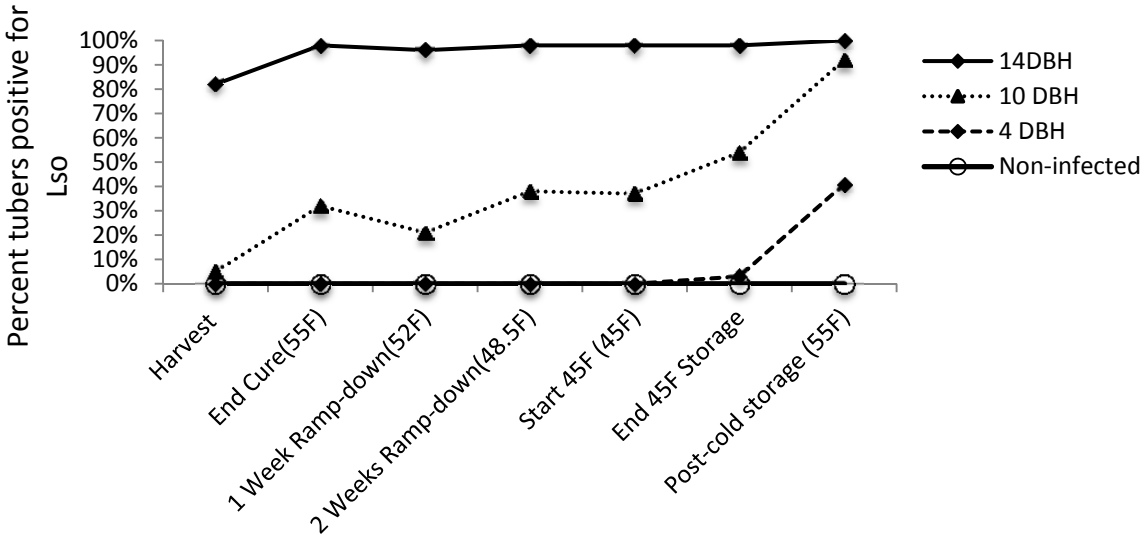
At each sampling approximately 100 mg tissue/tuber was removed for Lso quantification and about 150 mg tissue was stored for later biochemical analyses. Lso extractions and quantifications were performed at the Texas A&M AgriLife Research and Extension in Bushland, TX. A slightly modified DNeasy® Plant Mini Kit (QIAGEN) protocol was used to maximize yield. In summary, ~100 mg of tuber tissue was ground in a homogenizer. After the first set of buffers was added, the samples were incubated at 65°C for 15 min. Using an Applied Biosystems 7500 real-time PCR machine (Taqman probe) and comparative Ct method (ΔΔCt) Lso was quantified in relation to a calibrator and an endogenous 18s RNA control (Rashed et al. 2013).

Tissue samples collected at the end of curing and after storage were analyzed to detect changes in levels of reducing sugars, amino acids and phenolics. A total of 50 mg of tissue per tuber was placed into two microcentrifuge tubes. One of these aliquots was extracted in methanol (1:10 w/v, e.g. 50 mg tuber tissue extracted in 500 mL of methanol) for phenolic analysis using high performance liquid chromatography (Wallis et al. 2012). The other aliquot was extracted in PBS buffer (1:10 w/v) for amino acid analysis via derivatization and gas chromatography using a commercially available kit (Rashed et al. 2013). Additional PBS buffer extract was used to analyze fructose and glucose levels by ion-exchange HPLC (Rashed et al. 2013).

Results and Discussion

Lso was detectable immediately after harvest in majority (82%) of the tubers, albeit in low liters, from plants infested 14 days before harvest (Figure 1). By the end of the curing period (3 weeks at 55°F), Lso was detectable in 98% of the tubers, and Lso titer level showed an increasing trend (Figure 1). Likewise, for the tubers infested 10 days before harvest, the percentage of Lso-positive tubers increased from 5% to 32% during the curing stage when tubers were exposed to the ambient temperature of 55°F. Although tubers infected 4 days before harvest did not test positive for Lso until after storage, Lso was detectable in a considerable percentage of those tubers (41%) post-storage (Figure 1A). This indicates that Lso can reach tuber tissue within 4 days of infestation though remaining at undetectable levels.

A significant difference in titer was detected among tubers from different infestation treatments (Repeated Measure Mixed Effect Model: $F_{2, 99.9} = 66.9$, $P < 0.001$). Overall, Lso titer showed a significant increase throughout the pre-storage and the storage practices ($F_{6, 106.2} = 22.61$, $P < 0.001$). Although the rate of titer increase appeared to be lower at colder temperatures (Figure 1A,B), more studies are needed to confirm this possibility. This is because Lso titer may continue to increase over time regardless of thermal conditions. However, pattern of increase in Lso titer varied significantly among different treatments as revealed by the significant treatment-by-sampling time interaction ($F_{12, 106.9} = 14.38$, $P < 0.001$) (Figure 1B).



A.

B.

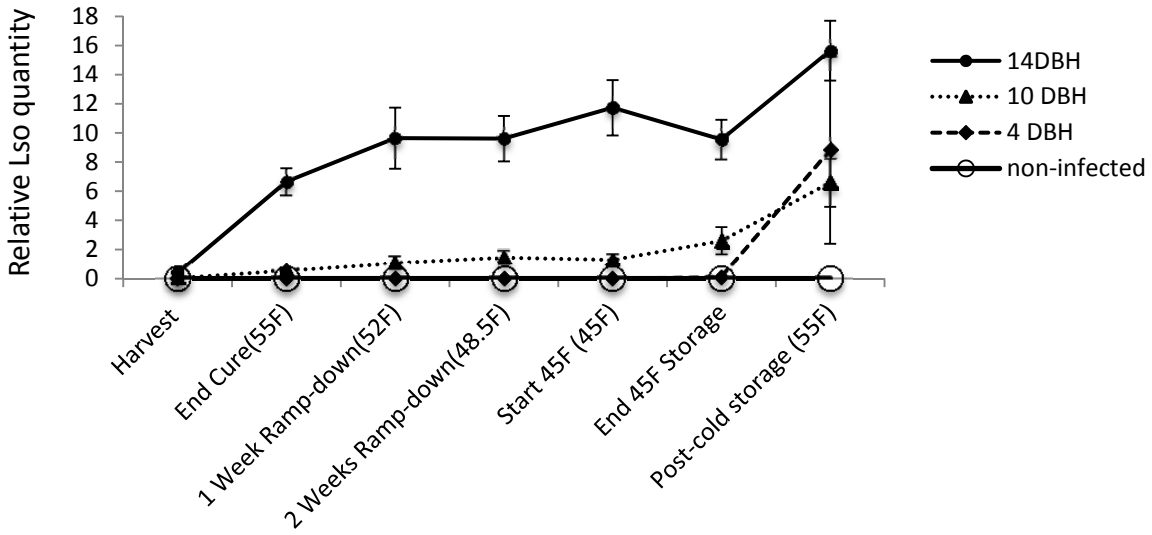


Figure 1A,B: Lso development within harvested tubers pre- and post-storage. In all infestation treatments both percentage Lso-positive tubers (A), and Lso titer increased over time (B). Controls always tested negative for Lso.

A significant increase in carbohydrate concentrations was also detected during storage (MANOVA: Pillai's Trace, $F_{3, 217} = 31.7$, $P < 0.001$). While no statistical difference was detected in sucrose concentrations ($P = 0.285$), both glucose ($P < 0.001$) and fructose ($P < 0.0001$) levels were elevated in the samples which were taken at the end of the holding period when compared to samples collected prior to temperature ramp-down stage (Figure 2). A similar pattern was also observed in the uninfected controls. Although the overall sugar concentrations across sampling dates were not affected by the time of infection in the field (Pillai's Trace, $F_{6, 436} = 1.91$, $P < 0.078$), the pattern of change in the sugar levels was influenced by the duration of infection as revealed by the significant treatment-by-sampling date interaction (Pillai's Trace, $F_{6, 436} = 5.88$, $P < 0.001$; Figure 2). Unexpectedly, however, at the end of storage levels of fructose and glucose were negatively correlated with Lso concentrations; this observation requires further investigation. It is important to note that Russet Norkotah potatoes are

typically not used for processing and inherently accumulate reducing sugars at these lower storage temperatures.

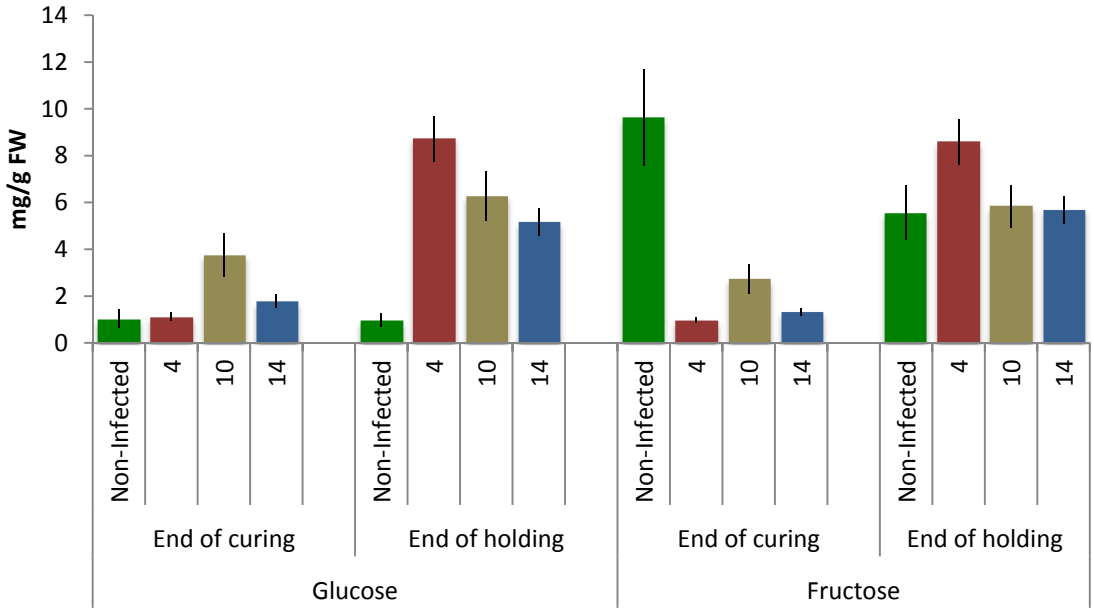


Figure 2: Changes in carbohydrate concentrations during temperature ramp-down and holding temperature across the three infestation dates conducted 4, 10, and 14 days before harvest.

With the exception of glutamine ($F_{1, 215} = 0.645$, $P < 0.423$), the remaining 18 amino acids were affected by the sampling time (all $P < 0.040$). However, only 9 of the quantified amino acids in non-infected controls changed significantly during storage. Alanine, glycine, ornithine, and tyrosine were the only amino acids not affected by the time of infection in the field ($P > 0.067$). Lucine, isoleucine, valine, and proline were among the 9 amino acids that responded positively to Lso titer at the end of the holding stage (Pearson correlation, $P < 0.030$).

There was a significant increase in the phenolic activity levels during storage (Pillai's Trace, $F_{10, 210} = 32.7$, $P < 0.001$). There was also a significant increase in phenolic activity levels in the uninfected control tubers. The increase in the phenolic activity of non-infected controls (and infected tubers) might have been in response to the damage that was inflicted via sampling. However, it is known whether disease severity is associated with elevated levels of phenolics. Figure 3 illustrates changes in chlorogenic acid during ramp-down and holding stages in the three infestation treatments. Changes in phenolics were also significantly affected by the time of infection in the field (Pillai's Trace, $F_{20,422} = 4.2$, $P < 0.001$; Figure 3). Phenolic activity levels were also higher in tubers infested earlier (Pillai's Trace, $F_{20,422} = 4.2$, $P < 0.001$). With the exception of 2 of the flavanoid hexoside derivatives, all phenolic compounds were correlated with Lso titer at the end of storage (Pearson correlation, $P < 0.030$).

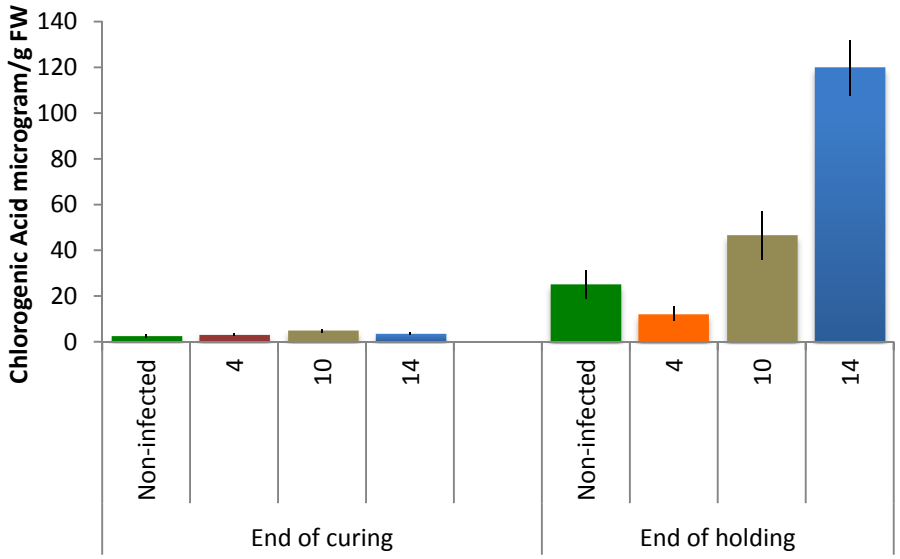


Figure 3: Changes in phenolic compounds during ramp-down stage and holding temperatures in tubers from control plants and plants infested 4, 10, and 14 days before harvest.

Results indicate that Lso continues to impact harvested tubers after harvest. Additional years of observations, a better understanding on the impact of sampling on tuber physiology, and storage temperature on Lso and ZC symptom development are needed. There are some indications suggested that Lso development within tubers would become slower in lower temperatures. Thus, some of the other post vine-kill practices, such as a pre-harvest curing period to promote skin set, which leaves tubers exposed to higher temperatures prior to storage, may also promote pathogen development. More studies are needed to provide recommendations that would reduce tubers exposure to higher temperatures after vine-kill and prior to storage holding temperatures to minimize the impact of late-season ZC infections on potato physiology and subsequently its marketability.

Acknowledgements

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References

Munyaneza, J. E. 2012. Zebra chip disease of potato: Biology, epidemiology and management. Am J Pot Res. 89: 329-350.

Rashed, A., Wallis, C. M., Paetzold, L., Workneh, F., and Rush, C. M. 2013. Zebra Chip disease and potato biochemistry: Tuber physiological changes in response to '*Candidatus* Liberibacter solanacearum' infection over time. Phytopathology, 103: 419-426.

Wallis, C. M., Chen, J., and Civerolo, E. L. 2012. Zebra chip-diseased potato tubers are characterized by increased levels of host phenolics, amino acids, and defense–related proteins. Physiol Mol Plant Pathol 78: 66-72.

Impact of Vine-kill on Lso and Zebra Chip Symptom Development in Tubers Following Late Season Infestations

Rush[1], C. M., Workneh[1], F., Paetzold[1], L., Olsen[2], N. L., Henne[1], D. C., and Rashed[3], A. [1]Texas A&M AgriLife Research; [2]University of Idaho, Idaho Falls, ID 83402; [3]University of Idaho, Aberdeen Research and Extension Center, Aberdeen, ID 83210.

Abstract

Experiments were conducted to determine the impact of vine kill on zebra chip development in potatoes infested with psyllids late in the season (near harvest). Caged potatoes were infested with bacteriliferous pyllids 7 and 2 days before, and 2 and 7 days after they were sprayed with chemicals possessing slow (Reglon mix) and fast (Sulfuric acid) vine-kill properties. Over 85% of potatoes infested 7 days before vine kill developed zebra chip, regardless of whether it was fast or slow vine kill, while those infested 2 days before vine kill had nearly 50% of tubers with zebra chip symptoms with no significant difference between the slow and fast-kill chemicals. However, none of the plants infested 2 days or 7 days after vine kill developed zebra chip. The study showed that infestation of potatoes with psyllids as late as 2 days before vine kill could result in significant zebra chip development in tubers. It appears that the disease continues to develop in tubers in the soil after vine kill.

Introduction

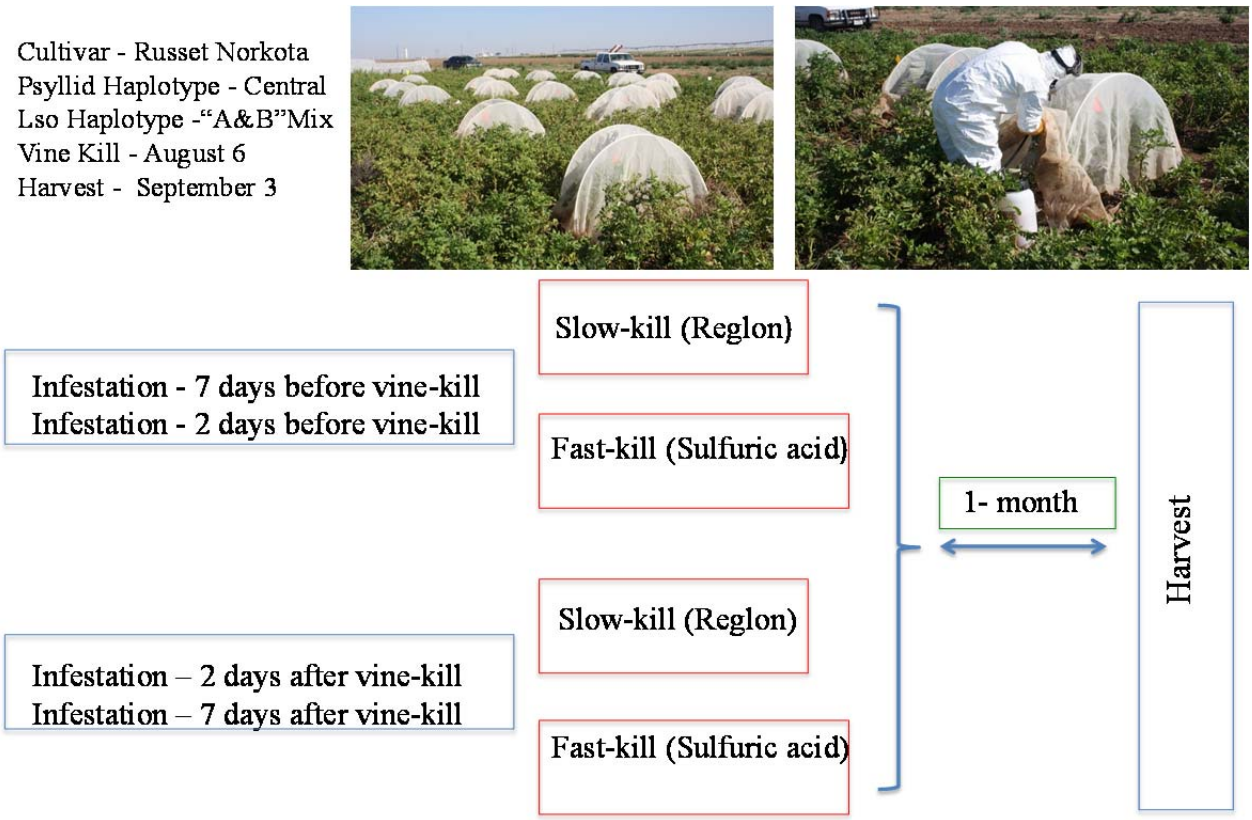
Late season infestation of potato fields by psyllids has recently been identified as a potential risk of zebra chip (ZC) development in tubers during transit or storage. Rashed et al. (2014) found that 40% of tubers harvested from potatoes infested with psyllids 4 days prior to harvest tested positive for the pathogenic bacterium '*Candidatus* Liberibacter solanacearum' (Lso) in storage, although they were neither symptomatic nor tested positive for Lso at harvest. This has enormous implications for seed and commercially stored processing potatoes, with regard to ZC development. Furthermore, many growers kill the vines several weeks prior to the intended harvest dates to allow the skins to set to reduce the likelihood of bruising and rot during storage. After vine-kill and before harvest, tubers may stay in warm soils for several weeks, in an environment conducive for multiplication of the bacterium. If Lso reaches the tubers after psyllid feeding, shortly before vines are killed, ZC could potentially develop. Although psyllids can transmit Lso within hours of feeding (Buchman et al., 2011), the likelihood of this scenario happening is unknown. Because the impact of vine-kill on translocation and multiplication of Lso in the plant had not been studied, study was initiated to determine the impact of vine kill on Lso translocation to tubers, and subsequent development of ZC in tubers from plants infested shortly before and after vine kill.

Materials and Methods

Potatoes (*cv* Russet Norkota) were planted at 9-in spacing on 30-in beds on the 10th of April 2014 under a center-pivot irrigation system at the Texas A&M AgriLife Research Station at Bushland, TX. After emergence, plots of four adjacent plants were selected and covered with a cage to serve as an experimental plot. The experiment contained 13 treatments in three replications, which included 4 psyllid infestation periods, two vine kill chemicals,, a non-vine-killed but infested positive control with

each infestation period, and a non-infested negative control. The cages were infested 7 or 2 days before vine kill (7DBVK) or 2DBVK) and 2 or 7days after vine kill (2DAVK or 7DAGK) (Fig. 1). Two types of chemicals were used in killing the vines: Reglon and Aim mix (32 and 3 oz/acre, respectively) for a slow-kill treatment, and 97% sulfuric for a fast-kill treatment. The chemicals were sprayed with a knapsack sprayer until runoff. Each plant in treatment cages (except the negative control) was infested with 6 bacteriliferous psyllids on each prescribed day (Fig. 1) and tubers were harvested 4 weeks after vine kill. Tubers from each treatment cage (4 plants/treatment) were dug and 3 tubers per plant were randomly selected, sliced at the stolon attachment end and scored for ZC severity on a 0 to 3 scale, where 0 is no apparent discoloration and 3 is severely discolored (Fig. 2, inset). The sliced tubers then were tested for Lso using qPCR (Wen, et al., 2009).

Fig. 1. Schematic representation of psyllid infestation treatment protocols



Results and Discussion

Plants which received the fast-kill treatment wilted and collapsed immediately with no detectable

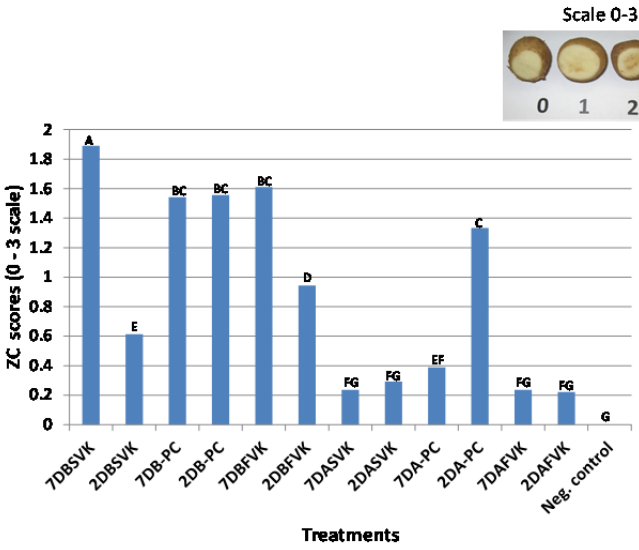


Fig. 2. Relationship between time of psyllid infestation in relation to vine kill and tuber zebra chip severity.

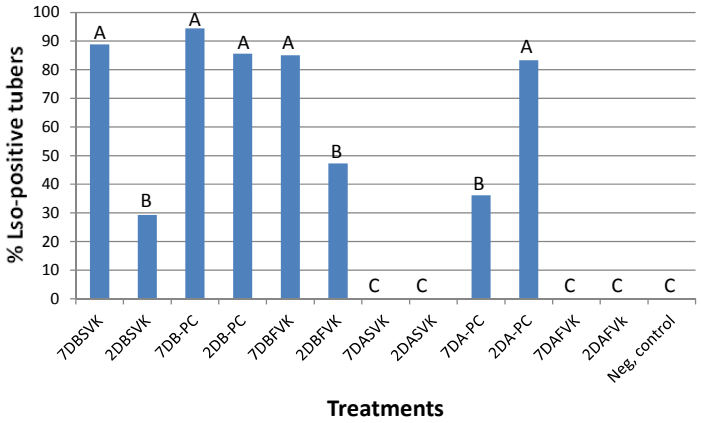


Fig. 3. Relationship between time of psyllid infestation and % tubers with Lso.

green tissue remaining. However, those which received the slow-kill treatments wilted and desiccated gradually, over a five day period. Although the leaves began to wilt within hours after treatment application, the stem tissues remained green for several days. One of the focal points of this study was to determine whether psyllids could still feed on these green stems and transmit Lso after the slow vine kill treatment, even though the leaves had completely dessicated.

Average visual scores of tuber symptom severity for all treatments ranged from near 0 to 1.9 on a

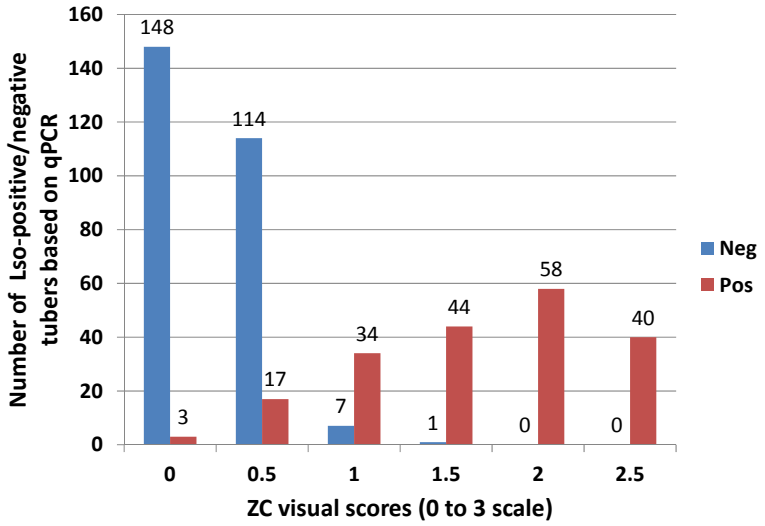
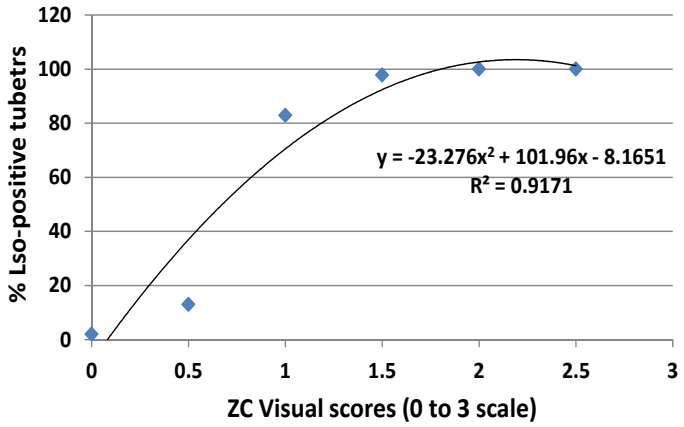


Fig. 4. Numbers of Lso-positive and negative tubers in each zebra chip visual-score category.

0 to 3 scale (Fig. 2). ZC severity levels from all treatments could generally be grouped into three categories. Treatments which received psyllid infestation 7-days before vine kill (7DBVK) had the highest ZC scores (> 1.5), regardless of whether it was fast or slow-vine kill,



Fi.g.5. Relationship between zebra chip severity scores and % Lso-positive tubers.

with ZC severity levels comparable to the positive controls (infested but non-vine killed). Over 85% of the tubers in this group tested positive for Lso (Fig. 3). The next group included those which were infested 2-days before the vine kill (2DBSVK and 2DBFVK), having ZC severity scores between 0.5 and 1. Nearly 30 and 50% of the tubers tested positive for Lso, respectively, for the slow and fast vine kill, but the difference the two was not significant (Fig. 3).
The last group included 2-days and-7 days after vine kill (2DASVK, 2DAFVK, 7DASVK, 7DAFVK). All treatments in this group (except the positive controls) had less than 0.5 ZC severity scores and there was no significant difference between this group and the negative control. Some of the sliced tubers from these treatments had slight browning (<0.3 score) but did not test positive for Lso (Fig. 3), indicating that ZC visual scores of ≤0.5 are unreliable as indicators of disease. However, there was a strong correlation (curvilinear) between visual scores >0.5 and Lso titer (Figs. 4 and 5).
In summary, the study showed that Lso can translocate from a feeding psyllid to tubers in $\underline{<}$ 2 days, and that zebra chip continues to develop in these infected tubers after vine kill but before harvest.

Acknowledgements
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References
Buchman, J. L., Sengoda, V. G., and Munyaneza, J. E. 2011. Vector transmission efficiency of Liberibacter by Bactericera vockerelli (Hemiptera: Triozidae) in zebra chip potato disase:effects of psyllid life stage and inoculation access period. J. E. Entomol. 104:1486-1495.

Rashed, A., Workneh, F., Gray, J., Paetzold, L., and Rush, C. M. 2014. Zebra chip disease development in relation to plant age and time of infection of '*Canididatus* Liberibacter solanacearum' infection. Plant Dis. 98:24-31.

Wen, A., Mallik, I., Alvarado, V. Y., Pasche, J. S., Wang., X., Li, W., Levi, L., Lin, H., Scholthoff, H. B., Mirkov, T. E., Rush, C. M., and Gudmestad, N. C. 2009. Detection, distribution, and genetic variability of '*Candidatus*' Liberbacter species associated with zebra complex disease of potato in North America. Plant Dis. 93:1102-1115.

Potato Psyllid Scouting App Development (PsyllidScout)

Bradshaw, J. D.

University of Nebraska-Lincoln, Panhandle Research and Extension Center, Scottsbluff, NE 69361

Abstract

Extension could be seen as facing a crossroads in communication. Our now digitally-connected world is quickly evolving tools and technologies that guide our communications and decisions across a vast relational network. Why should our data not do the same? Reported here is the 2015 update on a project that aims to both simplify data collection and stream it in real-time. This project will provide a tool to integrate a fast potato-psyllid scouting procedure, data archive, and a point-map of treatment decisions. The application uses a binomial sampling method for potato/tomato psyllid monitoring as its core decision function (Butler and Trumble 2012). This sampling plan and delivery strategy attempts to borrow from the success of other binomial or "speed scouting" sampling plans (Giles et al. 2003, Hodgson et al. 2004, Butler and Trumble 2012). This project aims to simplify scouting procedures and reduce management costs.

Introduction

The way in which we approach pest monitoring is at a crossroads. The definition of "community" has evolved considerably in recent years; from a world dominated by face-to-face interactions and traditional mass media to today's more personalized social networking websites. Connectivity through cell phones, more specifically "smartphones" with internet capabilities, has played a pivotal role in this transition with adoption rates surpassing those set by early internet users. In addition to this connectivity, the mobile nature of smartphones allow "on-the-go" access of information during normal daily activities.

Given this movement towards wireless information delivery and increased use of social media platforms, Extension programs need to transform the way in which information is delivered and how we interact with stakeholders. Recent advances in mobile technologies and modes by which we access data have paved the way for more efficient, real-time approaches to connect pest monitoring and management information with pest management practitioners over wide geographic areas. Without easily-accessible information and IPM recommendations agricultural producers will fall back on non-sustainable, reduced-risk approaches that include prophylactic use of pesticides.

The goal of this project is to expand the accessibility of the existing potato/tomato psyllid monitoring program by using mobile technologies and web-based tools. Simply building a static website on a mobile device is not enough and does not adequately facilitate real-time decision making. In addition, a positive user-experience leads to increased use and rapid integration of relevant stakeholder inputs. Therefore, a major undertaking of this project is to define elements needed to sustain user interests in this monitoring system. We use open source software to develop the core program, which will ultimately lead to a more sustainable software platform that not only adapts to changing technologies, but also easily integrates added-value features with minimal cost.

Materials and Methods

A beta (i.e., development) version of a potato psyllid management mobile-compatible application (PsyllidScout) is in available for use (see Fig. 1, http://em-dev002.unl.edu/testpsyllid/index.php). It is has been developed as a web-based application (available from any mobile or desktop device) with the hopes of developing a mobile-OS specific apps for Apple and Android markets in the near future. The

Figure 1. Diagrammatic representation of some of the capabilities of PsyllidScout.

platform is developed on the Drupal content management service and the data are managed through a MySQL database. The interface design presents the user with two main fields – a scouting field (with its stepwise function outlined in the yellow box) and an interactive sample data archive (outlined in the blue box). Activating the "Start scouting" feature will guide the user through the binomial sequential sampling plan for the potato psyllid in potatoes (Butler and Trumble 2012). As detailed in the app, the user, essentially, observes the upper canopy of a plant and taps "yes" for the presence (the indicators default to "no") of potato psyillid egg, nymphs, or adults for a series of plants. These demographic data are summed together to feed into the sampling plan. The reason for collecting the demographic data is to facilitate the monitoring of psyllid population development for use in general survey collection of treatment efficacy evaluation. The sampling process is continued as directed by the application. These data are then recorded and archived by the software. Additionally, on the main screen the user can select to view currently infested fields by insect stage. Tapping on "view details" will reveal all of the seasonality data for all stages for that selected field. The user can use these data to evaluate the effectiveness of their treatment actions in those fields. Finally, as researchers update the scouting plan (or as users provide feedback) the formulae or other features can be seamlessly updated similarly to other apps.

Results and Discussion

The next step in PsyllidScout development is to develop an "action map". Currently, a map, such as the one to the right, just displays the registered fields. However, the intent for the map as we continue to develop and aggregate users will be to display a color-coded map of treatment recommendations. These recommendations are the outputs from the sampling procedure in the yellow box above (treat, do not treat, resample in 1 week). These recommendations could be represented on the map as color-coded pins. For example, the map pins to the right could indicate field locations for *all users* and red could equal a "treat" recommendation and blue pins could equal a "do not treat" recommendation. These recommendations could then be archived and displayed for the last sampling results for all locations and might also be archived so the progression of treatment recommendations could be tracked throughout the season. With such a tool the user could then see which fields in the area are at an economic threshold and how that threshold may or may not be spatially encroaching on the user's location. This could allow a farm manager greater warning in planning for treatment supplies and logistics.

References

Butler DC and JT Trumble. 2012. Spatial dispersion and binomial sequential sampling for the potato psyllid (Hemiptera: Triozidae) on potato. *Pest Management Science* 68(6): 865-869.

Giles KL, DJ Jones, TA Royer, NC Elliott, and SD Kindler. 2003. Development of a sampling plan in winter wheat that estimates cereal aphid parasitism levels and predicts population suppression. *Journal of Economic Entomology* 96(3): 975-982.

Hodgson EW, EC Burkness, WD Hutchinson, and DW Ragsdale. 2004. Enumerative and bionomial sequential sampling plans for soybean aphid (Homoptera: Aphididae) in soybean. *Journal of Economic Entomology* 97(6): 2127-2136.

Interactions of Potato Psyllids, Plant Virus, and '*Candidatus* Liberibacter solanacearum'

Prager, Sean M.[1], Wallis, Christopher[2], Trumble, John T. [1]
[1]Department of Entomology, University of California, Riverside, Riverside, CA
[2] USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier, CA

Abstract
Plant pathogens can influence many aspects of an insect's biology. This includes behavior, development, and efficiency as a vector. These factors are often studied as pairwise questions within a tripartite interaction between a plant host, a pathogen, and an insect vector. Here an expanded multi-partite system with two plant pathogens, a single insect vector, and a common host plant was studied. Using this model system consisting of '*Candidatus* Liberibacter solanacearum' (Lso), *Tobacco mosaic virus* (TMV), potato psyllids, and tomato plants, the influence of TMV infection on potato psyllids behavior and transmission of Lso was tested. Further, primary and defensive metabolites were examined to determine if shifts in host physiology were behind potential interactions.

Introduction
Typically the interactions of plants, pathogens, and vectors are considered in isolation. The three actors in these tripartite relationships are assumed to influence each other, but not to be influenced by additional factors. This assumption simplifies already complex scenarios. However, fields of crops such as potatoes or tomatoes are really mosaics, with multiple pathogens and insect pests.

It is well documented that pathogens can alter the behaviors of insects. This can be a direct effect of the pathogen on the insect, or it can result from physiological changes in the plants as a result of infection. Studies of these physiological changes typically focus on how a given pathogen influences its own vector. What is poorly understood and studied is how infection with a pathogen might influence another insect vector.

The various choices a vector makes with respect to feeding and oviposition has profound implications. Those plants on which the insect feeds are likely to be infected, while those that are avoided will not. Similarly, if an insect oviposits on a more suitable plant, it should result in more eggs, or more offspring developing. This will, in turn, result in greater pest densities in the field. Consequently, if a factor such as infection with a pathogen influences an insect vectors choices, it may alter the levels of infection and damage to a crop field. Here the combination of tomato (*Solanum lycopersicum*), *Tomato spotted wilt virus*, and the tomato-potato psyllid (*Bactericera cockerelli*) was used to determine if a pathogen other than Lso would induce physiological changes in plants that alter potato psyllid behavior, and Lso transmission.

Materials and Methods
To determine whether infection with TMV influences the attractiveness of tomato plants, a series of choice experiments were performed. In the experiments, 50 adult psyllids were placed in rectangular screen cages (Bioquip 36" jumbo cages, Bioquip, Rancho Dominguez, CA). Each cage contained one intact uninfected tomato plant, and one plant that had been mechanically infected with TMV. The number of psyllids on each plant was recorded daily for five days, after which the number of eggs on each plant was counted. The study was replicated 10 times.

Since infection with a pathogen can influence the suitability of a plant for insect development, a series of no-choice bioassays was also performed. No-choice bioassays were conducted by caging two male: female pairs (4 total) of post-teneral *B. cockerelli* onto a terminal leaflet of either an uninfected or

TMV-infected tomato plant using white 10.16 x 15.25 cm mesh sachet bags (JoAnn Fabric and Craft Stores, Hudson, OH). Psyllids were caged on plants for 48 h after which the psyllids were removed and the number of eggs counted. Plants were subsequently inspected daily to determine the number of eggs, small (1st or 2nd instar) nymphs, large (3th to 5th instar) nymphs, and adults. Counting proceeded until all individuals died, failed to hatch, or emerged as adults. The study was replicated 10 times.

In order to determine whether infection with TMV influences transmission of Lso, an additional set of no-choice bioassays was performed. These experiments were conducted using a similar method to the previous no-choice bioassays described above. However, following the 48 h exposure period, psyllids were stored in 70% ethanol, eggs were removed without excising the leaflet, and plants were retained for 14 days in a climate controlled rearing room at 27°C. Lso titers in both plants and insects were then evaluated via quantitative real-time PCR (qPCR) using the methods of Butler et al. (2011).

Since changes in psyllid biology relative to TMV infection are likely to have been mediated by changes in plant physiology, a series of biochemical analyses were performed to evaluate the influence of TMV infection on plant physiology. All bioassays were performed on plants grown under identical conditions to those use in other bioassays. Both TMV-infected and uninfected plants were examined. Primary (amino acids, sugars), and defensive (phenolics, terpenoids) metabolite levels were analyzed according to the previously described procedures of Rashed et al. (2013) and Wallis et al. (2008; 2012).

Results and Discussion

When potato psyllids were presented a choice of TMV infected or uninfected tomato plants, they choose to settle on the uninfected plants significantly more often than on plants infected with TMV (PERMANOVA: $F=5.14$; $df=1,18$; $p<0.05$, $R^2=0.22$; Fig. 1). Similarly, following the 96 h period, more eggs were laid on uninfected control plants than on plants infected with TMV (TTEST: $t=4.06$, $p<0.001$, $df=16.6$; Fig. 2). A similar pattern as observed in no-choice bioassays where once again more eggs were laid on uninfected plants (TTEST: $t=3.24$, $p<0.001$, $df=18.75$; Fig. 3). Despite the differences in oviposition rates, there were no significant differences in growth index (a measure of development) between plants infected with TMV and control plants (TTEST: $t=-1.11$, $p=0.29$, $df=12.6$; Fig. 4). Collectively, these results suggest that psyllids prefer healthy (uninfected) plants to those with TMV. The preference apparently leads to increased oviposition on these plants but these choices do not translate to better development. This is interesting because it suggests that, contrary to theory, females do not lay eggs on plants that are better for their eggs to develop.

In Lso transmission experiments, significantly fewer plants infected with TMV tested positive for Lso than control plants (Chi-square: $X^2=4.233$, $p<0.05$, $df=1$; Figure 5). It is unclear if these results are due to a change in the ability of plants infected with TMV to defend against Lso, or a result of the behavior of the vector. However, since psyllids settle and oviposit less often on plants infected with TMV, it is likely that they are also feeding less on these plants. Reduced feeding would be expected to result in less transmission of Lso, and would explain the observed patterns.

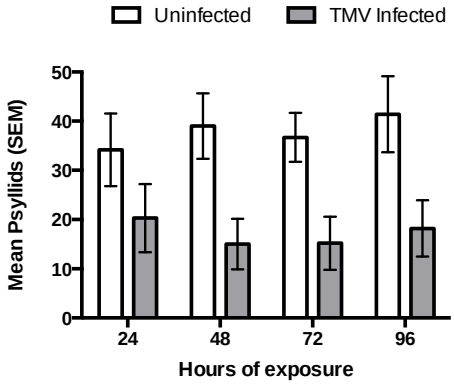


Figure 1: The mean potato psyllids settling on tomato plants infected with tobacco mosaic virus (grey bars), or on uninfected control plants (white bars), over a 96 hour period.

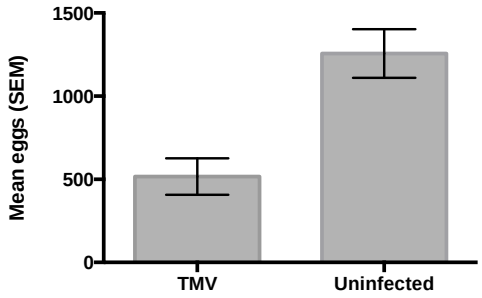


Figure 2: The mean number of eggs laid on plants with tobacco mosaic virus or on uninfected control plants, when presented a choice.

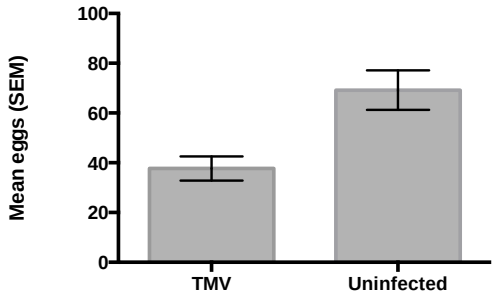
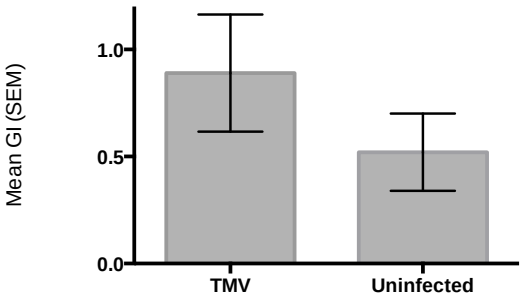


Figure 3: The mean number of eggs laid on plants infected with tobacco mosaic virus or on uninfected control plants in no-choice bioassays.

Figure 4: The mean growth index for plants infected with TMV and for uninfected control plants.



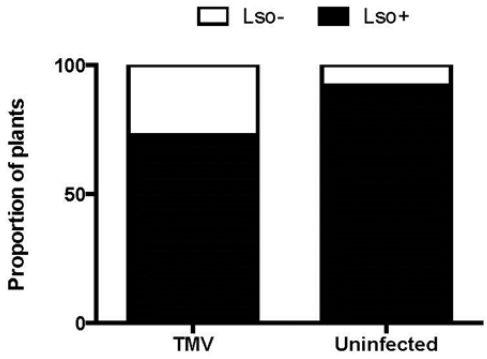


Figure 5: Proportion of plants infected with TMV and control plants that tested positive for Lso.

Finally, when plants infected with TMV and control plants were examined for differences in primary and defensive compounds, there were some important patterns revealed. Among the 15 amino acids examined, there was an overall difference between infected and control plants (MANOVA: Pillai's trace $\Delta = 0.975$; $F = 15.302$; $P = 0.001$). However, only a single amino acid, proline, differed individually. Similarly, there was a significant overall difference in phenolic compounds (MANOVA: Pillai's trace $\Delta = 0.975$; $F = 15.302$; $P = 0.001$), but again there were few consistent trends, with differences only observed in levels of caftaric acid, protocatechulic acid, quinic acid, and isoquercetin. In the analysis, six monoterpenoids were examined and an overall trend was observed (MANOVA: $\Delta = 0.946$; $F = 11.756$; $P = 0.001$), with the sole individual difference detected in levels of eucalyptol ($F=15.3$, $p<0.001$, $df=2, 23$). Interestingly, eucalyptol levels were higher in TMV-infected plants, and eucalyptol is a known repellent. This might explain some of the observed avoidance of infected plants, but eucalyptol is also insecticidal although no such effects were observed in our experiments. Finally, there was a significant overall difference in the levels of sugars between control and TMV-infected plants (Overall: $F = 5.727$; $P = 0.011$; Fig. 5). This was associated with specific differences in both fructose ($F = 6.144$; $P < 0.022$) and glucose ($F = 11.996$; $P = 0.002$). These results indicate more sugar in uninfected control plants. Since psyllids feed primarily on sugars distributed through the phloem, a plant with higher levels of sugars would be more attractive to psyllids. This increase in sugar may therefore explain the many of the patterns observed in the various bioassays. Psyllids probably prefer to feed on uninfected plants that produce more sugar. This preference would, in turn, result in more settling and more oviposition on those plants. Finally, because feeding is reduced on TMV-infected plants, there is reduced transmission of Lso in infected plants.

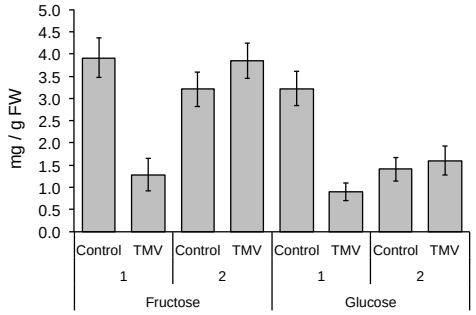


Figure 6: The mean (SEM) levels of sugars in TMV infected and uninfected control plants. Two sets of plants were examined for each of glucose and fructose.

Potato fields are often subject to multiple infections. For example, tomatoes and potatoes can be infected with both TMV and Lso. This study indicates that infection with TMV will in fact alter the dynamics of Lso infection and vector behavior. As a consequence, Lso is likely to spread differently in fields that are also being infected with TMV. Understanding how these patterns of disease spread will have implications for management decisions. TMV has recently been proposed as a delivery mechanism for RNAi-based management of potato psyllids (Wuriyanghan & Falk, 2013). However, the studies found that plants containing RNAi treated with TMV has less oviposition. The results presented here indicated that TMV may be not be effective for delivery, as the psyllids will avoid infected plants. Although, they also suggest that attenuated TMV may be an effective management tool as a repellent. Finally, the association of eucalyptol and avoidance by the psyllids indicates that it too may be useful as a repellent.

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References
Butler, C.D., Byrne, F.J., Keremane, M.L., Lee, R.F. & Trumble, J.T. (2011) Effects of insecticides on behavior of adult *Bactericera cockerelli* (Hemiptera: Triozidae) and transmission of *Candidatus* Liberibacter psyllaurous. *Economic Entomology*, 104, 586–594.

Rashed, A., Wallis, C.M., Paetzold, L., Workneh, F. & Rush, C.M. (2013) Zebra chip disease and potato biochemistry: Tuber physiological changes in response to "*Candidatus* Liberibacter solanacearum" infection over time. *Phytopathology*, 103, 419–426.

Wallis, C.M., Chen, J. & Civerolo, E.L. (2012) Zebra chip-diseased potato tubers are characterized by increased levels of host phenolics, amino acids, and defense-related proteins. *Physiological and Molecular Plant Pathology*, 78, 66–72.

Wallis, C., Eyles, A., Chorbadjian, R., McSpadden-Gardner, B. Hansen, R., Cipollini, D., Herms, D. & Bonello, P. (2008) Systemic induction of phloem secondary metabolism and its relationship to resistance to a canker pathogen in Austrian pine. *New Phytologist*, 177, 767–778.

Wuriyanghan, H. & Falk, B.W. (2013) RNA Interference towards the potato psyllid, *Bactericera cockerelli*, Is induced in plants infected with recombinant Tobacco mosaic virus (TMV). *PloS one*, 8, e66050.

Effects of '*Candidatus* Liberibacter solanacearum' Infections on the Physiology of Tubers at Different Storage Temperatures

Wallis, C. M.[1], Rashed, A.[2], Workneh, F.[3], Paetzold, L.[3], and Rush, C. M.[3]
[1]USDA-ARS-SJVASC, 9611 S. Riverbend Ave, Parlier, CA 93631, USA; [2]University of Idaho, Aberdeen, ID 83210, USA; [3]Texas AgriLife Research, Amarillo, TX 79109, USA

Abstract
Zebra chip disease (ZC), associated with infection by the bacterium '*Candidatus* Liberibacter solanacearum' (Lso), is an emerging disease causing substantial economic loses for potato growers and processors in North America and New Zealand. In general, many Lso-infected tubers will be rejected by processors upon harvest due to conspicuous browning symptoms. However, when tubers are infected by Lso within two weeks of harvest no apparent symptoms may be present at harvest, but undesirable browning may develop while the tubers await processing in storage. This study was aimed to determine ZC symptom development, Lso titer changes, and associated physiological shifts in tubers from two different potato cultivars stored for 12 weeks that were asymptomatic at harvest but positive for Lso. In addition, to observe whether holding temperatures could affect ZC development, tubers were kept at three different storage temperatures (3°C, 6°C, or 9°C). Fresh and fried ZC symptoms were less severe in Red La Soda kept at 6°C than 3°C, and fresh ZC symptoms were less in Russet Norkotah kept at 6°C than 3°C. However, Lso titers were greater in tubers kept at 6°C and 9°C than those kept at 3°C. Physiological shifts were variable between tubers throughout the study, and no firm conclusions could be made about effects of temperature or time in storage on tuber metabolism. Taken together, these results demonstrate that ZC development is highly variable in tubers kept in storage, and this poses a problem using existing detection methods to cull stored infected tubers.

Introduction
Zebra chip disease (ZC), which is associated with infection by 'Candidatus *Liberibacter solanacearum*' (Lso) that is vectored by the potato psyllid *Bactericera cockerelli* (Sulc) (Hemiptera, Triozidae), is of concern to potato growers in North America due to its capacity to render tubers unmarketable. Specifically, ZC-affected tubers exhibit increased browning when freshly cut or fried to make potato chips. These symptoms were observed to be associated with changes in tuber physiology that occur following Lso infection (Wallis et al. 2012; Rashed et al. 2013; Wallis et al. 2014). In particular, increased phenolic and polyphenol oxidase levels would result in increased browning of ZC-affected tubers when freshly cut (Wallis et al. 2012), whereas increased amino acid and reducing sugar levels would result in increased browning in fried potato slices (Rashed et al. 2013; Wallis et al. 2014). Processors could reject Lso-infected tubers displaying ZC symptoms prior to storage or processing. However, both ZC symptoms and significant changes in physiology take roughly two to three weeks after infection to develop (Rashed et al. 2013), which opens up the possibility that tubers infected just prior to harvest could still be placed in storage. These late-season infected tubers could in turn allow Lso infections to proceed further, with symptoms apparent when stored tubers are finally processed. However, little is known about the development of ZC in stored tubers. It is plausible that certain storage conditions, such as low temperatures, could reduce ZC symptom progression and therefore mitigate losses due to ZC from reception of late-season Lso-infected tubers. Thus, this study was conducted with the aim to observe ZC symptom progression, Lso titers, and changes in ZC-associated tuber physiological changes of tubers infected one week prior to harvest and stored at three different

storage temperatures [3°C (37°F), 6°C (43°F), or 9°C (48°F)] for 12 weeks. These results should help to determine optimal long-term storage temperatures for tubers that are potentially Lso-infected.

Materials and Methods

Six each of caged Red La Soda and Russet Norkotah potato plants were inoculated in the field at Bushland, TX, by releasing six Lso-positive potato psyllids per cage and allowing feeding for one week. An equivalent number of cage plants were set aside for controls. Following the inoculation, all of the tubers were then harvested, had tissue samples (~ 300 mg of tissue) taken with a cork borer from the apical end, and placed in storage at 3° C, 6° C, or 9° C. Additional sampling using a cork borer was done two, four, eight, and twelve weeks later. An aliquot of each sample was used to analyze Lso titers according to the methods outlined by Rashed et al. (2013). The remaining samples were frozen and shipped to the USDA-ARS for biochemical analyses according to the procedures below. At 12 weeks, the tubers had fresh and fried symptoms assessed by making full slices at the apical ends. Disease severity was assest on a 0 (no symptoms) to 3 (severe symptoms) scale (Rashed et al. 2013). For chemical analyses, a total of 50 mg of tuber tissue per sample was placed into two microcentrifuge tubes. One of these aliquots was extracted for 2 days at 4°C in methanol (1:10 w/v, e.g. 50 mg tuber tissue extracted in 500 mL of methanol) for phenolic analysis by high performance liquid chromatography (HPLC) (Wallis et al. 2012). The other aliquot was extracted over 2 days at 4°C in PBS buffer (1:10 w/v) for amino acid analysis via derivatization and gas chromatography using a commercially available kit (Rashed et al. 2013). Additional PBS buffer extract was used to analyze fructose and glucose levels by ion-exchange HPLC or (Rashed et al. 2013). Statistical analyses were performed by SPSS ver. 11.0 (IBM, Armonk, NY). In brief, differences due to storage temperature for each cultivar in disease symptoms using univariate ANOVA. For differences in Lso titers, two-way ANOVAs were performed with weeks in storage, storage temperature, and the interaction as factors. For compound levels, two-way ANOVAs were performed with infection status, storage temperatures, and the interaction as factors. Each sampling time was analyzed separately for compound level analyses. Significant ANOVAs were followed by multiple comparison tests when appropriate. For simplification, individual levels of compounds with the same compound class (amino acids, sugars, or phenolics) were summed together for analysis. This provided an overview of physiological shifts in tubers while in storage.

Results and Discussion

Storage temperature significantly affected fresh (F = 6.694; P = 0.003; N = 45) and fried (F = 5.198; P = 0.010; N = 45) symptom severity for Lso-infected Red La Soda tubers (Fig. 1). Storage temperatures also affected fresh symptom severity for Russet Norkotah tubers (F = 3.661; P = 0.034; N = 45), but did not significantly affect fried symptom severity for Russet Norkotah tubers (F = 1.345; P = 0.272; N = 45) (Fig. 1). Fresh symptom severity for both cultivars was greater at 3°C than at higher temperatures (Fig. 1). However, fired symptom severity was less affected by storage temperature, with the 6°C storage temperature having reduced severity for Red La Soda tubers than those kept at 3°C or 9°C. Lso titers varies significantly due to storage temperature for Red La Soda tubers (F = 3.209; P = 0.043; N = 180), but did not vary significantly be week sampled (F = 1.225; P = 0.302; N = 180) nor was there a storage temperature by week interaction (F = 0.645; P = 0.739; N = 180) (Fig. 2). Lso titers were significantly affected by storage temperatures (F = 4.222; P = 0.016; N = 176) and sample week (F = 2.442; P = 0.049; N = 176) for Russet Norkotah, and the interaction was not significant (F = 1.593; P = 0.131; N = 176) (Fig. 2). For both potato cultivars, tubers kept at 9°C had greater Lso titers than those kept at 3°C. Red La Soda tubers kept at 6°C also had greater titers than those kept at 3°C. For Russet

Norkotah, samples from week 12 had greater titers than those at weeks 0, 2, and 4. Likewise, samples from week 8 had greater titers than those at weeks 0 and 4.

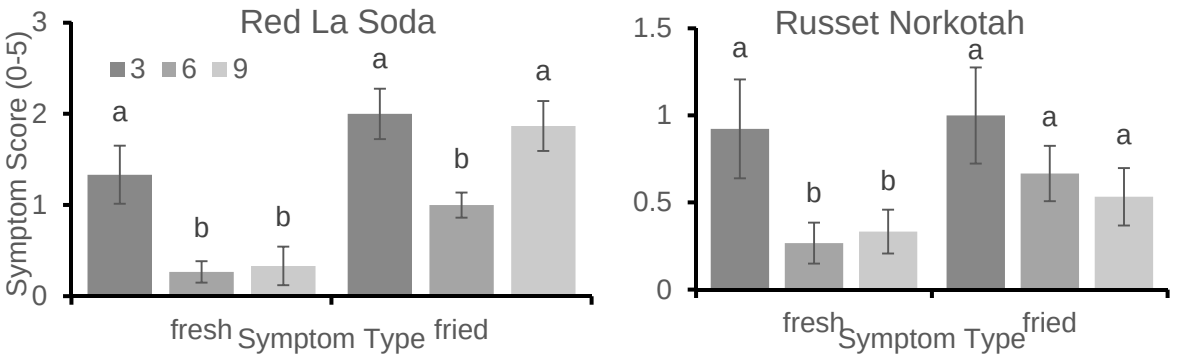

Figure 1. Mean (± SE) fresh and fried symptom severity for Red La Soda and Russet Norkotah tubers assessed after 12 weeks of storage at 3°C, 6°C, or 9°C. Letters represent significant differences due to LSD tests.

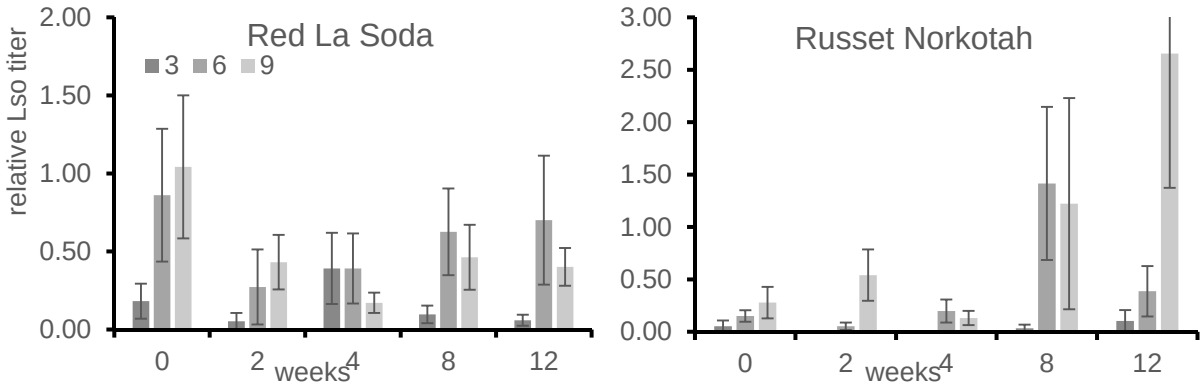

Figure 2. Mean (± SE) Lso titers over the duration of the study from Red La Soda and Russet Norkotah tubers stored at 3°C, 6°C, or 9°C.

Regarding host chemistry, Red La Soda tuber amino acid levels were not generally significantly affected ($P > 0.05$) by storage temperature, infection status, or the interaction, with the exception of storage temperature on samples taken 8 weeks after harvest ($F = 6.360$; $P = 0.003$; N = 57), which showed greater levels of amino acids in tubers kept at 3°C than those at higher temperatures (Fig. 3). Russet Norkotah tuber amino acids levels also were generally unaffected ($P < 0.05$) by storage temperature, infection status, or the interaction with the exception of storage temperature on samples taken 8 weeks after harvest ($F = 4.020$; $P = 0.024$; N = 59), which also showed greater levels of amino acids in tubers kept at 3°C than those at higher temperatures (Fig. 3). Likewise, initial (0 weeks) amino acid levels in Russet Norkotah varies due to assigned temperature (so initial levels were different) ($F = 8.024$; $P = 0.010$; N = 58), and a significant interaction also was observed ($F = 5.165$; $P = 0.009$; N = 58). Reducing sugar (fructose and glucose) levels in Red La Soda tubers were consistently significantly greater in non-infected controls than Lso-infected tubers after the initial sampling ($F = 68.729$; $P < 0.001$; N = 60 for week 2; $F = 37.510$; $P < 0.001$; N = 60 for week 4; $F = 5.647$; $P = 0.021$; N = 57 for week 8; and $F = 14.769$; $P < 0.001$; N = 52 for week 12) (Fig. 4). Also for Red La Soda tubers, sugar levels were significantly greater ($F = 24.984$; $P < 0.001$; N = 60) in tubers kept at 3°C than the other temperatures four weeks after harvest, and greater ($F = 5.936$; $P = 0.005$; N = 52) in tubers kept at 3°C

than 9°C at twelve weeks after harvest. There were significant interactions at four (F = 4.725; P = 0.013; N = 60) and twelve weeks (F = 4.183; P = 0.021; N = 52) after harvest for Red La Soda tubers. For Russet Norkotah tubers, sugar levels were significantly greater in non-infected than Lso-infected tubers eight (F = 7.394; P = 0.009; N = 56) and twelve (F = 5.003; P = 0.031; N = 46) weeks after harvest (Fig. 4). There was a significant interaction for Russet Norkotah sugar levels two weeks after harvest (F = 6.801; P = 0.002; N = 60).

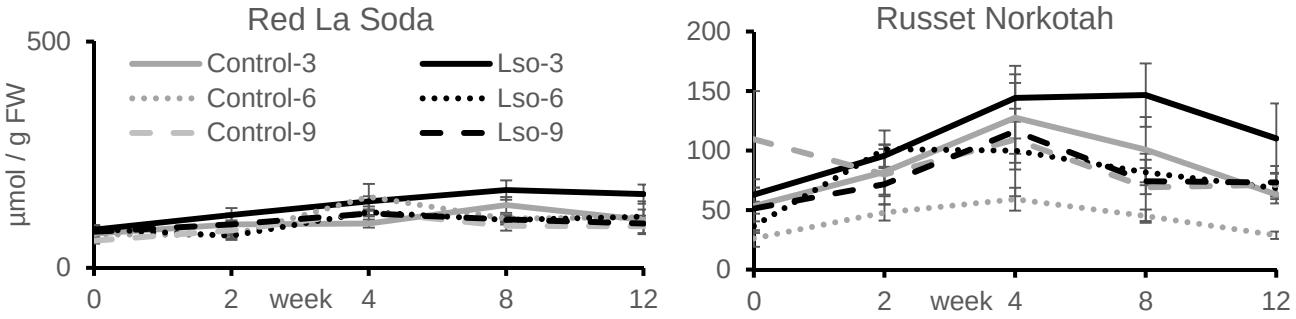

Figure 3. Mean (± SE) amino acid levels for non-infected or Lso-infected Red La Soda or Russet Norkotah tubers placed in storage at 3°C, 6°C, or 9°C over time.

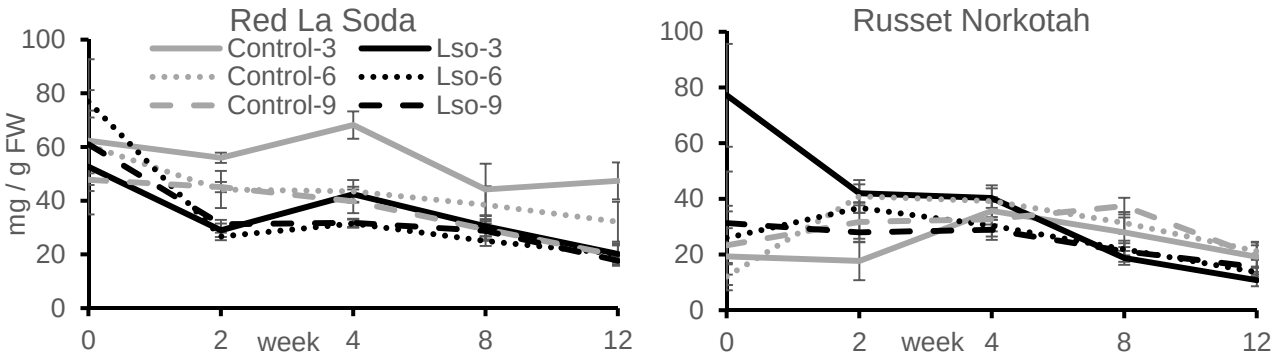

Figure 4. Mean (± SE) reducing sugar (fructose and glucose) levels for non-infected or Lso-infected Red La Soda or Russet Norkotah tubers placed in storage at 3°C, 6°C, or 9°C over time.

Regarding phenolic levels, both chlorogenic acid and flavonoid levels in Red La Soda tubers generally did not significantly differ, although there was significantly more flavonoids in Lso infected tubers at harvest (F = 32.756; P < 0.001; N = 60) and a significant interaction in chlorogenic acid levels at week eight (F = 3.845; P = 0.028; N = 57) (Fig. 5). For Russet Norkotah tubers, phenolic levels also did not generally significantly differ, albeit storage temperature affected flavonoid levels at eight (F = 7.504; P = 0.001; N = 59) after harvest, with greater levels in tubers kept at 3°C than both 6°C and 9°C (Fig. 5). There were also significant interactions initially after harvest (F = 3.256; P = 0.046; N = 60) and after two weeks (F = 3.491; P = 0.038; N = 60) for chlorogenic acid levels in Russet Norkotah tubers. To conclude, these results determined that tubers should be kept at 6°C instead of 3°C to limit the development of fresh and fried symptoms, albeit conclusions were clearer for Red La Soda than Russet Norkotah tubers. Paradoxically, Lso titers were lower at 3°C than at other temperatures, but this could be an artifact of the sampling process whereby only the apical end was taken throughout the experiment. Regarding host chemistry, amino acids in generally appeared greater in infected tubers than those non-infected whereas sugar levels where greater in non-infected controls, albeit this was not always significant. It could be that in storage the processes tubers can use to increase sugar content to combat

pathogen-induced stresses are compromised, and Lso consumption of sugars led to a net decline in sugar levels over time. Finally, levels of phenolics remained generally unaffected by infection or storage temperatures in this study, in contrast to previous studies where levels increased greatly over infection duration, albeit when phenolics were assessed in freshly-harvested tubers (Rashed et al. 2013; Wallis et al., 2014). Taken together, the data from this project suggests changes in tubers infected late in the season can be quite variable. Because ZC symptoms development and associated phytochemical changes is variable in storage, it would be challenging to detect Lso infected tubers in storage.

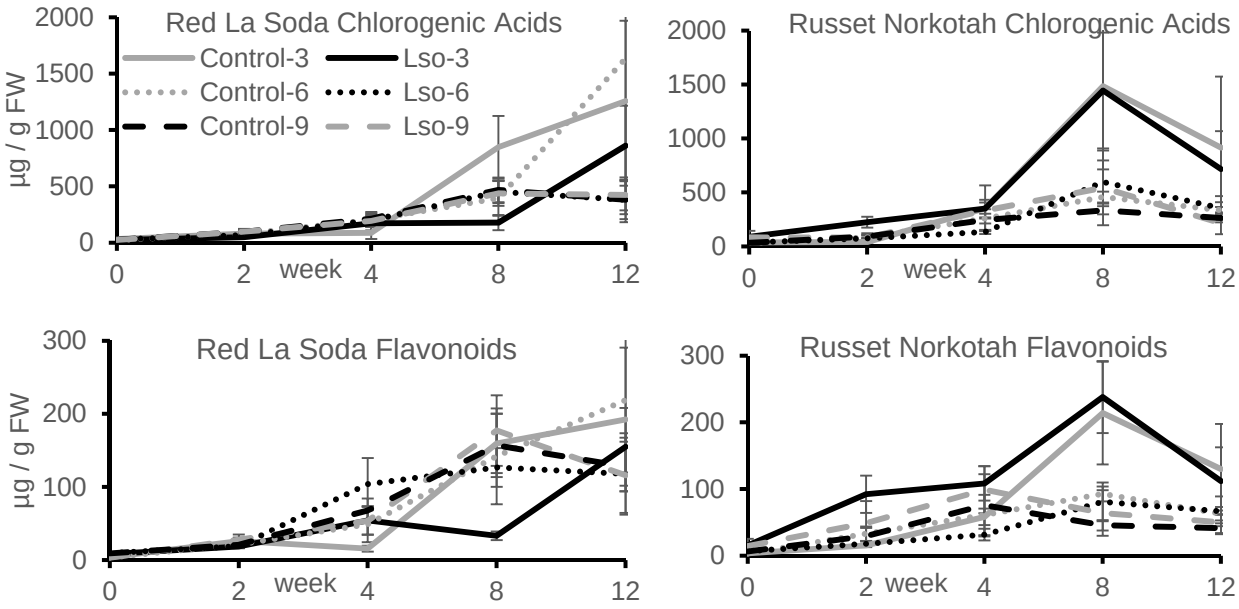

Figure 5. Mean (± SE) phenolic levels for non-infected or Lso-infected Red La Soda or Russet Norkotah tubers placed in storage at 3°C, 6°C, or 9°C over time.

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

Wallis, C. M., Chen, J., Civerolo, E. L. 2012. Zebra chip-diseased potato tubers are characterized by increased levels of host phenolics, amino acids, and defense–related proteins. Physiol. Mol. Plant Pathol. 78: 66-72.

Wallis, C. M., Rashed, A., Wallingford, A. K., Paetzold, L., Workneh, F., Rush, C. M. 2013. Similarities anddifferences in physiological responses to '*Candidatus* Liberibacter solanacearum' infection among different potato cultivars. Phytopathology 104:126-133.

Resistance Evaluation and Insecticide Rotation Programs for Control of Potato Psyllids

Trumble, J. T. and Prager, S. M. Department of Entomology, University of California, Riverside, Riverside, CA

Abstract

Control of zebra chip disease currently is highly dependent on chemical control of the potato psyllid vector. For most growers, effective control requires the repeated use of a relatively small set of compounds. Repeated use of a limited set of insecticides is likely to result insects developing resistance. In this study psyllids from Texas, the Pacific Northwest and California were evaluated for resistance development to spirotetramat and abamectin in the laboratory. In addition, field studies were conducted to evaluate various insecticide rotations containing new and alternate materials that may be useful in control of psyllids. The availability of such new materials will allow for use of a greater variety of modes of action and can therefore reduce risk of resistance developing.

Introduction

Under the current conditions of potato production, management of zebra chip disease is accomplished almost exclusively through management of the potato psyllid vector. Psyllid control is accomplished with repeated insecticide applications with a relatively limited set of materials that are used by most operations in most years. For example, Guenther et al. (2012) reported that abamectin was being used on as much as 67% of fields in Texas and 42% in Kansas and Nebraska. Similarly, spirotetramat was used on 100% of fields in Texas, Kansas, and Nebraska. This frequent and repeated use increases the likeliness of resistance development (Prager et al. 2013), which poses a threat to management strategies for potato psyllids and zebra chip. Further, because of the psyllids' threat to non-potato crops and varying levels of adoption of successful insecticide management strategies, some crops are still treated with broad-spectrum insecticides that are both harmful to the environment and workers.

In this proceeding, we report the results of two sets of experiments intended to partially address these issues. In a first set of experiments, we compared insecticide susceptibility to abamectin and spirotetramat for three colonies of potato psyllids. The colonies were collected in Texas in 2006 and 2012/2014, and in the Pacific Northwest. The psyllids collected in 2006 are known to be generally susceptible to most of the pesticides used. However, by 2012, high levels of resistance had developed to imidacloprid in Texas populations (Prager *et al.* 2013). This led to an even greater reliance on the remaining compounds. One goal of this research was to determine the potential resistance development for two of these remaining pesticides. A second goal was to test insecticidal rotation programs featuring new and alternative compounds that have the potential to supplement and replace some commonly used materials, allowing for greater rotation of modes of actions, and reduced risk of resistance development.

Materials and Methods

Insecticide resistance trials were performed using the previously established methods of Prager *et al*. (2013). In brief, these consist of treating tomato plants with a maximum field rate of each specific insecticide to be investigated. In addition, a series of serial dilutions from the maximum field rate were also evaluated. Four rates of each insecticide were tested along with a control treatment containing no pesticide. Bioassays are conducted by placing 20 second or third instar nymphs onto treated plants either following insecticide application (for systemic compounds) or 24 hours prior to application (for contact insecticides). Nymphs were subsequently examined every day until they died or reached

adulthood. This protocol was used to examine both abamectin and spirotetramat on a minimum of 100 nymphs from each of the Texas 2006, Texas 2012/2014, and Pacific Northwest colonies.

Field trials were conducted at the University of California's South Coast Research and Extension Center in Irvine California. Seed sets of variety "Atlantic" potatoes were transplanted into experimental plots 1 row wide (5-ft centers) by 40 ft long separated by a buffer row. The potatoes were drip irrigated (water pH 7.2 - 7.5). Seven different rotational strategies (Table 1) were examined, and each was replicated 4 times. Insecticide applications were made at twilight using commercial application equipment.

During the growing season, two field counts were performed. In each instance, the top third of five plants in each treatment were examined for psyllids at each of the adult, nymphal, and eggs stages (after Butler and Trumble, 2012). At the end of the season (approximately 110 days), plants were 'vine killed' using diquat and allowed to reside in the soil for an additional two weeks. After this period, four tubers were collected from five plants in each treatment plot, for a total of 80 tubers per treatment. Tubers were returned to the laboratory, and two slices from the middle of each tuber were fried using a method that approximates commercial chip production. Chips were scored for zebra chip disease on a scale that ranges from zero to three; zero indicated no symptoms while three indicates extreme symptoms.

Table 1: Insecticide rotational strategies examined in 2014 field trials.

Treatment	Compound	Formulation	Rate(s) (ml/ha)
Control	Untreated Control	Untreated Control	-
No Neonic	Novaluron	Rimon	878
	Abamectin	Agri-mek	585
	Cyazypyr	Exirel	987
	Spinetoram	Radiant	585
Chemical 1	Thiamathoxam	Platinum	190
	Novaluron	Rimon	877
	Tolfenpyrad	Torac	1535
	Abamectin	Agri-mek	8
Chemical 2	Cyazypyr	Verimark	987
	Novaluron	Rimon	877
	Abamectin	Agri-mek	585
Chemical 3	Thiamathoxam	Platinum	190
	Spirotetramat + Alcohol Ethoxylate	Movento + Ventre	365, 8 (ml/L)
	Sulfoxaflor	Closer	365
	Abamectin	Agri-mek	585
IPM	Thiamathoxam	Platinum	190
	Spirotetramat + Permethrin	Movento + Pounce	365, 585
	Abamectin	Agri-mek	858
	Pymetrozine	Fulfill	5402
Methomyl/Permethrin	Methomyl	Lannate 2.4 LV	3508
	Permethrin	Pounce 3.2	585

Results and Discussion

Resistance bioassays

Psyllids collected from the Weslaco area of south Texas in 2012 and 2014 demonstrated resistance development to spirotetramat. Even at the maximum field rate 100% mortality was not achieved, and the reduction in efficacy was relatively consistent across all rates (Figure 1). Importantly, this same colony was found to have substantially decreased susceptibility to imidacloprid, and showed indications of early resistance to thiamethoxam (Prager et al., 2013).

By comparison, bioassays conducted with spirotetramat revealed that in both the Pacific Northwest and the Texas 2006 colonies, all rates greater than ¼ of maximum field rate result in near complete mortality (Figure 1). The lowest rate of 1/8 maximum field rate was also effective for psyllids from these colonies with over 90% mortality, but the reduced efficacy would likely lead to rapid selection for resistance.

In contrast to the results from resistance bioassays with spirotetramat, none of the colonies tested showed any indications of resistance to abamectin, with near 100% mortality at all rates examined (Figure 2).

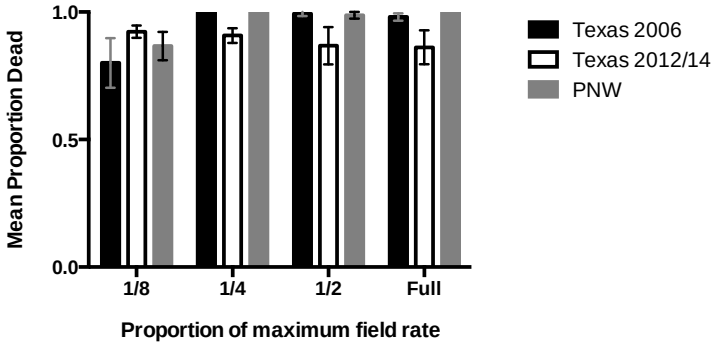

Figure 1: The mean proportion of psyllid nymphs that survived to adult when reared on tomato plants treated with spirotetramat at one of four rates.

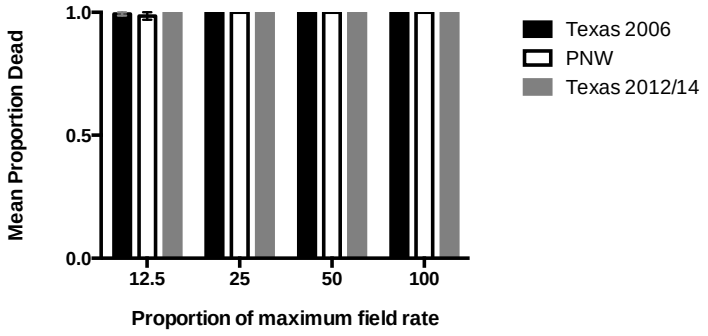

Figure 2: The mean proportion of psyllid nymphs that survived to adult when reared on tomato plants treated with abamectin at one of four rates.

Field Studies

Two field counts were taken for each insecticide rotation strategy. In the first field count (Figure 3), there was a significant overall difference in both the number of eggs (X^2=49.9, df=11, 226, p<0.001) and nymphs (X^2=64.7 df=11, 226, p<0.001). However, when examined further, the only significant differences from the untreated control treatments were in the methomyl/permethrin treatment. All other treatments were statistically similar to the control, and significantly different from the

methomyl/permethrin treatment. In the second field count (Figure 4), taken later in the season, there was again a significant overall difference in both eggs (X^2=39.4, df=11, 226, p<0.001) and nymphs (X^2=178.44, df=11, 226, p<0.001). When individual treatments were compared relative to the untreated control treatments, only the methomyl/permethrin differed with respect to eggs, which were reduced. When the number of nymphs was compared to the control, the methomyl/permethrin, chemical 2, and chemical 3 treatments all differed from control. All three treatments had significantly more nymphs. The methomyl/permethrin also had significantly more nymphs when compared to the other treatments.

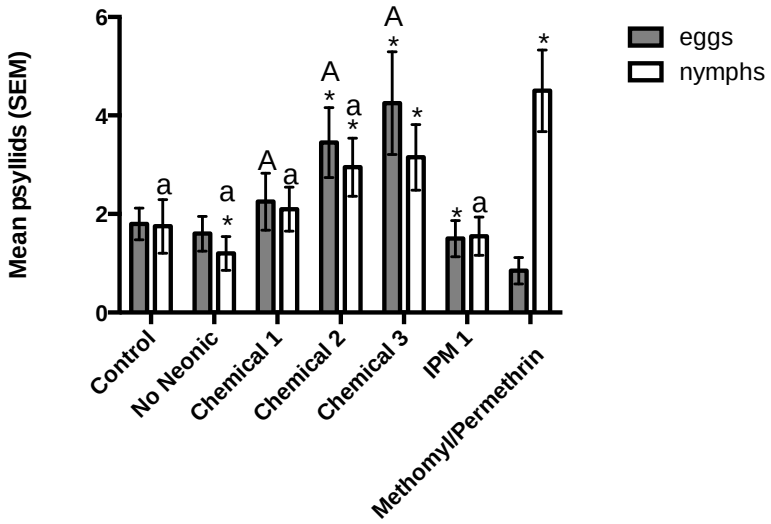

Figure 3: The mean number of *B. cockerelli* eggs and nymphs in each treatment during the early season field count. Letters indicate significant differences with the methomyl/permethrin treatment,; asterisks indicate significant difference with the control treatments.

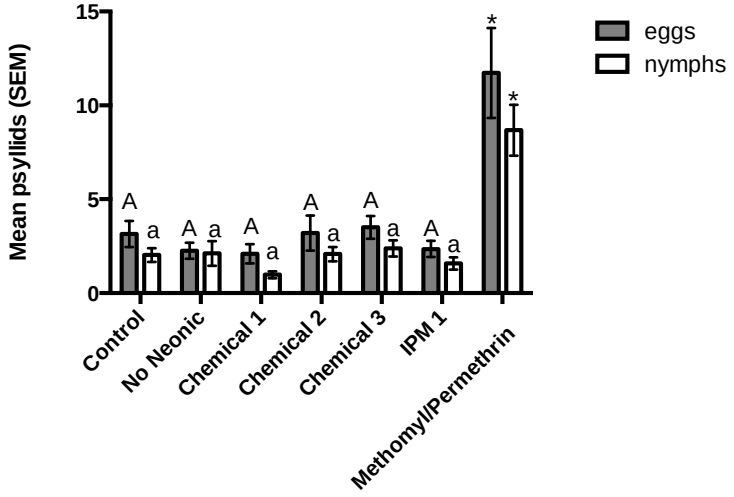

Figure 4: The mean number of *B. cockerelli* eggs and nymphs in each treatment during the early season field count. Letters indicate significant differences with the methomyl/permethrin treatment; asterisks indicate significant difference with the control treatments.

Following harvest, tubers were collected from plants in each treatment and fried to evaluate zebra chip disease. Overall, there was no significant difference in the percentage of zebra chip positive tubers in any treatment (Figure 5, X^2=4.6, d.f.=6, 248, p=0.6).

The results of these field studies indicate that when incorporated into an insecticide rotation program, compounds such as cyazypyr, sulfoxaflor, and tolfanpyrad offer alternatives to some currently used materials. However, they also indicate that while potato psyllid density can be held to a relatively low level though insecticides, some zebra chip will still be present. Additionally, the substantial increase in nymphs and eggs in those plots treated with methomyl and permethrin indicate that use of

broad-spectrum insecticides will lead to increased psyllid densities. This pattern was also observed by the Trumble group in potato fields at the same location in 2011 and in bell pepper fields in 2014. It is unclear if this pattern resulted from reduced numbers of beneficial insects or from hormologosis and actual increases in psyllid development. In either case, growers should be careful of making repeated applications of broad-spectrum insecticides, saving them as a last resort or avoiding use altogether.

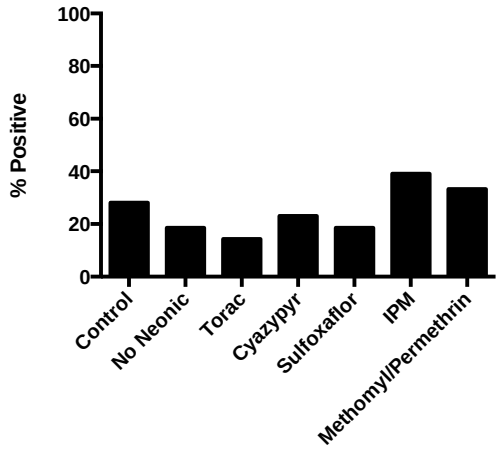

Figure 5: The percent of tubers that exhibited zebra chip symptoms (scores of 1,2 or 3).

The combined laboratory resistance and field studies presented here have important implications for potato psyllid management. First, they indicate that in some regions, especially south Texas, resistance is developing to commonly used insecticides. This is seemingly not the case in California and the Pacific Northwest where psyllid management has been less intense. Our results also indicate alternative insecticides can be used in place of many of the common materials to which resistance is developing. We suggest that growers consider incorporating these materials and rotate them with current products to reduce the risk of resistance development. Finally, we note use of broad-spectrum insecticides may lead to increased potato psyllid density, and recommend minimal use and additional sampling if they are applied.

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References

Butler, C.D. and Trumble, J.T. 2012. Spatial dispersion and binomial sequential sampling for the potato psyllid (Hemiptera: Triozidae) on potato. Pest Management Science 68: 865-869.

Guenthner, J., Goolsby, J., and Greenway, G. 2012. Use and cost of insecticides to control potato psyllids and zebra chip on potatoes. Southwestern Entomologist, 37: 263–270.

Prager, S.M., Vindiola, B., Kund, G.S., Byrne, F.J., and Trumble, J.T. 2013. Considerations for the use of neonicotinoid pesticides in management of *Bactericera cockerelli* (Šulk) (Hemiptera: Triozidae). Crop Protection 54: 84–91.

Prager, S.M., Vindiola, B., Kund, G.S., Byrne, F.J., and Trumble, J.T. 2013. An update on resistance and the use of neonicitinoids to manage zebra chip disease and potato psyllids. Proceedings of the 13th Annual SCRI Zebra Chip Reporting Session, Nov. 3-6, San Antonio TX.

The Mitochondrial Genome of the Potato Psyllid *(Bactericera cockerelli)*

Lopez, A., Hail, D., and Bextine, B. The University of Texas at Tyler, Tyler, Texas 75799 USA

Abstract
Zebra chip of potato is caused by the pathogen '*Candidatus* Liberibacter solanacearum' and is transmitted to plants by the potato psyllid (*Bactericera cockerelli*). While the vector is native to the United States and northern Mexico, the pathogen range has expanded north and south from this range over the past ten years. Several studies have used the mitochondrial gene Cytochrome Oxidase I (COI) and applied molecular biology techniques to understand the population genetics of the species. Unfortunately, most published phylogenies are based on single nucleotide polymorphisms (SNP's) within this single gene sequence. In this study, the complete mitochondrial genome for several potato pyllid populations from North America: Texas, Nebraska, California, Washington, northwest and southwest, were sequenced using next generation Illumina's sequencing technology. The gene content included: 2 ribosomal RNAs (16S and 12S rRNAs); the 22 tRNAs and the genes that encode for the 13 proteins: COI-III, Cytb, ND1-6, ND4L, ATP6, and ATP8. Through analysis of whole mitochondrial genome, potato psyllid populations from Texas, Nebraska (often referred to as "central biotype") and southwestern were determined to be most closely related. Divergent from this clade were the Washington and California populations (often referred to as "western biotype"). The northwestern population seems to be a new population that has arisen from the western biotype. It is evident that the use of the whole mitochondrial genome, instead of one gene, improves the understanding of the relationships among the different potato psyllid populations as well as with other closely related species by providing greater depth.

Introduction
The potato psyllid (*Bactericera cockerelli* sulc.) is a phloem feeder (Order: Hemiptera) of economic importance because it affects the production of potatoes (Munyaneza, 2010). This insect is also consider an important agricultural pest because of its ability to transmit a plant pathogen, '*Candidatus* Liberibacter solanacearum' which causes the zebra chip disease (Munyaneza, 2010). Although, the potato psyllid is native to the United States and northern Mexico (Buchman et al., 2012; Nachappa et al., 2012), its geographical range has increased, including the northwestern and western region of the US, some countries in Central America and New Zealand (Nachappa et al., 2012).

The application of genetic diversity studies has provided useful insights for understanding patterns of genetic variation of individuals and populations by using genetic markers (Ekblom and Wolf, 2014; Allendorf et al., 2013) such as mitochondrial genes. Numerous studies have used the mitochondrial gene Cytochrome Oxidase I (COI) to identify genetic relationships between species, characterize variation, and classify individuals into strains, subspecific clades or haplotypes (Liu et al., 2006). In the case of the potato psyllid, gene analysis have identified two potato psyllid biotypes, populations that demonstrate biological and phenological differences from morphologically identical forms (Headrick 2011), by identifying a single nucleotide polymorphism (SNP) within an amplified COI fragment of 544 bp long (Liu et al., 2006). Also, high resolution melting analysis and DNA sequencing data of COI gene has determined the existence of potato psyllid haplotypes, populations that show differences on a single chromosome or a mitochondrial DNA gene (Allendorf et al., 2013), that correlate to the central, western, northwestern and southwestern geographical regions of the United States (Swisher et al., 2012). However, the use of a single gene has limited the correct genetic differentiation of the species and has

proved to be of little help. For example, Powell et al. (2012) reported an amplified 3,025 bp fragment of the mitochondria genome containing part of the Cytochrome B (CytB) gene, the complete NADH Dehydrogenase subunit 1 (NAD1) and the complete large subunit rRNA sequence. The results of this analysis revealed that from all seven potato psyllid populations evaluated, within this specific amplicon, they were 98% similar; demonstrating how poorly this amplicon is for genetic variation (Powell et al., 2012).

The mitochondrial genome is one of the most genomic resources used for systematic entomology (Cameron 2014). The Animal mitochondrial DNA (mtDNA) is abundant in the tissues (Li et al., 2012), is relatively small, haploid and generally maternally inherited (Allendorf et al., 2013). Also, presents a fast rate of evolution (Li et al., 2012); and it usually undergo no recombination, reason why is useful for reconstruction of phylogenies (Allendorf et al., 2013). In insects, mitochondrial DNA (mtDNA) is usually a small double-stranded circular molecule of 14-20 kb in length (Li et al., 2011) that codes for two ribosomal RNAs (16S and 12S rRNA); 22 tRNAs and encodes 37 genes including 13 proteins: COI-III, Cytb, ND1-6, ND4L, ATP6, and ATP8 (Friedrich and Muqim 2003; Chai et al., 2012; Cameron and Whiting 2008). Additionally, the insect mitogenome has at least one sequence known as the A+T-rich region, which includes some initiation sites for transcription and replication of the genome (Chai et al., 2012). The study of more than one mitochondrial gene can give more information for systematic approaches, as it can be applied for family, populations and biogeographic studies (Cameron 2014). Therefore, in this study, the complete mitochondrial genome for the potato psyllid and the different potato pyllid populations from North America: Central, Western, Northwestern and Southwestern, were analyzed by DNA sequencing data.

Materials and Methods

DNA extraction. Five adult potato psyllids coming from the different geographical regions of the United States: central biotype (Texas and Nebraska), western biotype (California and Washington), northwestern haplotype and southwestern haplotype, were processed, first for mitochondrial isolation and then, for DNA extraction. For an efficient separation and high yield of mitochondria, the Qproteome Mitochondria Isolation Kit (Qiagen) was used, following the supplementary protocol. After isolation of the whole mitochondria, mtDNA was extracted from the resulting mitochondrial fraction, which was treated as a bacteria culture using the DNeasy blood and tissue kit (Qiagen). The mitochondria DNA extractions of all six potato psyllids populations were sequenced using next generation sequence: MiSeq (Illumina's sequencing).

*Sequence assembly and annotation.*The assembly of the mitochondria sequences of the different potato psyllid populations was done using the bioinformatics software Geneious version 7.1.7 (Biomatters Limited, 2014). Protein-coding genes, rRNA genes and the tRNA genes were identified using the web server MITOS which automatic annotates metazoan mitocondrial genomes (Bernt et al., 2013). ARWEN, a tRNA software detection for metazoan mitochondrial sequences (Laslett and Canbäck 2008) and DOGMA, an automatic annotation for annotating plant chloroplast and animal mitochondrial genomes (Wyman et al., 2004), were used to confirm the presence of the tRNA genes in the genome. Also, with the assembled sequences, a tree was built using Geneious version 7.1.7 (Biomatters Limited, 2014) applying the Tamura-Nei Distance Model and Neighbor-Joining Method.

Results and Discussion

The Illumina's sequencing technology data resulted in a 419 coverage. The gene content observed in the potato psyllid mitochondria genome of all six potato psyllid populations were arranged as in the ancestral insect mitochondrial genome, including: the two ribosomal RNAs (16S and 12S rRNA); the genes that encodes for $tRNA^{alanine}$, $tRNA^{cysteine}$, $tRNA^{aspartic\ acid}$, $tRNA^{phenylalanine}$, $tRNA^{glycine}$, $tRNA^{histidine}$, $tRNA^{isoleucine}$, $tRNA^{lysine}$, $tRNA^{leucine1}$, $tRNA^{leucine2}$, $tRNA^{methionine}$, $tRNA^{aspargine}$, $tRNA^{proline}$, $tRNA^{glutamine}$, $tRNA^{arginine}$, $tRNA^{serine1}$, $tRNA^{serine2}$, $tRNA^{threonine}$, $tRNA^{valine}$, $tRNA^{tryptophan}$, $tRNA^{tyrosine}$ and $tRNA^{glutamic\ acid}$. As well as, the 13 protein coding genes: atp6, atp8, cob, cox1, cox2, cox3, nad1 nad2, nad3, nad4, nad4L, nad5 and nad6.

Analysis of the mitochondrial genomes of the different potato psyllid populations: central biotype (Texas and Nebraska), western biotype (California and Washington), northwestern haplotype and southwestern haplotype; and *Paratrioza sinica* (the wolfberry psyllid) was best visualized in a phylogenetic tree (Figure 1). *P. sinica* was used as the outgroup in our analysis because it is the most similar organism with a full mitochondrial genome available in NCBI GenBank. While the diagram was consistent with the information already known about the potato pysllids populations from individual genes, full mitochondrial analysis revealed regions of the mitochondrial genome that differed significantly. From our more advanced analysis with greater data richness, the northwestern population arose from a well geographically distributed western population to become a new isolated population that has been the product of genetic island effect. Our results are consistent with the hypothesis that populations belonging to the central (Texas and Nebraska) and western (California and Washington) region of the United States are biotypes because of their biological differences, such as survivorship, growth index and developmental time (Swisher et al., 2013). The northwestern and southwestern haplotypes are considered haplotypes because only genotypic differences have been identified by using a single mitochondrial gene (Swisher et al., 2013). While some additional data was collected which strengthens genotypic variation, no biological data was collected which can upgrade these populations to biotype. The variations between the Central and Southwestern psyllids was very small, indicating that these may not be truly different populations.

Future investigations into northwestern haplotype variations will be very interesting from a gene conservation prospective. It is evident that this population has been isolated from the others for a long time, which is obvious due to the physiography of the Pacific Northwest and Columbia Basin. Also, haplotyping results from archived psyllids collected from the northwestern region have shown that this population has potentially been present in this region longer than the western population (Swisher et al., 20013). Since the northwestern and western populations are present in the same area, the latter results suggest that the northwestern population is native to this area, while the western population has migrated (Swisher et al., 20013). Moreover, migration of the western population to the Pacific Northwest and Columbia Basin can lead to gene flow between these two populations, resulting in a very important source of genetic variation. Unfortunately, since mtDNA acts as a single locus because does not undergoe recombination, is not useful for describing genetic population structure within species. Nevertheless, there can be considerable differences in terms of genetic variation (Allendorf et al., 2013). This genetic variation seen in the northwestern haplotype is mostly due to natural selection and the means to adapt to this climatic zone.

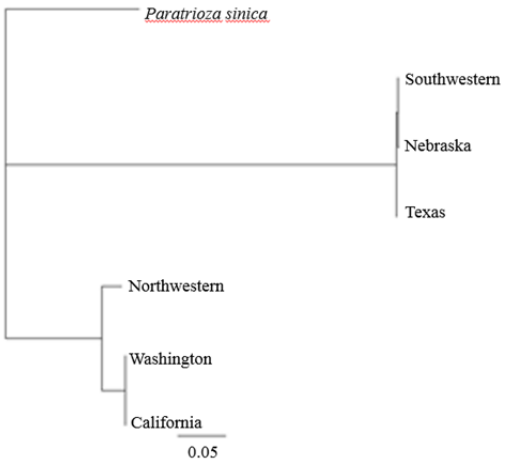

Figure 1. A view of the relationships of the different potato psyllids populations: Central (Texas and Nebraska), Western (California and Washington), Northwestern and Southwestern; and *Paratrioza sinica*, the wolfberry psyllid, used as an outgroup.

Acknowledgements

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References

Allendorf, F. W., Luikart, G. and Aitken, S. N.. 2013. Conservation and the Genetics of Populations. Second edition. Wiley-Blackwell, Malaysia. 602 pp.

Bernt M., A. Donath, F., Jühling, F., Externbrink, C., Florentz, G., Fritzsch, J., Pütz, M., Middendorf, P. F., and Stadler, P.F. 2013. MITOS: Improved de novo Metazoan Mitochondrial Genome Annotation. Molecular Phylogenetics and Evolution, 69(2):313-319.

Biomatters. 2014. Geneious v7.1.7. www.geneious.com.

Buchman, J., Fischer, T. W., Sengoda, V. G., and Munyaneza, J. E. 2012. Zebra Chip Progression: From Inoculation of Potato Plants with Liberibacter to Development of Disease Symptoms in Tubers. Am. J. Pot Res 89:159-168.

Cameron, S.L. 2014. How to sequence and annotate insect mitochondrial genomes for systematic and comparative genomics research. Systematic Entomolgy 39: 400-411.

Cameron, S. L., and Whiting, M. F. 2008. The complete mitochondrial genome of the tobacco hornworm, *Manduca sexta*, (Insecta: Lepidoptera: Sphingidae), and an examination of mitochondrial gene variability within butterflies and moths. Gene 408: 112-123.

Chai, H-N., Du, Y-Z., and Zhai, B-P. 2012. Characterization of the Complete Mitochondrial Genomes of *Cnaphalocrocis medinalis* and *Chilo suppressalis* (Lepidoptera: Pyralidae). Int. J. Biol. Sci. 8(4): 561-579.

Ekblom, R., and Wolf, J.B.W. 2014. A field guide to whole-genome sequencing, assembly and annotation. Evolutionary Applications 7(9): 1026-1042.

Friedrich, M., and Muqim, N. 2003. Sequence and phylogenetic analysis of the complete mitochondrial genome of the flour beetle *Tribolium castanaeum*. Molecular Phylogenetics and Evolution 26:502-512.

Headrick, D. 2011. A dictionary of Entomology. Second edition, CSIRO Publishing, 1526 pp.

Laslett, D., and Canbäck, B. 2008. ARWEN, a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics 24:172-175.

Li, H., Liu, H., Shi, A., Stys, P., Zhou, X. and Cai, W. 2012. The Complete Mitochondrial Genome and Novel Gene Arrangement of the Unique-Headed Bug *Stenopirates* sp. (Hemiptera: Enicocephalidae). PLoS ONE, 7(1): e29419. doi:10.1371/journal.pone.0029419.

Li, H., Gao, J., Liu, H., Liu, H., Liang, A., Zhou, X., and Cai, W. 2011. The Architecture and Complete Sequence of Mitochondrial Genome of an Assassin Bug *Agriosphodurs dohrni* (Hemiptera: Reduviidae). Int. J. Biol. Sci, 7(6): 792-804.

Liu, D., Trumble, J.T., and Stouthamer, R. 2006. Genetic differentiation between eastern populations and recent introductions of potato psyllid (*Bactericera* cockerelli) into western North America. Entomologia Experimentalis et Applicata 118: 177-183.

Munyaneza, J. 2010. Psyllids as vectors of Emerging Bacterial Diseases of Annual Crops. Southwestern Entomologists, 35(3): 471-477.

Nachappa, P., Levy, J., and Tamborindeguy, C. 2012. Transcriptome analyses of *Bactericera cockerelli* adults in response to "*Candidatus* Liberibacter solanacearum" infection. Mol Genet Genomics 287: 803-817.

Powell, C. M., King, J., and Bextine, B. R. 2012. Cytochrome B Sequences of Potato Psyllids, *Bactericera cockerelli* (Sulc), from North and Central America. Southwestern Entomologist, 37(4): 521-523.

Swisher, K.D, Munyaneza, J. E., and Crosslin, J. M. 2013. Temporal and Spatial Analysis of Potato Psyllid Haplotypes in the United States. Environ. Entomol. 42(2): 381-393.

Swisher, K. D., Munyaneza, J. E., and Crosslin, J. M. 2012. High Resolution Melting Analysis of the Cytochrome Oxidase I Gene Identifies Three Haplotypes of the Potato Psyllid in the United States. Environmental Entomology, 41(4): 1019-1028.

Wyman, S.K., Jansen, R.K., Boore, J.L. 2004. Automatic annotation of organellar genomes with DOGMA. Bioinformatics 20(17): 3252-3255.

Chasing Zebra Chip in the Columbia Basin and Northeast Oregon: Research Updates

Rondon, S.I., and Hamm, P.B. Oregon State University, Hermiston Agricultural Research and Extension Center (HAREC), Hermiston, OR 97838

Abstract

Zebra chip is a relatively new disease in the Columbia Basin that causes significant yield and tuber quality losses. Since 2011, our team has developed an integrated pest management program focusing on monitoring for the insect, detection of Lso in insects and plants using improved techniques, understanding the ecology of this insect on and off season, haplotyping, epidemiology of the disease, landscape ecology and control methods. An area-wide potato psyllid monitoring program was adopted in 2012 in Umatilla and Morrow counties in Oregon, and a year later, in Union and Baker counties. All sites were selected to provide representative sampling within each production area. Also, a new high fidelity PCR protocol was developed to help detect Lso in potato tubers and insects. Overwintering studies were designed to investigate potato psyllid populations in alternative hosts and to determine population dynamics. Bittersweet nightshade (*Solanum dulcamara*) was found to host potato psyllids during the winter months and sustain potato psyllid populations during the off season. Other studies have shown that potato psyllids clearly choose potatoes over the other crops, and probably, presence of volunteers drive potato psyllids presence on those other crops. Another study offered some insight about the positive effect of thiamine (vitamin B1) application on potato resistance to Zebra chip. This is the first time that thiamine was tested using potatoes as a model crop. Efficacy, residual and sub-lethal effects of several chemicals were tested against potato psyllids. The immediate toxicity of the pesticides could help prevent the spread of Zebra chip. Pesticides with a quick knockdown would be effective at reducing the amount of Lso in fields. Techniques for monitoring potato psyllid populations have been polished and effectively integrated into current integrated pest management programs. Outreach efforts have been helpful to growers and field consultants to educate them on the risk of Lso based on efforts mentioned above to better manage this disease, reduce inputs while reducing the potential for Zebra chip disease spread in the region.

Introduction

Zebra chip, caused by the bacterium '*Candidatus* Liberibacter solanacearum' (Lso) is a relatively new disease in the Columbia Basin that causes significant yield and tuber quality losses. Since 2011, a number of fields and potato cultivars (e.g. Russet Norkotah, Umatilla Russet, Alturas, Russet Ranger, a red cultivar, and Pike) have been confirmed to be infected but damage has been reported to be overall minor though some issues have developed during processing. Potato psyllids and disease have been monitored since their arrival to the region. Thus, the following information is provided as an update of new developments and tools for monitoring both the vector and the bacterium in our area and to help manage this disease.

Since 2011, our team has developed an integrated pest management program focusing on detection and monitoring, understanding of the ecology of this insect on and off season, haplotyping, epidemiology of the vector and disease, landscape ecology and control methods.

Area-wide monitoring program. The area-wide potato psyllid monitoring program was adopted since 2011 in Umatilla and Morrow counties in Oregon, and a year later, in Union and Baker counties. Psyllid samples were collected from potato plant foliages by operating an inverted leaf blower vacuum device for 3 to 5 minutes near the field edge. You can see a demonstration of the technique in the following YouTube

video https://www.youtube.com/watch?v=dLpI3jkCjXQ ; Alpha scent sticky cards were also used. Thirty five (n=35) commercial fields were included in the weekly monitoring of potato psyllid populations in Umatilla and Morrow; six (n=6) commercial processing potato production fields in Baker County and four (n=4) commercial Oregon certified seed potato fields in Union county. All sites were selected to provide representative sampling within each production area. Four hundred forty miles were driven each week from April until mid-September. After the 2011 outbreak year, in Umatilla and Morrow counties, potato psyllids numbers in 2012, 2013 and 2014 were lower than previous years. Figure 1 shows 2013-2014 data; both years numbers are low and as season progressed, potato psyllids numbers increased aspotato fields are being harvested. In 2014, only one potato psyllid from Umatilla-Morrow counties tested positive for Lso. Please refer to the weekly report for trap-specific details which are available online at http://oregonstate.edu/dept/hermiston/trap-reports. All potato psyllid samples collected in Union and Baker counties the same year have been subjected to PCR analysis and all test results have been negative. Please refer to the weekly report for trap-specific details which are available online at http://extension.oregonstate.edu/union/potato-aphid-reports-current. Samples collected were shipped to the OSU-HAREC Irrigated Agricultural Entomological Laboratory for species identification confirmation; PCR analysis to determine presence of Lso was performed at the OSU- HAREC Plant Pathology Diagnostic Laboratory.

Survey of potential overwintering sites. This study was designed to investigate potato psyllid populations in alternative hosts and to determine population dynamics during three consecutive years (2011 -2013). In 2011, a bittersweet nightshade (*Solanum dulcamara*) was found to host potato psyllids during the winter months (Murphy et al. 2013). The fact that this plant is a perennial compelled us to look for signs of psyllid populations surviving through the winter in association with this unwanted plant. Overwintering of potato psyllid populations was confirmed in both southwestern Idaho and the lower Columbia Basin. Potato psyllid populations were numericaly different in each location (Figure 2). More recently, other weeds such as bindweed (Fam. Convolvulacea) and Lycium (Fam. Solanaceae) have been found to host psyllids as well, but their importance as hosts is yet to be confirmed (Thinakaran et al. 2014).

Better detection method for Lso. This bacterium has been difficult to confirm at times when typical tuber symptoms were present. Likewise, being able to test individual insects, with a high level of confidence, to confirm the presents of Lso has been lacking. A new high fidelity PCR technique was developed that increased detection on this disease in potato tubers by 30-40% over conventional PCR (Cating et al. 2015).

Haplotypes. A variety of psyllid haplotypes occur in the region. In Umatilla and Morrow counties, northwestern and western haplotypes were dominant. Further east, in Union and Baker counties close to the Idaho border, the northwestern haplotype was predominant early in November-December followed by western haplotype in early spring. In commercial fields in the same area, western haplotype is dominant until a latter arrival of the southwestern haplotype (Rondon et al. 2015). This area seems to be a critical "hub" of multiple haplotypes of potato psyllids arriving in the area.

Other studies. There are anecdotal reports of potato psyllids in other crops such as wheat and corn, but their presences is speculated as probably due to the occurrence of volunteer potatoes in those crops. Therefore, in order to determine potato psyllid host preference, natural population of potato psyllids selected from potatoes, corn, weeds, and corn planted with volunteer potatoes, and weeds

planted with volunteer potatoes. Potato psyllids clearly picked potatoes over the other crops, however, they were also found in corn and wheat planted with volunteer potatoes raising the status of volunteer potatoes as a potential alternate host of psyllids and Lso since a percentage of volunteers tested positive for the Lso (Murphy et al. in progress). Another study offered some insight about the effect of thiamine (vitamin B1) application on potato resistance to Zebra chip. This is the first time that thiamine was tested using potatoes as a model crop. Results from this study suggest that thiamine applications increased potato plant immune system helping plant fight against the bacteria. Results of final molecular studies are still pending.

Efficacy, residual and sub-lethal effects of several chemicals was tested against potato psyllids. Pesticides were tested in the greenhouse and in the field. The immediate toxicity of the pesticides could help prevent the spread of Zebra chip. Pesticides with a quick knockdown would be effective at reducing the amount of Lso in a field.

Conclusions

Techniques for monitoring potato psyllid populations have been polished and effectively integrated into current integrated pest management programs. Outreach efforts have been helpful to growers and field consultants to scale down potential for Zebra chip disease spread.

Acknowledgements

The authors thank Darrin Walenta, Brian Charlton, and Robert Cating for psyllid collecting and laboratory analysis, Alexzandra Murphy, Erik Echegaray and Amber Vinchesi, Posdoctoral Scholars, and Matthew Klein and Amelia Jordan, graduate students and members of Rondon's lab. Thanks to the Oregon Potato Commission, Washington Potato Commission and Agricultural Research Foundation for funding.

References

Cating, R.A., Rondon, S.I., and Hamm, P. B. 2015. High-fidelity PCR improves the detection of 'Candidatus *Liberibacter* solanacearum' in potato tubers. Am. J. Potato Res. (Accepted, in print).

Murphy, A.F., Rondon, S.I., and Jensen, A. 2013. First report of potato psyllids, *Bactericera cockerelli*, overwintering in the Pacific Northwest. Am. J. Pot. Res. 9 (3): 294. Online 10.1007/s12230-012-9281-0.

Rondon, S.I., Wohleb, C.H., Hamm, P.B., Walenta, D.L., Charlton, B.A., Reitz, S. and Cating, R. 2015. Potato psyllids colonizing Oregon and Washington: regional monitoring incidence of the pathogen and management options. Potato Expo, Florida. Poster presentation.

Thinakaran, J., Munyaneza, J.E., and Cooper, R. 2015. Matrimony vine/goji berries potential overwintering host for potato psyllid in Pacific Northwest. *In* Washington/Oregon Potato Conference. 27-29 Jan. Kennewick, WA.

Figure 1. Population dynamics of potato psyllids near commercial potato fields in Umatilla and Morrow counties (2013-2014).

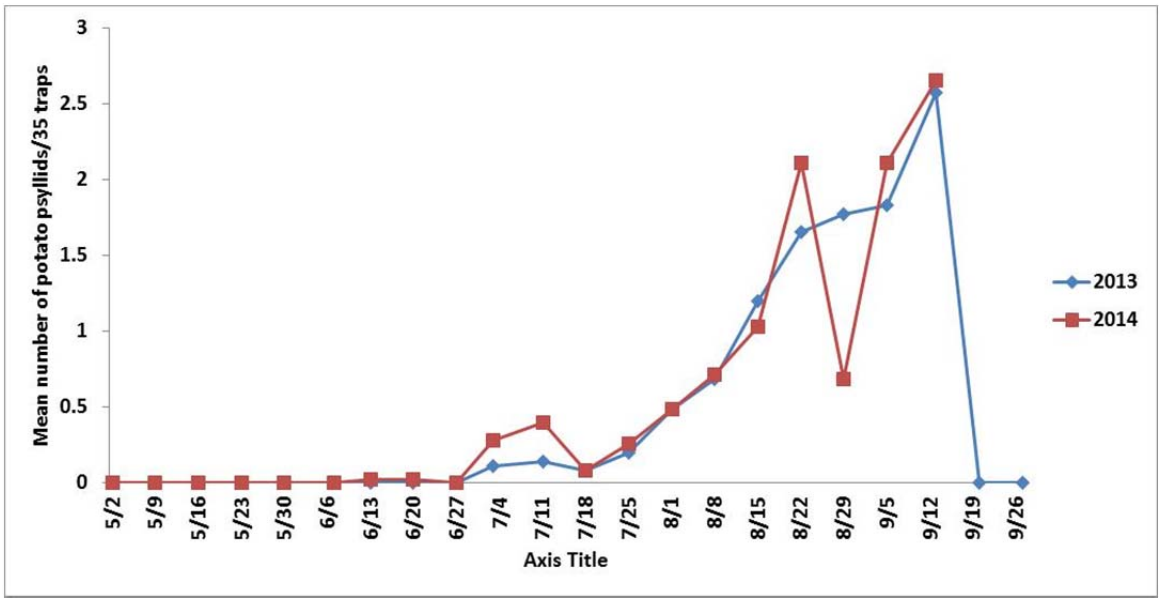
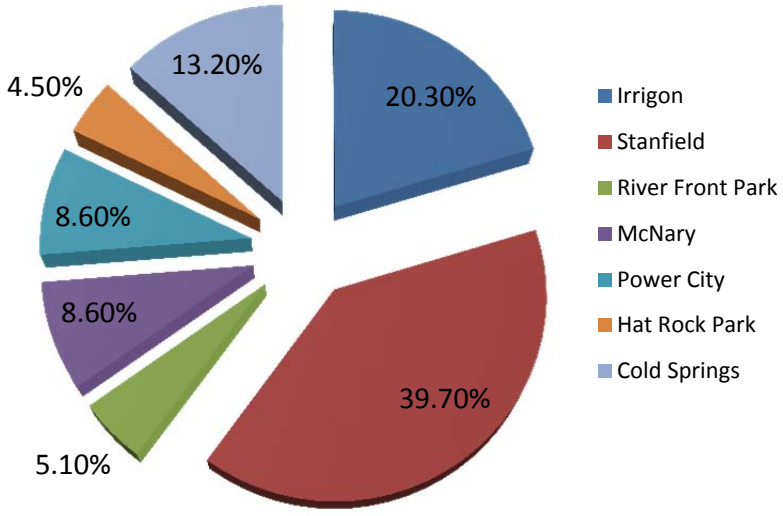


Figure 2. Potato psyllids distribution in the lower Columbia Basin (Nov.2012-Apr.2013) in *Solanum dulcamara* L.



Effect of Haplotype on the Transmission of '*Candidatus* Liberibacter solanacearum' in Eight Potato Cultivars

Shjerve, R.A., Johnson, C.P., Wen, A., Johansen, V.G., and Gudmestad, N.C. Department of Plant Pathology, North Dakota State University, Fargo, ND 58108

Abstract

Zebra chip caused by the bacterial pathogen, '*Candidatus* Liberibacter solanacearum' (Lso) which is vectored by the potato psyllid (*Bactericera cockerelli*) is a devastating disease of potatoes. The pathogen itself has been found to have two forms in the USA, haplotype A and haplotype B (Nelson et al. 2011). Seed transmission of the pathogen from infected plants to daughter tubers is of great concern due to the possibility of Lso infected plants growing from infected seed tubers and becoming a source of inoculum (Pitman et al. 2011). In this study, we infected and recorded the transmission of the ZC bacterium to daughter tubers in eight potato varieties: Alturas, Chieftain, Dakota Pearl, Russet Burbank, Pike, Modoc, Snowden, and Russet Norkotah. Each of these varieties was infected with Lso by psyllids with either Lso haplotype A or B and then allowed to continue to grow and produce daughter tubers. The daughter tubers were collected and placed into storage until replanting, and once planted, the samples were compared for differences between the tubers infected with each haplotype. Samples were compared in several ways including percentage of emergence, delay of emergence, number of days the plant lived, and percentage of emerged plants found to be Lso positive. All tubers collected from the emerged plants were also collected and compared in two categories, mass of tubers from emerged plants and visual symptoms of zebra chip.

Introduction

'Candidatus Liberibacter solanacearum' (Lso) is the bacterial causal agent of the potato zebra chip (ZC) disease. Since, when it was first reported in northern Mexico in 1994, the disease has been identified in New Zealand and throughout the United States (Liefting et al. 2008; Bech 2008). The Lso bacterium has two different haplotypes, denoted as haplotype A and haplotype B, which differ slightly from each other in the 16s-ISR-23s region (Wen et al. 2009; Nelson et al. 2011). Previously, two studies were published reporting conflicting results on the success of seed transmission of the bacteria. In a study conducted in the USA, there was a drastic decrease of emergence when the mother plants of these tubers were infected with Lso by psyllids, with only 44% emergence for Ranger Russet and 18% for Russet Norkotah (Henne et al. 2010). When testing for the presence of Lso however, they only had 1 emerged plant and 2 seed tubers test positive for the bacterium. In a study conducted in New Zealand, it was reported that over 90% of the tubers collected from Lso haplotype A infected plants did emerge and that only 3.4% of the tubers showed symptoms commonly associated with zebra chip (Pitman et al. 2011). They also reported that 70% of the plants tested positive for Lso, leading to the concern that seed tubers collected from a plant that had been infected with zebra chip could serve as a source of secondary inoculum in fields. The purpose of this study, was to determine if there are any differences in tuber transmission and tissue detection of Lso between haplotype A and B among 8 potato varieties: Alturas, Modoc, Chieftain, Dakota Pearl, Russet Burbank, Russet Norkotah, Snowden and Pike.

Materials and Methods

Plant Infection and Tuber Harvest. Clip cages with 2 psyllids, both infected with either haplotype A or B, were attached to a single leaf on each healthy potato plant for 7-10 days and then carefully removed to ensure the psyllids remained within the cage. After inoculation the plants were allowed to continue to

grow for approximately 2 months until plants were ready for tuber harvest. Harvested daughter tubers were placed in storage for a minimum of 3 months before replanting. Prior to planting the harvested tubers, a small portion of each tuber was cut from the stolon end to evaluate for the presence of visual ZC symptoms and to supply tissue for DNA extraction. Once planted, the tubers were monitored daily to determine the frequency of emergence. Plant heights were recorded beginning 16 DAP until emerged plants were ready for tuber harvest (approx. 11 weeks). Tubers were originally planted in square 10.5cm×10.5cm×12.5cm pots, then transplanted to round 20.5cm×14.5cm pots to allow for further plant growth. Once the potato plants had naturally senesced, tubers were collected, weighed and cut to evaluate for visual symptoms of ZC.

Potato DNA Extraction. DNA extractions were performed by taking 250mg of each tuber and petiole collected and extracted using the FastDNA SPIN Kit (MP Biomedical, Santa Ana, CA).

QPCR Testing. QPCR testing of DNA extracted samples for presence of Lso and to determine haplotype was performed according to previously established protocols (Wen et al. 2013) with the LightCycler 96 system (Roche Diagnostics, Indianapolis, IN).

Results and Discussion

Chieftain, Dakota Pearl, Modoc and Pike were removed from analysis due to insufficient emergence in one or more of the infection groups: Lso haplotype A, B, or healthy. Across the remaining potato varieties Alturas, Russet Burbank, Russet Norkotah, and Snowden, there were no significant difference found between Lso haplotype A and B in any data collected.

Emergence, Height and Plant survival. There was a significant difference in the delay of emergence between non-infected healthy control seed tubers and seed tubers infected with Lso (both haplotype A and B) in varieties Alturas, Russet Burbank, and Snowden ($\alpha = 0.05$; Figure 1). There was also a significant difference in emergence frequency between non-infected seed tubers and seed tubers infected with Lso in Alturas and Snowden ($\alpha = 0.05$; Figure 2). Plant survival was significantly reduced in Lso infected seed tubers, regardless of haplotype, compared to non-infected seed tubers in the potato varieties Alturas, Snowden, and Russet Burbank ($\alpha = 0.05$; Figure 3). Plant height was significantly decreased between non-infected seed tubers and Lso infected seed tubers in varieties Alturas and Russet Burbank ($\alpha = 0.05$; Figure 4).

Frequency of Lso Detection in Emerged Plants. The frequency of plants with detectable levels of Lso ranged from 0% to 30% and 0% to 50% for haplotype A and B respectively, and was not significantly different among potato varieties.

Daughter Tubers Harvested from Lso Infected Seed Tubers.

There were no significant differences in the mass of daughter tubers produced from non-infected seed tubers and Lso-infected seed tubers in any variety except Russet Norkotah ($\alpha = 0.01$). Positive visual symptoms were observed in progeny tubers of the varieties Russet Burbank, Russet Norkotah, and Snowden. Harvested tubers from the varieties Russet Norkotah and Snowden tested positive for Lso; Russet Norkotah for haplotype B only and Snowden for both haplotype A and B.

In this study, the 1st of 3 trials, we found that there were no significant differences between seed tubers infected with Lso haplotype A and B in delay of emergence, percentage of plants emerged, plant

survival, plant height, frequency of Lso detection in emerged plants, or mass of harvested progeny for potato varieties Alturas, Russet Burbank, Russet Norkotah, and Snowden. There were, however, significant differences observed between non-infected seed tubers and infected seed tubers for varieties Alturas, Snowden and Russet Burbank for delay of emergence and plant survival, Alturas and Snowden for percentage emerged, Alturas and Russet Burbank for plant height, and Russet Norkotah for mass of harvested progeny tubers. The varieties Chieftain, Dakota Pearl, Modoc, and Pike were removed from analysis for this study, but will be added if applicable once all three trials of the experiment are completed.

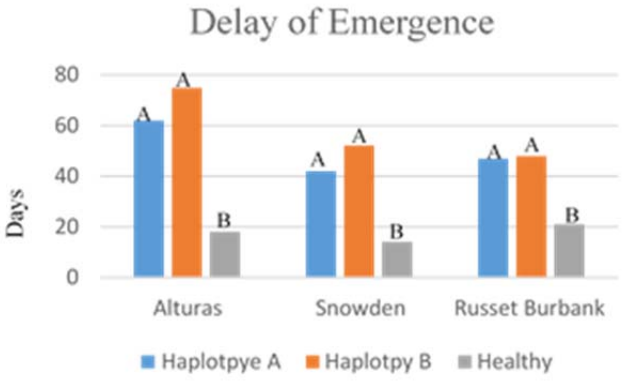


Figure 1. Delay of Emergence. Compares the average number of days after planting until emergence. Alturas (p<0.001, 0.05), Snowden (p<0.001, 0.01), and Russet Burbank (p=0.006, <0.001) were the only varieties to have a significant difference. Significant differences are denoted by letter for each variety.

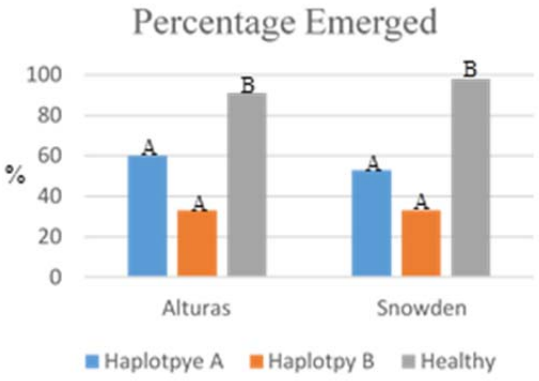


Figure 2. Percentage Emerged. Compares the percentage of tubers planted that emerged during the experiment. Alturas (p= 0.02, 0.005) and Snowden (p= <0.001, <.0.001) were the only varieties to have a significant difference. Significant differences are denoted by letter for each variety.

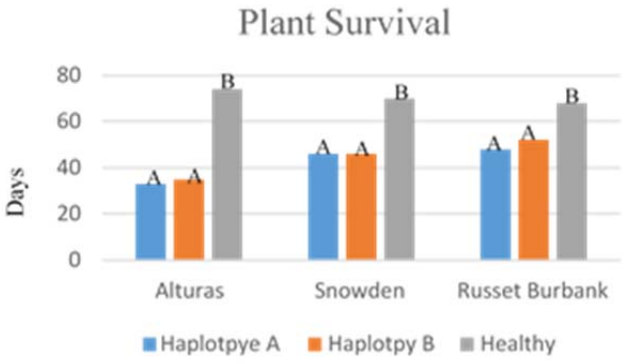


Figure 3. Length of Plant Life. Compares the average number of days plants survived after emergence. Alturas (p<0.001, 0.01), Snowden (p=0.002, 0.05), and Russet Burbank (p=0.009, <0.001) were the only varieties to have a significant difference. Significant differences are denoted by letter for each variety.

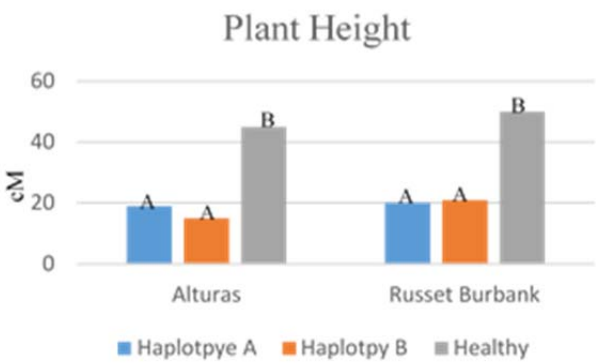


Figure 4. Plant Height. Compares the average tallest height of each plant that emerged during the experiment. Alturas (p<0.001, 0.05) and Russet Burbank (p<0.001, <0.001) were the only varieties to have a significant difference. Significant differences are denoted by letter for each variety.

References

Bech, R. 2008. 'Candidatus Liberibacter' spp. As putative causal agent of zebra chip of potatoes. *USDA-APHIS, SPRO DA-2008-32*, July 14, 2008.

Henne D.C., Workneh, F., Wen, A., Price, J.A., Pasche, J.S., Gudmestad, N.C., and Rush, C.M. 2010. Characterization and Epidemiological Significance of Potato Plants Grown from Seed Tubers Affected by Zebra Chip Disease. Plant Dis. 94: 659-665.

Liefting, L.W., Perex-Egusquiza, Z.C., and Clover, G.R.G. 2008. A new '*Candidatus* Liberibactoer' species in *Solanum tuberosum* in New Zealand. Plant Dis. 92:1474.

Nelson, W.R., Fisher, T.W., and Munyaneza, J.E. 2011. Haplotypes of "*Candidatus* Liberibacter solanacearum" suggests long-standing separation. Eur. J. Plant. Pathol. 130:5-12.

Pitman, A.R., Drayton, G.M., Kraberger, S.J., Genet, R.A., and Scott, I.A. (2011) Tuber transmission of *'Candidatus* Liberibacter solanacearum' and its association with zebra chip on potato in New Zealand. Eur. J. Plant Pathol. 129:389-398.

Wen, A., Mallik, I., Alvarado, V.Y., Pasche, J.S., Wang, X., Li, W., Levy, L., Lin, H., Scholthof, H.B., Mirkov, T.E., Rush, C.M., and Gudmestad, N.C. 2009 Detection, distribution and genetic variability of '*Candidatus* Liberibacter' species associated with zebra complex disease of potato in North America. Plant Dis. 93:1102-1115.

Wen, A., Johnson, C.P., and Gudmestad, N. C. 2013. Development of Conventional and Real-Time PCR for Detection and Genotyping of '*Candidatus* Liberibacter solanacearum'. Proceedings of the 13th Annual SCRI Zebra Chip Reporting Session. Pp 68-72.

Effect of '*Candidatus* Liberibacter solanacearum' Haplotype on Potatoes

Levy, J.[2], Mendoza, A.[1], Scheuring D.[1], Koym J.[3], Huot O.[1], Ibanez F.[1], Yao, J. [1], Pierson, E[2], Miller, J.C.[2], Tamborindeguy, C.[1] Department of Entomology, [2] Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843, USA, [3] Texas A&M AgriLife Research Center Lubbock, TX

Abstract
Potato psyllids, *Bactericera cockerelli*, vector '*Candidatus* Liberibacter solanacearum' (Lso), a Gram-negative bacterium, the causative agent of potato zebra chip (ZC) disease, a plant disorder responsible for millions of dollars in losses. The existence of two Lso haplotypes (A and B) in the USA infecting solanaceous plants was reported. The geographic distribution of Lso haplotypes and their pathogenicity might explain inconsistencies reported by scientists related to disease development and severity, and the outcome of infected plants. For instance, the disease in New Zealand, where only haplotype A has been detected so far, presents some particularities compared with the disease observed in the USA, such as difficulties detecting Lso in plant samples or percentage of tuber germination from infected plants. The objective of this research was to test if differences in ZC symptoms could be related to Lso haplotype. We found that both Lso haplotypes can cause ZC but LsoA appears to induce milder symptoms (less pathogenic) in potato plants and tubers.

Introduction
The potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae) is an emergent pest in the Western half of the USA, Central America and New Zealand (Crosslin et al., 2010). It is a threat to all solanaceous crops because of the damage caused by its feeding and because it transmits the Gram negative bacterium '*Candidatus* Liberibacter solanacearum' (Lso), the causal agent of the zebra chip (ZC) disease of potato (Munyaneza 2007, Hansen et al., 2008, Liefting et al. 2008, Liefting et al., 2009).

Zebra chip was discovered in Mexico in 1994 and reported in Texas in 2000 (Crosslin et al., 2010). Damages caused by ZC were estimated to $33.4 million annually for 2006-2008 in Texas (CNAS 2009). Today, ZC is an emergent disease affecting the western half of the USA including Oregon, Washington and Idaho, Mexico, Central America and New Zealand (Crosslin et al., 2010).

In the past, variations in ZC symptom development and severity were observed but remained unexplained. Recently, the existence of different Lso haplotypes was reported (Nelson et al. 2011), which might explain, at least some of this observed variation. It appears that two Lso haplotypes exist in the USA (LsoA and LsoB) and that psyllids can harbor each haplotype or both haplotypes at the same time. Both haplotypes have been shown to be transmitted to plants and cause ZC. It remains to be shown if differences in disease or symptom development can be associated to infection by different haplotypes. To answer this question, psyllid colonies originated from a single Lso uninfected colony were infected with each Lso haplotype separately (LsoB and LsoA) or with both haplotypes at the same time (LsoAB) in the Tamborindeguy laboratory (Fig 1) (Yao and Tamborindeguy, 2015).

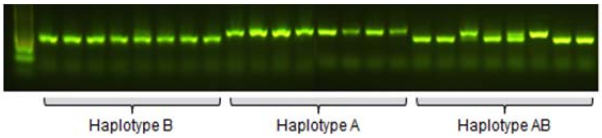


Figure 1: Haplotype test on individual psyllids from *B. cockerelli* colonies.

Materials and Methods

Bactericera cockerelli colonies carrying each Lso haplotypes were maintained on tomato. Lso presence and haplotype detection were performed regularly as in Nachappa et al. (2011), and Yao and Tamborindeguy (2015).

Solanum tuberosum 'Atlantic' were planted in January 15th 2014 in the Texas A&M AgriLife Research Station in Weslaco, TX, USA, and in May 16th 2014 in Barrett Farm in Springlake, TX, USA. Eight tubers per cage were panted; each cage was assigned one of the 5 treatments: control (no insects), Lso-free insects, LsoA-infected insects, LsoB-infected insects, and LsoAB-infected insects. Between eight and nine weeks after planting, when most of the plants were at flowering stage, two insects were transferred to each plant on organza bags, except in the control cage. The insects were given a one-week inoculation access period (IAP). After the IAP, leaves with the organza bags were removed. Tubers were harvested on April 28th 2014 and on September 28th 2014, respectively

After harvest, total number of tubers per plant, total tuber weight per plant and average tuber weight were measured. All tubers from each plant were tested for ZC symptoms. From each tuber, a chip was fried to detect ZC. ZC symptoms were scored from 1 to 6, with 1 representing no stripping or darkening and 6 representing very dark chips. The remaining of the tuber was kept in the cold room to break dormancy. Tubers were then allowed to germinate. Percentage of tubers germinating was recorded and presence of Lso was tested in sprouts by PCR.

Results and Discussion

Based on frying results, 7 out of 8 plants and 8 out of 8 in the LsoB and the LsoA treatments show ZC symptoms in the Weslaco trial, respectively. As expected, none of the tubers from the control cage or the Lso-free psyllid treatments tested positive for ZC. Unexpectedly, none of the tubers in the LsoAB-treatment showed ZC symptoms (Figure 2). The insects from the LsoAB colony are regularly

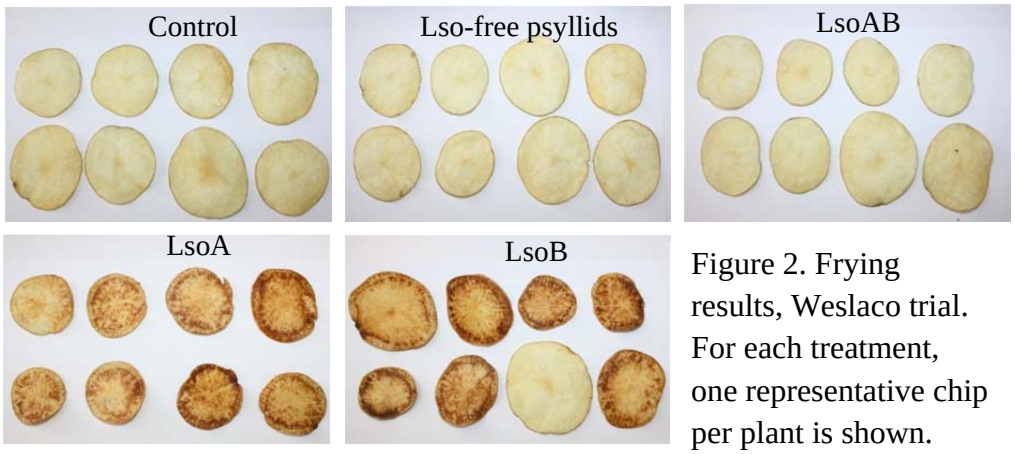


Figure 2. Frying results, Weslaco trial. For each treatment, one representative chip per plant is shown.

tested for presence of Lso and they are used regularly for plant infection. The causes of the absence of infection in this treatment remain unknown.

No differences in total number of tubers per plant (F=1.639, df=4, P=0.186), in total weight (F=1.646, df=4, P=0.185), or in average tuber weight (F=2.61, df=4, P=0.0521) were found among the treatments. Only differences in ZC grading score were found (F=34.23, df=4, P<0.001). LsoA- and LsoB-infected plants had significantly higher ZC. On average the control treatments and the LsoAB treatment scored 1 for ZC symptoms while LsoA and LsoB scored 3.

A similar experiment was carried in Springlake. Based on frying results, all plants in the infected treatments, except one plant in the plant in the LsoA treatment tested positive for ZC in the Springlake trial. All plants in the LsoAB treatment developed ZC symptoms which confirmed that the colony used for this treatment inoculates Lso. None of the plants in the control treatments appeared to be infected (Figure 3).

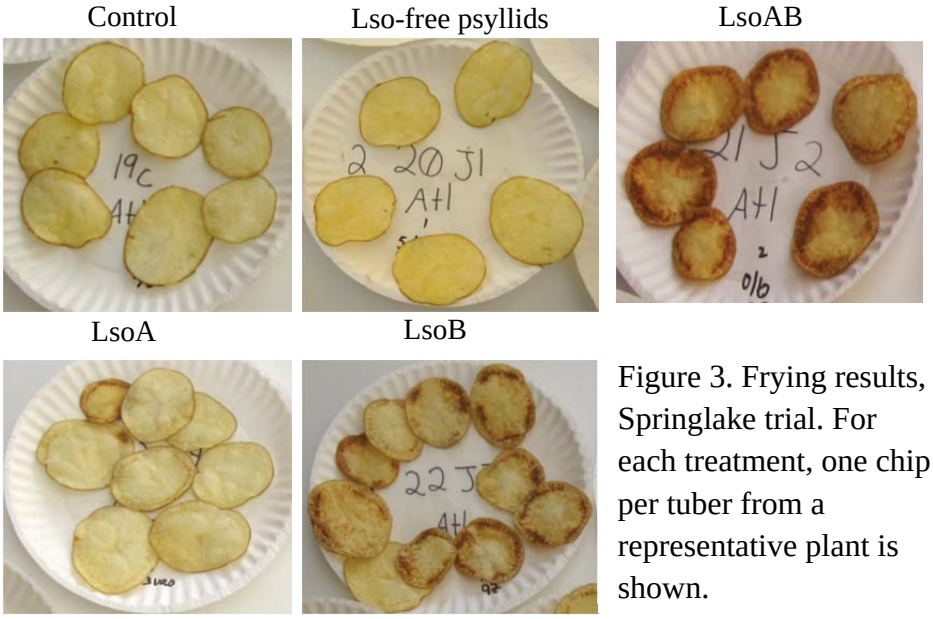


Figure 3. Frying results, Springlake trial. For each treatment, one chip per tuber from a representative plant is shown.

As in Weslaco, no differences in tuber counts (F=0.718, df=4, P=0.587), or total tuber weight (F=2.016, df=4, P=0.122) were found in the Springlake trials. However, there were significant differences among treatments on average tuber weight (F=6.4, df=4, P<0.001). Average tuber weight in infected plants was significantly lower than average tuber weight from plants that were infested with Lso-free psyllids. ZC scores were significant different among treatments (F=32.62, df=4, P<0.001). All infected treatments scored significantly higher for ZC grading than the uninfected controls. Interestingly, LsoB- and LsoAB- infected plants developed stronger ZC symptoms than LsoA-infected plants, which might suggest a difference of pathogenicity between LsoA and LsoB haplotypes.

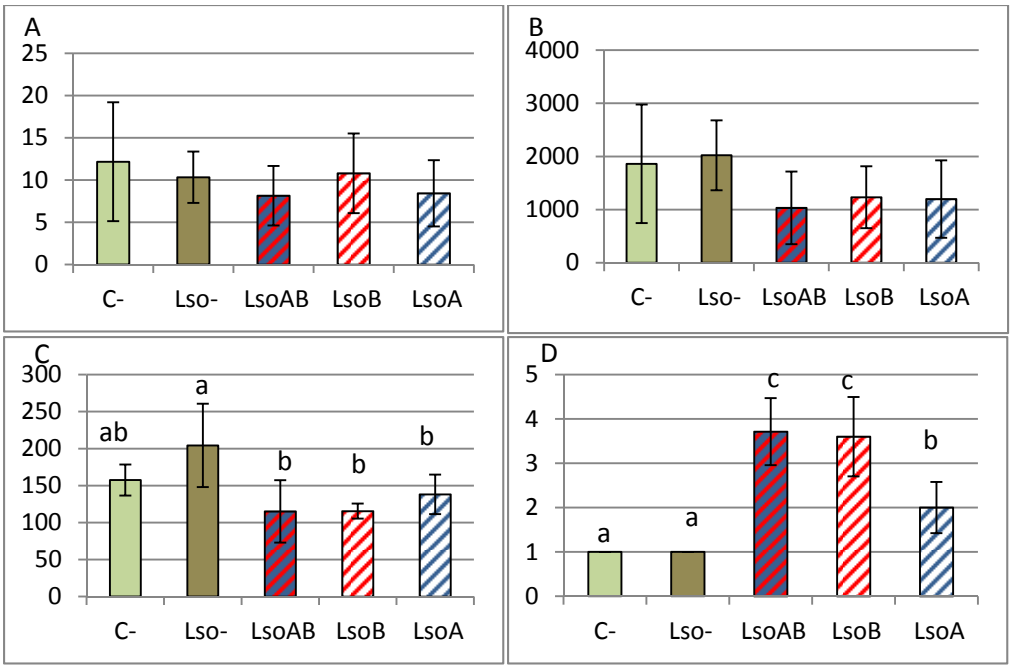


Figure 4. Effect of Lso haplotype on A: Number of tubers per plant; B: Total tuber weight per plant (in grams); C: Average tuber weight per plant (in grams); and D: ZC grading based on frying test.

Tubers from the Weslaco trial were kept in the cold room until November to break dormancy. Tubers were then kept at room temperature and sprouting was scored. Interestingly, none of the Lso-B infected tubers sprouted, while tubers from two of the LsoA-infected plants sprouted. High sprouting rate was measured for Lso-uninfected plants. Plants from the Springlake trial will be removed from the cold room in the near future.

Overall, these results confirmed that LsoA and LsoB haplotypes alone or in co-infection can be vectored by potato psyllids and can cause ZC disease in potato. Preliminary data indicate that LsoA-infected tubers might have higher sprouting rate than LsoB-infected tubers. These results seem to indicate that LsoA might be less pathogenic than LsoB.

Acknowledgements
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References
CNAS (2009) Economic Impacts of Zebra Chip on the Texas Potato Industry. http://cnas.tamu.edu/publications/Zebra%20Chip%20Impacts%20Final%20Jan%2009.pdf.

Crosslin, J.M., J.E. Munyaneza, J.K. Brown and L.W. Liefting (2010). Potato zebra chip disease: A phytopathological tale. Plant Health Prog. doi:10.1094/PHP-2010-0317-01-RV.

Hansen, A.K., J.T. Trumble, R. Stouthamer and T.D. Paine (2008). A new Huanglongbing Species, '*Candidatus* Liberibacter psyllaurous,' found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). Appl. Environ. Microbiol. 74: 5862-5865.

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

Liefting, L.W., P.W. Southerland, L.I. Ward, K.L. Paice, B.S. Weir and G.R.G. Clover (2009). A new '*Candidatus* Liberibacter' species associated with diseases of solanaceous crops. Plant Dis. 92: 208-214.

Munyaneza, J.E., J.M. Crosslin, and J.E. Upton. (2007). Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with "zebra chip," a new potato disease in southwestern United States and Mexico. J. Econ. Entomol. 100: 656-663.

Nachappa, P., J. Levy, E. Pierson and C. Tamborindeguy (2011). Diversity of endosymbionts in the potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), vector of zebra chip disease of potato. Curr. Microbiol. 62: 1510-1520.

Nelson, W., T. Fisher and J. Munyaneza (2011). Haplotypes of 'Candidatus Liberibacter solanacearum' suggest long-standing separation. Eur J Plant Pathol 130: 5-12.

Yao, J. and C. Tamborindeguy (2015). Infection by the different haplotypes of 'Candidatus Liberibacter solanacearum': influences on their vector, Bactericera cockerelli, and tomato host. J. Econ. Entomol. In review.

Assessing Transmission of '*Candidatus* Liberibacter solanacearum' Haplotypes through Seed Potato

Swisher, K.D. and Munyaneza, J.E.

United States Department of Agriculture- Agricultural Research Service, Wapato, WA 98951 USA

Abstract
Conflicting data has previously been reported concerning the impact of zebra chip disease transmission through seed tubers. These discrepancies may be due to the experimental design of each study, whereby different pathogen haplotypes, insect vector haplotypes, and potato plant varieties were used. In support of this, it has been suggested that the pathogen haplotypes induce different levels of zebra chip severity in potato. To address these factors that could have contributed to the conflicting results, we are conducting a large experiment to explore the impact of two '*Candidatus* Liberibacter solanacearum' (Lso) haplotypes, three *Bactericera cockerelli* psyllid haplotypes, and eight potato varieties on zebra chip disease transmission through tubers. Initial results suggest that both Lso haplotypes A and B are capable of inducing severe disease symptoms, regardless of the psyllid haplotype used to vector the Lso. Based on our preliminary results, it is not likely that pathogen haplotype or insect vector haplotype is the reason for the previous conflicting results.

Introduction
Zebra chip disease has caused significant economic losses to commercial potato growers across the United States, Mexico, Central America, and New Zealand. The disease has been attributed to the bacterium, '*Candidatus* Liberibacter solanacearum' (Lso) which is vectored to solanaceaous crops by the potato psyllid, *Bactericera cockerelli*. An understanding of the disease biology for identifying treatment and prevention options has been complicated by the finding of multiple Lso and psyllid haplotypes. Five haplotypes of Lso are currently known, including two (Lso A and Lso B) that are found in potato (Nelson et al. 2013). Currently, only Lso A has been reported in New Zealand, whereas both Lso A and B have both been found in the United States. Four haplotypes of the potato psyllid are currently known, including the Central, Western, Northwestern, and Southwestern haplotypes (Swisher et al. 2014). All four haplotypes have been identified in the United States, while only the Western haplotype has been reported in New Zealand.

The current understanding of zebra chip disease transmission has been hindered by conflicting results concerning the impact of Lso in seed tubers. A report by Pitman et al. (2011) suggests a significant number of zebra chip symptomatic tubers are capable of producing plants, many of which have asymptomatic foliar tissue but test positive for Lso by molecular methods. Contrary to this, it has been observed in the United States that zebra chip symptomatic tubers rarely produce plants and are epidemiologically unimportant when they do (Munyaneza, unpublished). These conflicting results could be due to differences in Lso haplotypes, psyllid haplotypes, and/or potato varieties. To assess these factors, we are currently conducting a large study to determine the impact of both Lso haplotypes A and B, as well as psyllid haplotypes Central, Western, and Northwestern, on eight different potato varieties.

Materials and Methods
Eight varieties of potatoes including Alturas, Russet Burbank, Russet Norkotah, Atlantic, Ranger Russet, Umatilla, FL 1867, and Pike were planted in 30 field cages each at the USDA-ARS research

farm in Moxee, Washington, as described in Munyaneza et al. (2008) and Buchman et al. (2011a,b). Ten different psyllid treatments were used including (1) Central psyllid with Lso A, (2) Central psyllid with Lso B, (3) Central psyllids with Lso A and B, (4) Western psyllid with Lso A, (5) Western psyllid with Lso B, (6) Western psyllids with Lso A and B, (7) Northwestern psyllid with Lso A, (8) Northwestern psyllid with Lso B, (9) Northwestern psyllids with Lso A and B, and (10) no psyllids. Each psyllid treatment was replicated three times on each potato variety. Psyllid and Lso haplotypes were confirmed prior to release by testing insects from each colony using high resolution melting analyses and conventional PCR, respectively, as described by Crosslin et al. (2011), Swisher et al. (2012), Cooper et al. (2015), and Wen et al. (2014). At tuber initiation stage, psyllids were released at the base of each potato plant for treatments 1-9. Psyllids were allowed to feed for two weeks before being eliminated by insecticide. At harvest, tubers were collected and a subset from each treatment was cut to score zebra chip disease severity. Portions of the cut tubers were stored for subsequent confirmation of Lso haplotypes by PCR as described in Wen et al. (2014).

Results and Discussion

Zebra chip disease severity in tubers was compared from each of the nine psyllid/Lso treatments and the no psyllid treatment control for the Alturas, Atlantic, Russet Burbank, and Russet Norkotah varieties. In all four varieties, severe symptoms were observed from samples inoculated with Lso A alone, Lso B alone, and with a dual infection of Lso A and B, regardless of the psyllid haplotype used to vector the Lso. An example of these severe symptoms can be seen in Alturas and Atlantic varieties (Figure 1 and 2, respectively). No zebra chip disease symptoms were seen in control tubers collected from cages with no psyllid or Lso pressure for any of the four varieties. From these results, it can be concluded that the Lso haplotype B does not produce disease symptoms that are more severe than Lso haplotype A in the Alturas, Atlantic, Russet Burbank, and Russet Norkotah varieties. These results suggest that the reported discrepancies concerning the importance of Lso transmission through seed potato are likely not due to differences in disease severity caused by Lso haplotypes A and B.

This study will be continued in the following field season, where zebra chip symptomatic tubers from each of the nine psyllid/Lso treatments and healthy tubers from the no psyllid control will be planted in large cages. Over the course of the growing season, observations will be made on emergence rates from diseased and healthy tubers and upon harvest, yield and disease symptoms will be recorded from the daughter tubers. Additionally, leaf tissue will be collected from the emerging plants, and molecular diagnostics will be conducted to determine if Lso is present. Testing of the leaf tissue will determine if the emerging plants could be a source of bacterium for the psyllid vectors to obtain and transmit.

Acknowledgements

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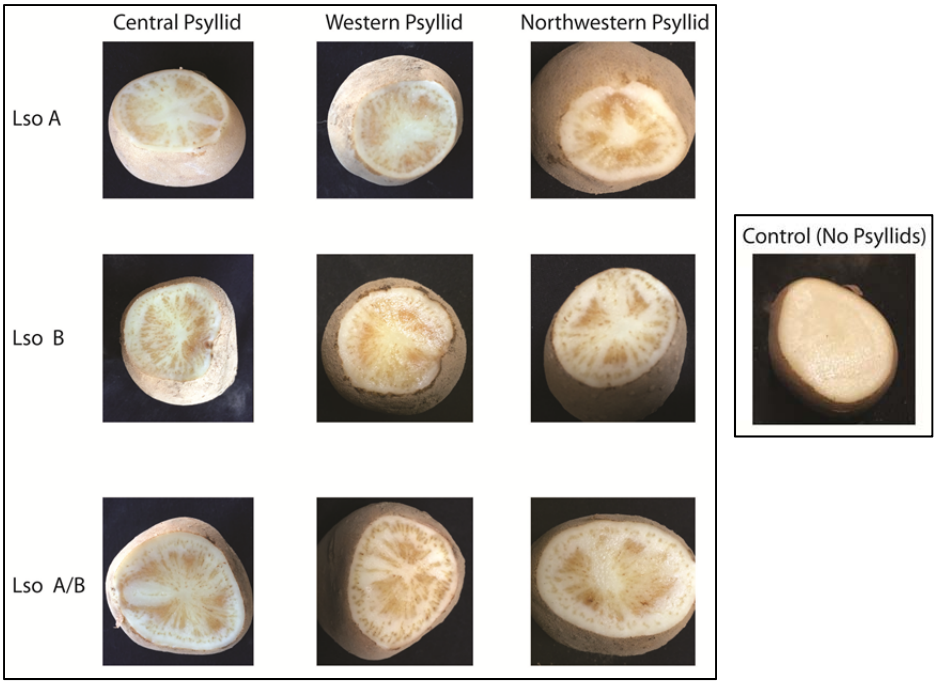

Figure 1. Severe zebra chip disease symptoms were recorded in the Alturas variety for all psyllid and Lso treatments (1-9). Columns depict psyllid haplotype used to vector the Lso pathogen, and rows depict the Lso haplotype used for zebra chip infection. Control tubers had no psyllid or Lso pressure.

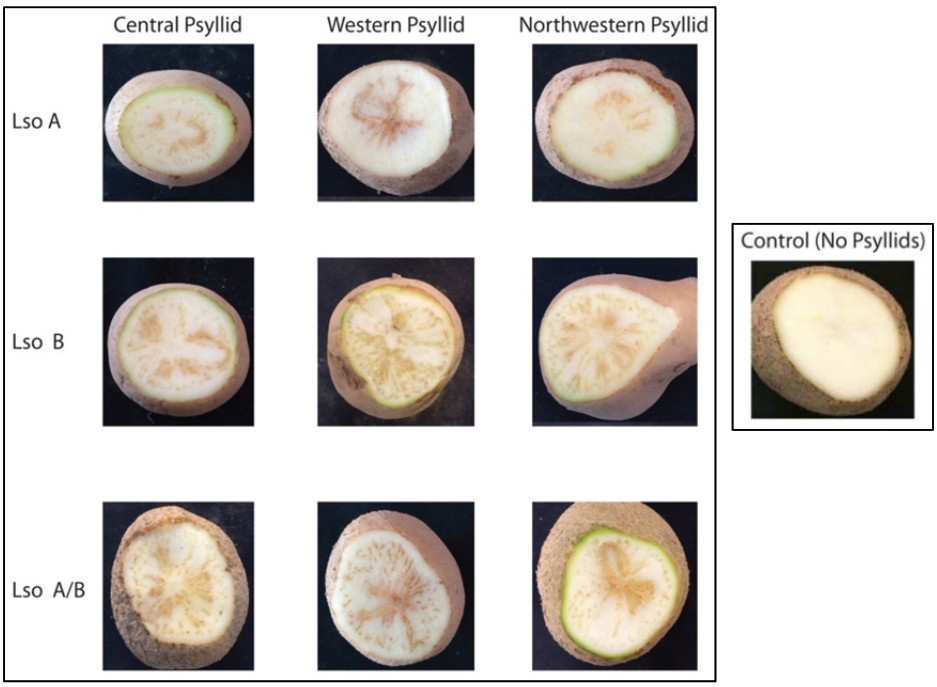

Figure 2. Severe zebra chip disease symptoms were recorded in the Atlantic variety for all psyllid and Lso treatments (1-9). Columns depict psyllid haplotype used to vector the Lso pathogen, and rows depict the Lso haplotype used for zebra chip infection. Control tubers had no psyllid or Lso pressure.

References

Buchman, J.L., B.E. Heilman, and J.E. Munyaneza 2011a. Effects of liberibacter-infective *Bactericera cockerelli* (Hemiptera: Triozidae) density on zebra chip potato disease incidence, potato yield, and tuber processing quality. J. Econ. Entomol. 104: 1783-1792.

Buchman, J.L., V.G. Sengoda, and J.E. Munyaneza. 2011b. Vector transmission efficiency of liberibacter by *Bactericera cockerelli* (Hemiptera: Triozidae) in zebra chip potato disease: effects of psyllid life stage and inoculation access period. J. Econ. Entomol. 104: 1486-1495.

Cooper, W.R., K.D. Swisher, S.F. Garczynski, T. Mustafa, J.E. Munyaneza, and D.R. Horton. 2015. Wolbachia Infection Differs among Divergent Mitochondrial Haplotypes of Bactericera cockerelli (Hemiptera: Triozidae). A. Entomol. Soc. Am. (*In press*).

Crosslin, J.M., H. Lin, and J.E. Munyaneza. 2011. Detection of '*Candidatus* Liberibacter solanacearum' in potato psyllids, *Bactericera cockerelli* Sulc, by conventional and real-time PCR. Southwest. Entomol. 36: 125-135.

Munyaneza J.E., J.L. Buchman, J.E. Upton, J.A. Goolsby, J.M. Crosslin, G. Bester, G.P. Miles, and V.G. Sengoda. 2008. Impact of different potato psyllid populations on zebra chip disease incidence, severity, and potato yield. Subtrop. Plant Sci. 60: 27-37.

Nelson, W.R., V.G. Sengoda, A.O. Alfaro-Fernandez, M.I. Font, J.M. Crosslin, and J.E. Munyaneza. 2013. A new haplotype of "*Candidatus* Liberibacter solanacearum" identified in the Mediterranean region. Eur. J. Plant Pathol. 135: 633-639.

Pitman, A.R., G.M. Drayton, S.J. Kraberger, R.A. Genet, and I.A.W. Scott. 2011. Tuber transmission of '*Candidatus* Liberibacter solanacearum' and its association with zebra chip on potato in New Zealand. Eur. J. Plant Pathol. 129: 389-398.

Swisher, K.D., J.E. Munyaneza, and J.M. Crosslin. 2012. High resolution melting analysis of the cytochrome oxidase I gene identifies three haplotypes of the potato psyllid in the United States. Environ. Entomol. 41: 1019–1028.

Swisher, K.D., D.C. Henne, and J.M. Crosslin. 2014. Identification of a fourth haplotype of the potato psyllid, *Bactericera cockerelli*, in the United States. J. Insect Sci. 14: 1-7.

Wen A., C. Johnson, N.C. Gudmestad. 2013. Development of a PCR assay for the rapid detection and differentiation of '*Candidatus* Liberibacter solanacearum' haplotypes and their spatiotemporal distribution in the United States. Am. J. Pot. Res. 90: 229-236.

Temporal and Spatial Variations of Psyllid Haplotype Occurring in Indigenous Vegetation of Texas

Paetzold, L.[1], Rashed, A.[2], Workneh, F.[1], and Rush C.M.[1]
[1]Texas A&M Agrilife Research, Bushland, TX 79012; [2]University of Idaho, Aberdeen Research & Extension Center, Aberdeen, ID 83210

Abstract
The potato psyllid (*Bactericera cockerelli*) is the vector of '*Candidatus* Liberibacter solanacearum' (Lso), the putative causal agent of potato zebra chip. Currently four distinct haplotypes of the insect (Central, Northwest, Southwestern, and Western) are recognized as occurring is the US. Knowledge of the dynamics of the different haplotypes in different locales is useful in understanding insect ecology, survival, and movement across regions. In 2012-2013, potato psyllids were collected from the Texas Panhandle (Bushland, Dalhart, and Olton) and southwest Texas (Andrews, Fort Stockton, and Kermit), using yellow sticky traps placed in natural vegetation and collected and replaced biweekly. DNA from the psyllids was extracted and tested for haplotype using high resolution melting point analysis (HRM). Three of the known haplotypes (Central, Southwestern, and Western) were detected in the survey. However, the vast majority of the psyllids were of the Central haplotype (84.4%) of which 2.7% tested positive for Lso with similar proportions of Lso type A&B. In some of the locations the proportion of individual psyllid haplotypes varied over time, which appeared to suggest seasonal displacement of one haplotype with another.

Introduction
The potato psyllid (*Bactericera cockerelli*) is the vector of *Candidatus* Liberibacter solanacearum (Lso), the putative causal agent of potato zebra chip (Munyaneza et al., 2007). For the last several years studies have been conducted to determine the survivability and the Lso status of potato psyllids in wild vegetation in the Texas Panhandle and parts of the southwestern Texas. Results of the surveys were reported previously by Arash et al. (2013). Recently, four haplotypes of the potato psyllid (Central, Northwest, Southwestern, and Western; Swisher et al., 2013) and two haplotypes the Lso (A and B; Wen et al., 2013) were discovered as occurring in the US. Knowledge of the dynamics of the different haplotypes in different locales is useful in understanding the psyllid ecology, survival, and movement across regions. The current project was initiated with a major objective characterizing psyllids collected from the natural vegetation over the last several years to understand the spatial and temporal variations of the individual psylld and the Lso haplotypes.

Materials and Methods
Psyllid collection: In 2012-2014, potato psyllids were collected from the Texas Panhandle (Bushland, Dalhart and Olton), West Texas (Andrews, Fort Stockton and Kermit), and South Texas (Pearsall), using yellow sticky traps placed in native pastures (surrounded by natural vegetation) and collected and replaced biweekly (Fig. 1).

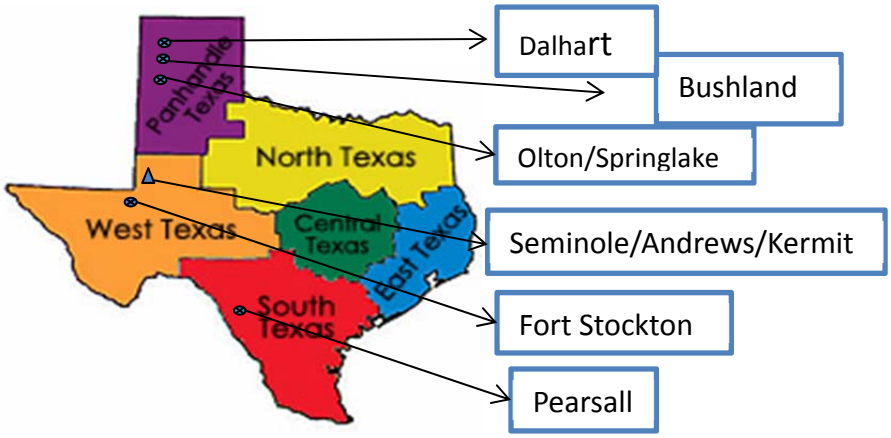

Fig. 1. Psyllid sampling locations in Texas.

DNA extraction: Potato psyllid DNA extractions were performed using QIAGEN DNeasy Blood and Tissue Kit.
Lso detection: The Lso pathogen detections were performed on a ViiA7 Real-Time PCR machine (ABI), using the primer pair LsoF/HLBr, Taqman Fast Advanced Master Mix, and custom Taqman probe HLBp (ABI). (Li et al., 2009).
Psyllid haplotype test: High Resolution Melt (HRM) was performed to distinguish among Central, Western and Southwestern haplotypes, using the primer pair CO1F1/CO1R1, and MeltDoctor HRM Master Mix (ABI). (Swisher et al., 2012). See Figure 2A (Aligned Melt Curves).
Lso haplotype test: SYBR Green was applied to distinguish between LsoA and LsoB, using two primer pairs LsoHA6f/LsoHA6r and LsoHB6f/LsoHB6r, and SYBR Select Master Mix (ABI). (Wen et al., 2013 proceeding). See Figure 2B (Melt Curve Plot).

Fig. 2A. High resolution melt (HRM) curves of different haplotypes.

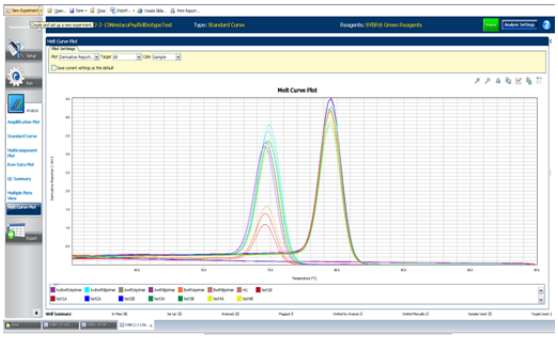

Fig. 2B. Differentiation of Lso haplotypes A & B using SYBR Green analysis.

Results and Discussion

Psyllid haplotypes. Overall, of the 1634 psyllids tested during the two-year survey period, the vast majority of them were the Central haplotype (84.4%) followed by the Southwestern (11.9%) and lastly the Western with 3.7%. In some of the locations, psyllid populations shifted in haplotype structure as the proportion one type haplotype was replaced by another. Although the overall haplotype structure was dominated by the Central type, occasionally the proportions of other haplotypes were greater. For

example, in Andrews and Bushland the Central haplotype was overtaken by either the Western or the Southwestern (Figs. 5 and 6).

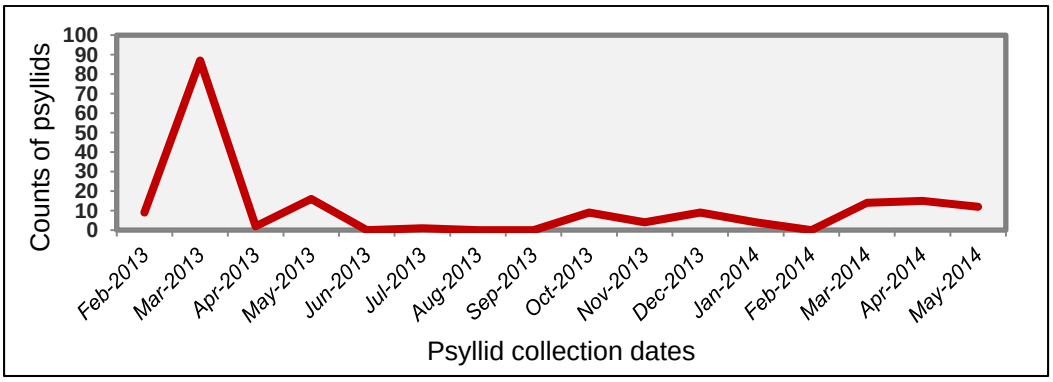

Fig. 3. Number of psyllids collected and tested from Andrews over time.

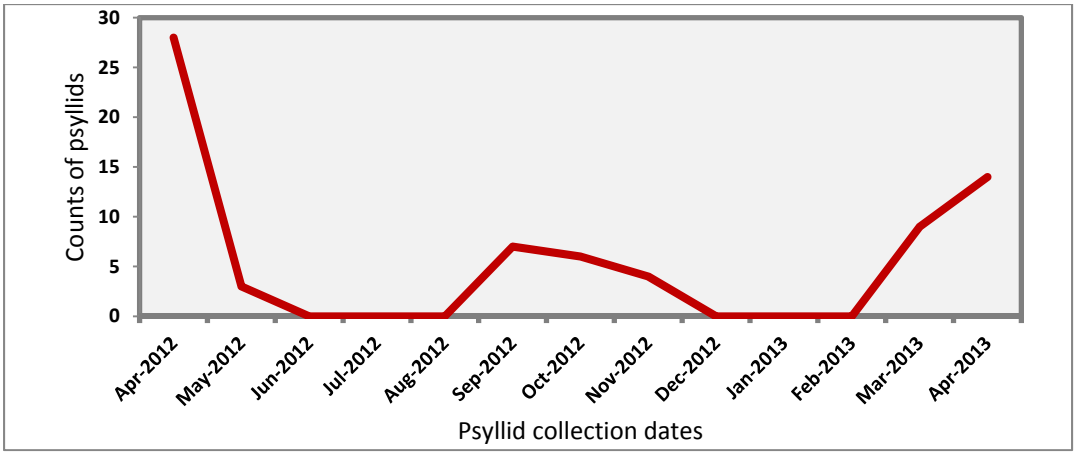

Fig.4. Number of psyllids collected and tested from Bushland over time.

There were also shifts in haplotype proportion in psyllids collected from other locations albeit not as substantial as that of Andrews and Bushland. Changes in haplotype proportion over time suggest that in wild ecosystems haplotype structures are not static but rather dynamic, which may be the result of influxes or departures certain haplotypes during a given season in reaction changes in environmental conditions.

Lso haplotypes. Approximately 2.7% the Central haplotype and 6.7% of the Western tested positive for Lso (Fig. 7). However, none of the Southwestern tested positive for the pathogen. Overall, about 2.5% of all psyllids tested positive Lso. 45.9% of the Lso in the Central haplotype were type A while 43% were type B. The rest (10.8%) were mixes of A&B (Table 1). 75% of Lso in the Western haplotype were A and rest (25%) was A&B mix. No type B by itself was detected in the Western psyllid haplotype.

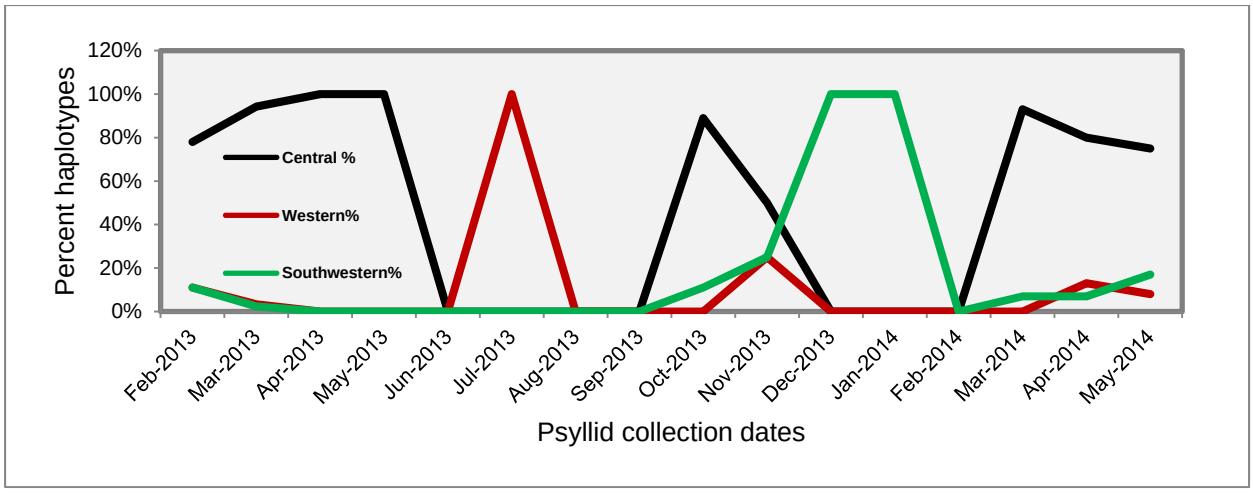

Fig. 5. Percentages of haplotypes collected and tested from Andrews over time.

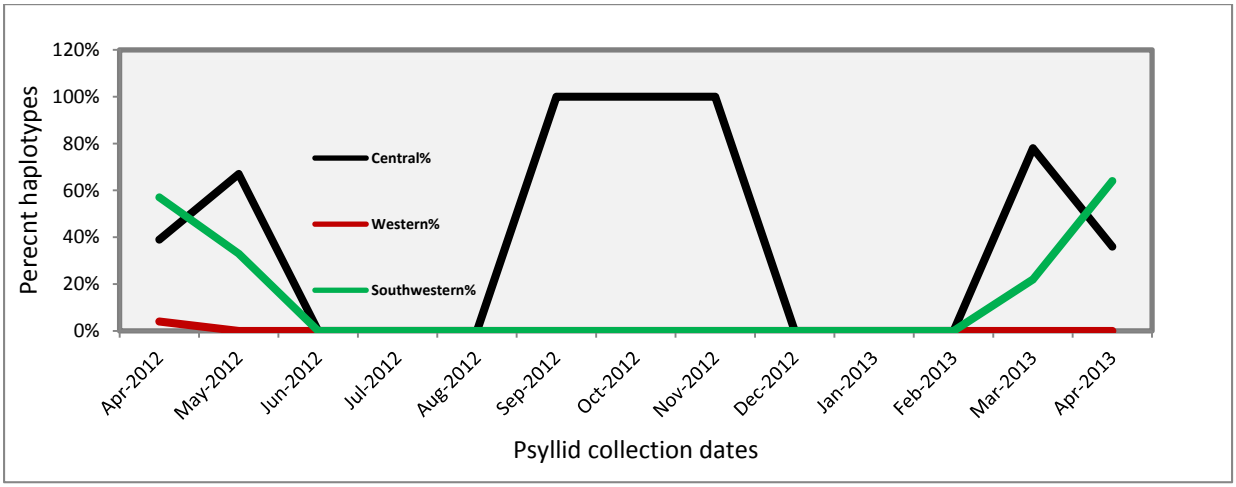

Fig. 6. Percentages of haplotypes collected and tested from Bushland over time.

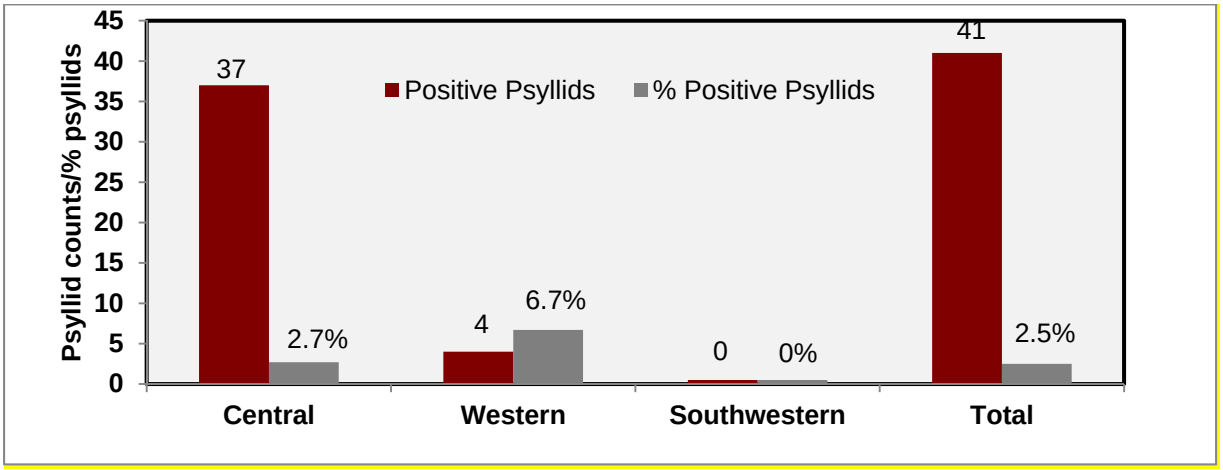

Fig. 7. Number and percentage of haplotypes which tested positive /negative for Lso.

Psyllid Haplotype	Lso Haplotype		
	Lso A	Lso B	Lso A+B
Central	17	16	4
Western	3	0	1
Southwestern	0	0	0

Table 1. Lso types in different psylid haplotypes.

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We thank Chelsea Archer, Jewel Arthur, Beau Bealmear, Becky Bryan, Jared Bull, Karina Camorlinga, Joshua Correa, Alisa Dubric, Jimmy Gray, Chelsey Mckee, Stephanie Pattison and Logan Strowmatt for collecting traps, counting psyllids, and testing psyllids.

References

Li, W., Abad, J.A., French-Monar, R.D., Rascoe, J., Wen, A., Gudmestad, N.C., Secor, G.A., Lee, I., Duan, Y., and Levy, L. 2009. Multiplex real-time PCR for detection, identification and quantification and quantification of '*Candidatus* Lieribacter solanacearum' in potato plants with zebra chip. Journal of Microbiological Methods 78 (2009) 59-65.

Munyaneza, J. E., Crosslin, J. M., and Upton, J. E. 2007. Association of *Bactericera cockerelli* with zebra chip, a new potato disease in southwestern United States. J. Econ. Entomol. 100:656-663.

Rashed, A., Paetzold, L., Archer, C., Pattison, S., Bralley, J. B., Gray, J., Workneh, F., and Rush, C. M. 2012. Potato psyllid population fluctuations in natural vegetation in relation to potato emergence time across Texas. Pages 58-62. In: The Proceedings of the 12th Annual SCRI Zebra Chip Reporting Session. F. Workneh and C.M. Rush eds., San Antonio, TX, Oct. 30 to Nov. 2.

Swisher, K.D., Munyaneza, J.E., and Crosslin, J.M. 2012. High Resolution Melting Analysis of the Cytochrome Oxidase I Gene Identifies Three Haplotypes of the Potato Psyllid in the United States. Environmental Entomology. 41(4):1019-1028.

Wen, A., Johnson, C., and Gudmestad, N. C. 2013. Development of Multiplex Conventional and Real-Time PCR for Detection and Genotyping of '*Candidatus* Liberibacter solanacearum'. Pages 68-72. In: The Proceedings of the 13th Annual SCRI Zebra Chip Reporting Session. F. Workneh and C.M Rush eds. San Antonio, TX, November 3-6, 2013.

Liberibacter Transmission Efficiency among Potato Psyllid Haplotypes

[1,2]Mustafa, T., [1]Munyaneza, J.E., [1]Swisher, K.D., [1]Horton, D.R., and [2]Zack, R.

[1]USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA 98951, USA
[2]Department of Entomology, Washington State University, Pullman, WA 99164, USA

Abstract
The potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a vector of the phloem-limited bacterium '*Candidatus* Liberibacter solanacearum' (Lso), the putative causal agent of zebra chip disease of potato. Little is known on the mechanisms by which the potato psyllid transmits Lso to potato. This lack of information is compounded by the recent discovery of at least four haplotypes of potato psyllid that often co-occur on potato crops in the Pacific Northwest. It is not known whether these haplotypes differ in their Lso transmission efficiency, which would necessitate haplotype-specific management approaches. The present study used the electrical penetration graph (EPG) technology to assess Lso transmission rates among the potato psyllids of three haplotypes. Transmission rates of Lso were not statistically different, suggesting that Lso transmission efficiency was similar among the haplotypes. Average Lso transmission rate was < 10% with <2 h inoculation access period, but increased to ~40% and 60% after 3 h and 24 h inoculation access periods, respectively.

Introduction
Zebra chip is an economically important disease of potato caused by the phloem-limited bacterium '*Candidatus* Liberibacter solanacearum' (Lso), and is vectored by the potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) (Munyaneza 2012). Zebra chip disease has caused millions of dollars in losses to the potato industry in the United States, Mexico, Central America, and New Zealand (Munyaneza 2012). Zebra chip was first reported in Mexico in 1994 and in 2000 in Texas (Munyaneza 2012). It was not until 2011 that the disease was first observed in the Pacific Northwest, the major potato growing region of the United States (Crosslin et al. 2012a,b). Little is known about psyllid-Lso interactions and the mechanisms by which the potato psyllid transmits Lso to potato. This lack of information is compounded by the recent discovery of at least four genetic variants, or haplotypes, of potato psyllid that often co-occur in potato fields in the Pacific Northwest. An innovative technology known as "Electrical Penetration Graph" (EPG) (Backus & Bennett 2009; Pearson et al. 2014) was used to compare Lso transmission efficiency among three psyllid haplotypes (Central, Western, and Northwestern), commonly found in potato crops in the Pacific Northwest.

Materials and Methods
Sources of Insects and Plants. Lso-infected potato psyllid colonies of three haplotypes (Central, Western, and Northwestern haplotypes) were established from psyllids collected from Weslaco, Texas (Central haplotype), southern California (Western haplotype), and Prosser, Washington (Northwestern haplotype) at the USDA-ARS facility in Wapato, WA, and maintained under laboratory conditions of 25±1 °C, 40±5% RH, with a photoperiod of 16:8 (L: D) h. Prior to conducting assays, the infection status was confirmed using conventional PCR (Munyaneza et al. 2010) and the haplotype status of each psyllid colony was confirmed by examination of mitrocondrial DNA (mtDNA) sequences, using high resolution melting analysis as described by Swisher et al. (2012, 2014a).

Certified disease-free potatoes (var. Atlantic) were grown from seed tubers obtained from CSS Farms Inc. (Colorado City, CO). The tubers were planted in 0.5-L pots (Kord Products, Toronto, Ontario, Canada) filled with a soil medium consisting of 86% sand, 13.4% peat moss, 0.5% Apex time release fertilizer (J. R. Simplot Co., Lathrop, CA), and 0.1% Micromax micronutrients (Scotts Co., Marysville, OH) in a greenhouse at USDA-ARS, Wapato, WA. Prior to conducting assays, the plants were tested for '*Ca*. L. solanacearum' by PCR (Munyaneza et al. 2010) to ensure they were free of the bacterium.

Assessing Lso transmission efficiency. Lso transmission efficiency for each of the three psyllid haplotypes was assessed by using electrical penetration graph (EPG) technology (Pearson et al. 2014). Adults from three Lso-infected psyllid haplotype colonies were collected and starved for 6 h at 4 °C. The psyllids were then attached to an insect electrode gold wire with a silver glue adhesive and were placed on the abaxial surface of the potato leaves for EPG recordings. The psyllids were given inoculation access periods of 1, 2, 3, 4, 5, 6, 12, and 24 h. After each access period, the psyllids were collected and tested for Lso by PCR to confirm infection. A total of 20 psyllids were used for each haplotype and for each inoculation access period. After the EPG recordings, the psyllid-exposed plants were transplanted outdoors under field cage conditions (Buchman et al., 2011) and monitored for 4 to 6 weeks for zebra chip disease symptom development (Sengoda et al., 2014). In addition, the plants were tested for Lso by PCR at the end of the experiment to confirm infection. Lso transmission rate was determined for each of psyllid haplotype and inoculation access period by dividing the number of Lso-infected plants by the number of Lso-positive psyllids.

Data Analysis. The Lso transmission rate was compared among the three haplotypes using the GLIMMIX procedure of SAS 9.3 (SAS Institute 2012).

Results and Discussion

Statistical analysis of Lso transmission rate among the psyllids of the three haplotypes (Fig. 1) showed that overall transmission of Lso to potato plants was not affected by the haplotype ($F_{2, 464}$= 0.90, P= 0.41). However, the statistical analysis showed that Lso transmission rate increased with the inoculation access time period ($F_{7, 464}$= 6.17, P= < 0.001). There was no haplotype by inoculation access period interaction indicating that the effects of inoculation access period on Lso transmission was similar among the three haplotypes ($F_{14, 464}$= 0.26, P= 0.99).

The present study examined Lso transmission efficiency among the three psyllid haplotypes by recording probing behaviors of the insects during selected inoculation access periods and then monitoring the inoculated plants for zebra chip symptoms and confirming Lso infection by PCR testing. Particular attention was paid to the time the psyllids spent probing in the phloem tissue. Lso is presumably inoculated into healthy plants during the phloem salivation phase, whereas the bacterium is acquired from infected plants during phloem ingestion (Pearson et al., 2014, Sandanayaka et al., 2014).

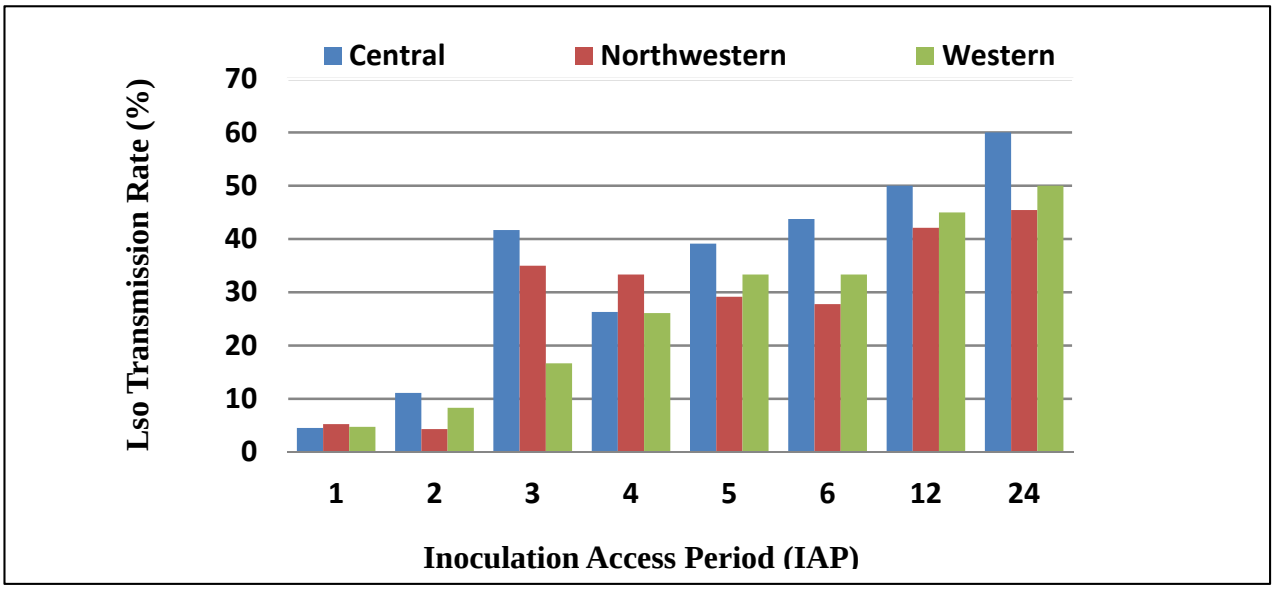

Figure 1. '*Candidatus* Liberibacter solanacearum' (Lso) transmission rate by potato psyllids of three haplotypes when feeding on potato.

Results showed that Lso transmission rates among the psyllids of the three psyllid haplotypes were not statistically different, suggesting that Lso transmission efficiency was similar among psyllids of the three haplotypes, regardless of inoculation access period (Fig. 1). However, Lso transmission rate for each of the psyllid haplotypes significantly increased with the inoculation access period (Fig. 1). Average Lso transmission rate remained low (< 10%) during the first two hours but significantly increased with 3 h inoculation access period (up to 40%) and was highest when the insects were allowed access to the plants for 24 h (up to 60%) (Fig. 1). An earlier report by Buchman et al. (2011) indicated that single potato psyllids (of Central haplotype) were effective in inducing zebra chip, with an average of Lso transmission rate of 47%, when given an inoculation access period as short as 6 h. That report is consistent with the results of the present study, during which an Lso transmission rate of 43% was observed for psyllids of Central haplotype when allowed access to the plants for 6 h. Results of the present study and those of Buchman et al. (2011) and Sandanayaka et al. (2014) clearly underscore the high risk of Lso infection by potato psyllid and substantial challenges to controlling this insect vector to manage zebra chip disease.

To date, Lso infection has been rare in Northwestern haplotype psyllids (the predominant haplotype in the Pacfic Northwest) and little zebra chip has been observed in the Columbia Basin after the 2011 potato growing season, prompting the question as to whether these psyllids are efficient vectors of Lso. Results of the present study clearly indicate that Northwestern haplotype potato psyllids are equally efficient in transmitting Lso to potato as Central and Western haplotype psyllids. The results also suggest that the recent low incidence of zebra chip observed in the Columbia Basin may be due to predominantly occurring Lso-free Northwestern haplotype psyllid populations. However, potato growers in the region should be cautious as the situation could change, especially if Lso ends up spreading throughout these psyllid populations through horizontal and vertical transmission. This could lead to zebra chip spreading further in the region and potentially causing serious damage to potato crops.

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References
Backus, E. A., and W. H. Bennett. 2009. The AC-DC correlation monitor: new EPG design with flexible input resistors to detect both R and emf components for any piercing sucking hemipteran. J. Insect Physiol. 55: 869-884.

Buchman, J. L., V. G. Sengoda, and J. E. Munyaneza. 2011. Vector transmission efficiency of Liberibacter by *Bactericera cockerelli* (Hemiptera: Triozidae) in zebra chip potato disease: effects of psyllid life stage and inoculation access period. J. Econ. Entomol. 104: 1486–1495.

Crosslin, J. M., P. B. Hamm, J. E. Eggers, S. I. Rondon, V. G. Sengoda, and J. E. Munyaneza. 2012a. First report of zebra chip disease and "*Candidatus* Liberibacter solanacearum" on potatoes in Oregon and Washington State. Plant Dis. 96: 452.

Crosslin, J. M., N. Olsen, and P. Nolte. 2012b. First report of zebra chip disease and "*Candidatus* Liberibacter solanacearum" on potatoes in Idaho. Plant Dis. 96: 453.

Sandanayaka, W. R. M., A. Moreno, L. K. Tooman, N. E. M. Page-Weir, and A. Fereres. 2014. Stylet penetration activities linked to the acquisition and inoculation of *Candidatus* Liberibacter solanacearum by its vector tomato potato psyllid. Entomol. Exp. Appl. 151: 170–181.

SAS Institute. 2012. SAS release 9.3 ed. SAS Institute, Cary, NC.

Sengoda, V. G., W. R. Cooper, K. D. Swisher, D. C. Henne, J. E. Munyaneza. 2014. Latent period and transmission of ''*Candidatus* Liberibacter solanacearum'' by the potato psyllid *Bactericera cockerelli* (Hemiptera: Triozidae). PLoS ONE, 9 (3): e93475. doi:10.1371/journal.pone.0093475

Swisher, K. D., J. E. Munyaneza, and J. M. Crosslin. 2012. High resolution melting analysis of the cytochrome oxidase I gene identifies three haplotypes of the potato psyllid in the United States. Environ. Entomol. 41: 1019-1028.

Swisher, K. D., V. G. Sengoda, J. Dixon, J. E. Munyaneza, A. F. Murphy, S. I. Rondon, B. Thompson, A. V. Karasev, E. J. Wenninger, N. Olsen, J. M. Crosslin. 2014. Assessing potato psyllid haplotypes in potato crops in the Pacific Northwestern United States. Am. J. Pot. Res. 91: 485-491.

Pearson, C. C., E. A. Backus, H. J. Shugart, and J. E. Munyaneza. 2014. Characterization and correlation of EPG waveforms of *Bactericera cockerelli* (Hemiptera: Triozidae): variability in waveform appearance in relation to applied signal. Ann. Entomol. Soc. Am. 107 (3): 650-666.

Munyaneza, J.E. 2012. Zebra chip disease of potato: biology, epidemiology, and management. Am. J. Pot. Res. 89: 329-350.

Assessing Reproduction of Potato Psyllid Haplotypes

[1,2]Mustafa, T., [1]Munyaneza, J. E., [1]Horton, D. R., [1]Cooper, W.R., [1]Swisher, K.D., and [2]Zack, R.S.

[1]USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA 98951, USA
[2]Department of Entomology, Washington State University, Pullman, WA 99164, USA

Abstract
Potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a serious pest of solanaceous crops in North and Central America and New Zealand. This insect vectors the bacterium '*Candidatus* Liberibacter solanacearum' that causes zebra chip disease of potato. So far, four distinct genetic populations, or haplotypes, of potato psyllid have been identified. Three of the haplotypes may co-occur in potato fields in the Pacific Northwest of U.S. Solanaceous weeds, including the perennial *Solanum dulcamara* (bittersweet nightshade), may provide refuge for psyllid populations which then migrate to potato crops. This study tested whether fecundity and fertility (% egg hatch) of potato psyllid were affected by host plant (*S. dulcamara* or potato) and whether these reproductive traits were similar among the three most common psyllid haplotypes in the Pacific Northwest: Northwestern, Central, and Western. The total female fecundity differed significantly among haplotypes, with an average lifetime fecundity of 1050, 877, and 629 eggs for Northwestern, Western, and Central females, respectively. Egg hatch was significantly reduced in psyllids reared on bittersweet nightshade (61.9%) versus potato (81.3%). Adult psyllids lived longer on nightshade than on potato, averaging 113.9 and 108.4 d on nightshade and 79.0 and 85.5 d on potato for males and females, respectively. Information from these studies will help growers develop more effective management strategies for zebra chip and its insect vector.

Introduction
Potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is economically detrimental to potato (*Solanum tuberosum* L.) and other crops in the Solanaceae (Wallis 1955). Damage is caused directly by its feeding activities, as well as by its vectoring of the bacterium '*Candidatus* Liberibacter solanacearum', the putative causal agent of zebra chip disease of potato (Munyaneza 2012). Potato psyllid is distributed in the United States, Mexico, and Central America (Pletsch 1947, Wallis 1955, Munyaneza 2012), and in New Zealand (Teulon et al. 2009). Four genetically distinct populations, or haplotypes, of potato psyllid have been identified so far (Swisher et al. 2012, 2014). Depending on the perceived centers of their geographic distributions, these haplotypes are referred to as "Central" (Texas and central U.S., eastern Mexico, and Central America), "Western" (southern California, Baja California, Mexico), "Northwestern" (Washington, Idaho, Oregon) and "Southwestern" (New Mexico, southern Colorado, north eastern Colorado). Currently, the only means to effectively manage zebra chip is by targeting potato psyllid for control (Munyaneza 2012). Potato psyllid management in Pacific Northwest potato crops is made more difficult due to the presence of these different psyllid haplotypes, which may differ in biological traits that determine their respective contributions to zebra chip epidemiology. The perennial bittersweet nightshade (*Solanum dulcamara* L.) has now been recognized to be a critical resource for potato psyllid overwintering in the Pacific Northwest (Jensen et al. 2012). Bittersweet nightshade is also a host of '*Ca*. L. solanacearum'(Murphy et al. 2014). Advances in understanding potato psyllid haplotype biology and in defining suitability of bittersweet nightshade for potato psyllids of each haplotype will help the potato industry in the Pacific Northwest make informed decisions about the need for psyllid controls as components of their pest management programs. The

objectives of the current study were to evaluate the host effects of bittersweet nightshade and potato on reproductive traits of three potato psyllid haplotypes commonly found in Pacific Northwest potato crops.

Materials and Methods

Sources of Plants and Insects. Potato (var. Russet Burbank) was grown from seed tubers (Skone and Connors Produce Inc., Warden, WA), and bittersweet nightshade was grown from cuttings collected locally (Wapato, WA), in 0. 5-L pots (Kord Products, Toronto, Ontario, Canada) filled with a soil medium consisting of 86% sand, 13.4% peat moss, 0.5% Apex time release fertilizer (J. R. Simplot Co., Lathrop, CA), and 0.1% Micromax micronutrients (Scotts Co., Marysville, OH) in a greenhouse at USDA-ARS, Wapato, WA.

Psyllid colonies of three potato psyllid haplotypes were established at the USDA-ARS facility in Wapato, and maintained on bittersweet nightshade or potato in the laboratory at 25 °C ± 1, 40±5% RH, with a photoperiod of 16:8 (L: D) h. Prior to conducting experiments, the plants and psyllids were tested for '*Ca*. L. solanacearum' by PCR (Munyaneza et al. 2010) to ensure they were free of the bacterium. Haplotype status of each of the psyllid colonies was confirmed by examination of mitrocondrial DNA (mtDNA) sequences, using high resolution melting analysis methods (Swisher et al. 2012, 2014a).

Fecundity, Egg Fertility, and Adult Longevity. Fecundity, egg fertility, and adult longevity of potato psyllids from each haplotype were assessed by rearing individual insects in small transparent cages as described by Mustafa et al. 2014 (Fig. 1), on either bittersweet nightshade or potato under conditions of 25 °C ± 1, 40±5% RH, and a photoperiod of 16:8 (L: D) h. Fifth instar nymphs of each haplotype were gently removed from the nightshade-reared and potato-reared colonies of psyllids with a soft painting brush and held individually in small cages to obtain virgin adults for the reproductive fitness assays. Newly-emerged adult psyllids were sexed, and male/female pairs were established in new cages containing a new clipping of the rearing host species. Lifetime fecundity and egg fertility, and adult longevity were monitored for a total of 12 pairs of psyllids per haplotype and host plant species. Number of eggs deposited per female was recorded under a binocular stereomicroscope (EMZ 13; Meija Techno America, San Jose, CA) every four days, starting with the first oviposition, so that the count was done before the eggs had a chance to hatch. Following each egg count, the psyllid pair was transferred to a new cage with new clippings of the appropriate host species. The process was repeated for each pair until the female died.

Figure 1. A small rearing cage used in all assays.

Adult longevity was estimated for each pair by determining the total life span duration (time from adult emergence to time of death) of both the male and female in each pair. Longevity of males was recorded only for the initial male in those pairs in which male death preceded female death (i.e., longevity was not recorded for replacement males).

Data Analysis. All statistical analyses were done using SAS (SAS Institute 2012). Effects of haplotype and host species on adult longevities were analyzed using a 3 x 2 factorial analysis of variance

(ANOVA). The count data (lifetime fecundity) were instead modeled assuming a negative binomial distribution. The analysis was done using the GLIMMIX procedure. Model fit was evaluated by use of the chi-square/df statistic provided by GLIMMIX (Littell et al. 2006). Probability of egg hatch was modeled as a binomial response (total eggs hatching/total eggs monitored) by use of PROC GENMOD. In all analyses, if the haplotype effect in the ANOVA was significant, haplotype means were compared using Tukey tests.

Results and Discussion

Fecundity. Mean lifetime fecundity was not affected by host species (Fig. 2B: $F_{1,66} = 0.02$, $P = 0.66$); mean egg production on either plant species was approximately 800 eggs per female when results are averaged over haplotype. Fecundity was affected by haplotype (Fig. 2C; $F_{2, 66} = 15.85$, $P < 0.0001$). Egg production was highest in the Northwestern and Western haplotypes.

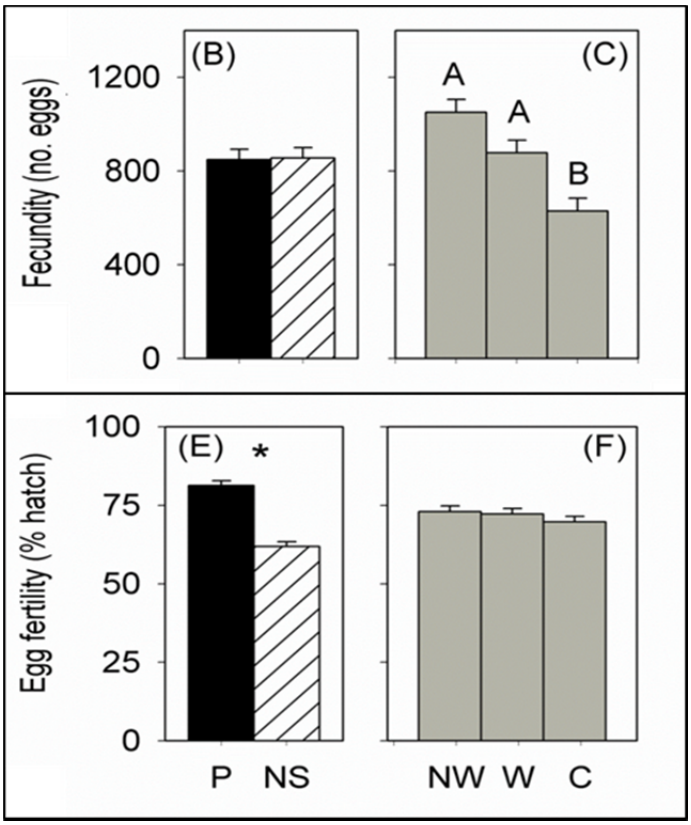

Figure 2. Mean (± SEM) lifetime fecundity (B&C) and percentage egg hatch (E&F) for the three haplotypes of potato psyllid reared on potato and nightshade host plant species.

Egg Fertility. Averaged over haplotype, percentage egg hatch was higher (by almost 20 percentage points) for eggs deposited on potato than eggs deposited on nightshade (Fig. 2E; $F_{1,66} = 83.3$, $P < 0.0001$). The three haplotypes exhibited overall similar rates of egg hatch (Fig. 2F: $F_{2, 66} = 0.11$, $P = 0.89$).

Adults Longevity. Host- and haplotype-associated patterns in adult longevity were similar between female and male psyllids (Fig. 3). Psyllids of both sexes showed a significantly longer life span on nightshade than potato (Figs. 3B&E: females, $F_{1, 66} = 10.35$; $P=0.002$; males, $F_{1, 66} = 23.7$, $P < 0.0001$). The increase in longevity on nightshade was about 23 and 35 d for female and male psyllids, respectively, averaged over haplotype (Figs. 3B&E). Haplotype effects were significant for male psyllids (Fig. 3F: $F_{2, 66} = 6.5$, $P = 0.003$), with males of the Northwestern haplotype having a significantly longer life span than males of the other two haplotypes (by 23-30 d).

Host plant did not affect fecundity of psyllids (Fig. 2B). Females of the Northwestern and Western haplotypes had higher lifetime fecundity than females of the Central haplotype (Fig. 2C). The absence of host effects has been found in other studies, most notably the study by Yang and Liu (2009), who showed that fecundity was similar for psyllids on eggplant (*Solanum melongena*) and bell pepper (*Capsicum annuum*). Average fecundity of potato psyllid was 1050, 877, and 629 eggs per female for the Northwestern, Western, and Central haplotypes, respectively. In sum, Northwestern and Western psyllids were more fecund than Central psyllids (Fig. 2C), and this trait could have an impact on

population growth rates under field conditions depending upon the relative abundance of the three haplotypes in any particular field.

Egg fertility was affected by host plant for each of the three psyllid haplotypes, with significantly higher percent egg hatch for psyllids reared on potato than those reared on bittersweet nightshade, irrespective of haplotype (Fig. 2E). The cause of the reduced egg hatch on nightshade is not yet known, but conceivably could include effects through either the male or female parent.

Previous studies showed that longevity of female potato psyllid ranged from 14.6 to 189 d on potato, and male longevity was reported averaging from 22 d on tomato to 64 d on potato (Yang and Liu 2009, Yang et al. 2010). During the present study, both male and female psyllids lived longer on bittersweet nightshade than potato, with an average life span of 108 and 85 d for females and 114 and 79 for males on nightshade and potato, respectively (Fig. 3). In addition, overall life span was higher for Northwestern psyllids than Western and Central psyllids (Fig. 3F).

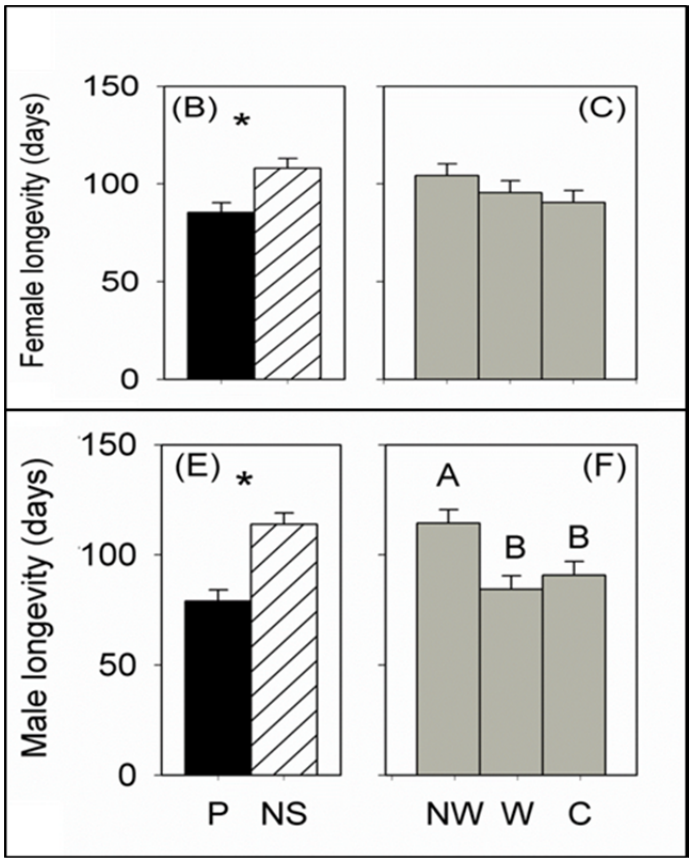

Figure 3. Mean (± SEM) longevity of adult female (B&C) and adult male (E&F) potato psyllids for three haplotypes of psyllid reared on two host plant species.

In summary, results of this study showed that there were significant differences in reproductive biology among the three haplotypes of potato psyllid found to co-occur in the Pacific Northwest of the United States. Collectively, our results indicate that a full understanding of psyllid population biology in any growing region will require knowledge of haplotype composition within the region, as well as diversity of the host plant complex in the growing region.

Acknowledgements

We thank Stacey Pettit, Millie Heidt, and Francisco de la Rosa for their technical assistance. Financial support for this research was partially provided by the Northwest Potato Research Consortium, USDA-ARS State Cooperative Potato Research Program, and USDA-NIFA-SCRI (Project #2009-51181-20176).

References

Jensen, A. S., S. I. Rondon, A. F. Murphy and E. Echegaray. 2012. Overwintering of the potato psyllid in the Northwest on *Solanum dulcamara*. Proceedings of the 12th Annual SCRI Zebra Chip Reporting Session, San Antonio, TX. (http://zebrachipscri.tamu.edu/files/2013/04/2012-Proceedings.pdf).

Littell, R.C., G.A. Milliken, W.W. Stroup, R.D. Wolfinger and O. Schabenberger. 2006. SAS for mixed models, second addition. SAS Institute, Cary, NC.

Munyaneza, J. E., T. W. Fisher, V. G. Sengoda, S. F. Garczynski, A. Nissinen, and A. Lemmetty. 2010. Association of "Candidatus Liberibacter solanacearum" with the psyllid *Trioza apicalis* (Hemiptera: Triozidae) in Europe. J. Econ. Entomol. 103: 1060-1070.

Munyaneza, J.E. 2012. Zebra chip disease of potato: biology, epidemiology, and management. Am. J. Pot. Res. 89: 329-350.

Murphy, A.F., R.A. Cating, A. Goyer, S.I. Rondon. 2014. First report of natural infection by '*Candidatus* Liberibacter solanacearum' in bittersweet nightshade (*Solanum dulcamara*) in the Columbia Basin of eastern Oregon. Plant Dis. 98: 1425.

Mustafa, T., D.R. Horton, W.R. Cooper, K.D. Swisher, R.S. Zack, and J.E. Munyaneza. 2014. Interhaplotype fertility and effects of host plant on reproductive traits of three haplotypes of *Bactericera cockerelli* (Hemiptera: Triozidae). Environmental Entomology (in press).

Pletsch, D.J. 1947. The potato psyllid *Paratrioza cockerelli* (Šulc) its biology and control. Montana Agr. Exp. Stn. Bull. 446.

Swisher, K. D., J. E. Munyaneza, and J. M. Crosslin. 2012. High resolution melting analysis of the cytochrome oxidase I gene identifies three haplotypes of the potato psyllid in the United States. Environ. Entomol. 41: 1019-1028.

Swisher, K. D., D. C. Henne, and J. M. Crosslin. 2014. Identification of a fourth haplotype of the potato psyllid, *Bactericera cockerelli*, in the United States. J. Insect Sci. (in press).

SAS Institute. 2012. SAS release 9.3 ed. SAS Institute, Cary, NC.

Teulon, D. A. J., Workman, P. J., Thomas, K. L. and Nielsen, M. C. 2009. *Bactericera cockerelli*: incursion, dispersal and current distribution on vegetable crops in New Zealand. New Zealand Plant Prot. 62: 136–144.

Wallis, R. L. 1955. Ecological studies on the potato psyllid as a pest of potatoes. USDA Tech. Bull. 1107.

Yang, X.B., and T.X. Liu. 2009. Life history and life tables of *Bactericera cockerelli* (Homoptera: Psyllidae) on eggplant and bell pepper. Environ. Entomol. 38: 1661–1667.

Yang, X-B, Y-M. Zhang, L. Hua, and T-X, Liu. 2010. Life history and life tables of *Bactericera cockerelli* (Hemiptera: Psyllidae) on potato under laboratory and field conditions in the lower Rio Grande Valley of Texas. J. Econ. Entomol. 103: 1729–1734.

Comparative Genomics Identification and Characterisation of Unique Orthologs in '*Candidatus* Liberibacter solanacearum'

Frampton, Rebekah A.[1,2], Thompson, Sarah M.[1,2], Johnson, Chris P.[3], Lu, Ashley[1,2], Sullivan, Kerry L.[1,2], Wen, Aimin[3], Pitman, Andrew R.[1,2], Crowhurst, Ross N.[4], Scott, Ian A. W.[1,2], Gudmestad, Neil C.[3], and Smith, Grant R.[1,2]

[1]The New Zealand Institute for Plant & Food Research Limited, Lincoln, New Zealand; [2]Plant Biosecurity Cooperative Research Centre, Canberra, Australia; [3]North Dakota State University, Fargo USA; [4]The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand

Abstract
Two genomes of '*Candidatus* Liberibacter solanacearum' (CLso) haplotype A, NZ1 from New Zealand and HenneA from Texas, were sequenced and assembled into five and seven contigs. The genomes were compared with the previously published haplotype B genome of CLso ZC1. The comparison revealed differences in the number and location of prophages, as well as several structural differences between the haplotype A and B genomes. Further comparisons of the coding sequences using OrthoMCL identified 63 regions specific to haplotype A and 55 to haplotype B. The majority of these coding sequences were annotated as hypothetical proteins. Many were associated with phage remnant regions and were smaller than 200 bp. The biological function of these coding regions remains to be determined, in particular whether they contribute to the differences in zebra chip disease symptoms seen in New Zealand and the USA.

Introduction
Over the past 20 years zebra chip disease has emerged as a significant problem for potato growers. The vector, the tomato/potato psyllid (TPP), *Bactericera cockerelli*, was first found in New Zealand in 2006 in capsicum and tomato glass houses from north of Auckland to Taupo (Gill, 2006). Two years later '*Candidatus* Liberibacter solanacearum' (CLso) was discovered in New Zealand, and proposed to be the causative agent of zebra chip disease (Liefting et al., 2008, Liefting et al., 2009). At the same time, '*Candidatus* Liberibacter phyllosarus', which is now generally regarded as a synonym of CLso, was reported in tomato and potato plants in the USA (Hansen et al., 2008).

The symptoms and severity of zebra chip are different in New Zealand than the USA (Berry et al., 2011, Pitman et al., 2010, Pitman et al., 2012). There are several factors that could be influencing this, including differences in the cultivars, cultivation methods, environment, as well as vector behaviour. Another potential reason is differences in the pathogen, CLso.

Analysis of the ribosomal region of CLso showed that two haplotypes (A and B) are associated with zebra chip disease and TPP; three other haplotypes (C, D, and E) have since been found to be associated with disease in carrot and celery in Europe (Nelson et al., 2012, Nelson et al., 2011, Teresani et al., 2014). The haplotypes are defined through 37 SNPs and seven small indels in the 16S rRNA, 16S/23S internal transcribed spacer (ITS) and 5S rRNA regions. It is unknown if the rRNA differences reflect wider differences in the genomes that could be related to differences in the biology of the haplotypes.

To investigate this aspect we have assembled and annotated two haplotype A genomes, one from New Zealand and one from southern Texas, and compared these two haplotype A genomes with the

previously published haplotype B genome (ZC1), sourced from northern Texas. This comparative analysis has identified coding sequences (CDS) unique to each haplotype.

Materials and Methods

NZ1: DNA was extracted from a single TPP with a high titre of CLso and whole genome amplified. A 300 bp library was prepared for 100 bp Illumina sequencing.

HenneA: DNA was extracted from two TPP with a high CLso titre and combined. The sample was sequenced on an Ion Torrent PGM.

CLso ZC1 (Lin et al., 2011) was used as the initial reference for iterative mapping and consensus calling of sequence reads and de novo contigs as described by Smith et al. (2013). The concatenated contigs from each assembly were aligned using progressiveMauve (Darling et al., 2010) in Geneious 6.1.6 (http://www.geneious.com, Kearse et al., 2012). CLso NZ1, HenneA, and ZC1 were annotated by RAST (Overbeek et al., 2013) for consistency. OrthoMCL (Li et al., 2003) was used to determine the core CLso orthologs through a reciprocal best blast analysis using the predicted coding sequences. A list of loci unique to haplotype A and haplotype B was generated following a further blastn analysis against the whole genome sequences.

Results and Discussion

Following sequencing both haplotype A genomes were assembled to a high standard and are summarized in Table 1. The CLso NZ1 genome is currently in five contigs, with a total size of 1.31 Mb and contains three prophages. CLso HenneA is currently in seven contigs, with a total size of 1.21 Mb. We were unable to assemble the repetitive regions of this genome; however, there is evidence for two prophages.

Table 1: Genomic characteristics of the three CLso genomes.

	NZ1	**HenneA**	**ZC1 (Lin et al. 2011)**
Haplotype	A	A	B
Contigs	5	7	1 (complete)
Size (Mb)	1.31	1.21	1.26
%GC	35.3	34.9	35.2
Prophage	3	evidence for 2	2
CDS (RAST)	1236	1225	1246

A large-scale comparison of the three genomes using progressiveMauve showed three major structural rearrangements between the two haplotypes (Figure 1). First, the prophages are in different locations, and the level of similarity between the prophages is low compared with the rest of the genome. ZC1 has two prophages located at 200 and 1,200 kb, while NZ1 has prophages at 550, 800, and 950 kb. Second, a large block of the genome (pink) is not in the same location in the haplotype A genomes compared with the haplotype B. This pink block is currently a separate contig in both haplotype A assemblies. The third difference is an inversion shown by the green block on the underside of the axis in ZC1 compared with both haplotype A genomes.

As the major differences between the genomes were between haplotype A and haplotype B, we used OrthoMCL to further analyse the differences between the haplotypes. OrthoMCL uses a reciprocal best blast approach to cluster CDS that have a high level of similarity. A total of 180 haplotype A orthologs and 208 haplotype B singletons were identified using this analysis. A nucleotide blast analysis of these

regions using the whole genomes (including the non-coding sequences), confirmed 63 regions as specific to haplotype A and 55 to haplotype B (Table 2).

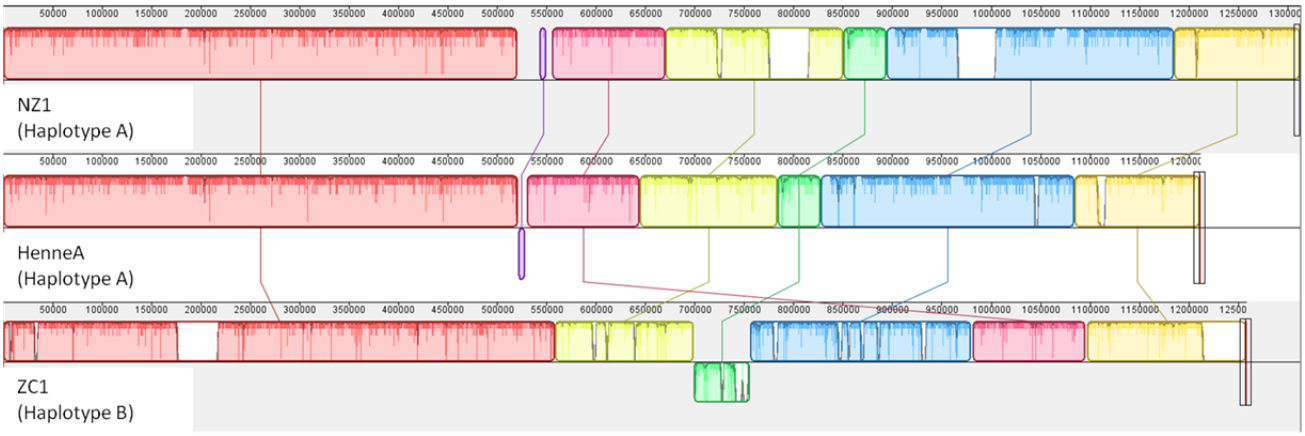

Figure 1. progressiveMauve alignment of CLso haplotype A strains; NZ1 and HenneA, and haplotype B strain ZC1. NZ1 has been used as the reference genome. The coloured blocks represent regions of the genomes with high similarity to each other. There are differences in the prophage number and location between the two haplotype A genomes and ZC1 (indicated by the white spaces within blocks and a gap between blocks in ZC1 and NZ1). There is also a rearrangement (pink block) and inversion (green block) in the haplotype A genomes compared to the haplotype B.

The regions were found throughout the genomes with several clusters in phage remnant regions (regions of the bacterial genome, which may be the result of previous lysogenic/ lytic phage activity) in both ZC1 and NZ1. The majority of the regions were annotated as hypothetical proteins. A large number are also predicted to be phage related (based on the annotation or due to their proximity to CDS with a phage related function) (Table 2). Another complicating factor is the small size (less than 200 bp) for many of the regions.

Table 2. Summary of CLso haplotype A and haplotype B specific regions.

	Haplotype A (NZ1 and HenneA)	**Haplotype B (ZC1)**
Unique CDS	63	55
Hypothetical proteins	48	54
Phage related	51	25
Less than 200 bp	30	31

A CDS with a Gene Ontology (GO) attribute of DNA replication was also found to be specific for CLso ZC1. Other CDS specific for haplotype A had GO attributes of translation, DNA modification, DNA methylation, protein transport, alcohol dehydrogenase, membrane, and metabolic processes. Further bioinformatic and functional analyses need to be carried before we can determine the biological role (if any) these CDS have in the physiology of CLso and if they relate to the differences in disease seen between New Zealand and the USA.

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References

Berry, N., Thompson, S., Taylor, N., Wright, P., Shah, F., Walker, M., Beard, S., Jorgensen, N., Butler, R., Scott, I., et al. (2011). The impact of *Ca.* Liberibacter infected seed tubers on potato production in New Zealand. *Proc 11th Ann 2011 Zebra Chip Report Sess*, 178–182.

Darling, A. E., Mau, B., and Perna, N. T. (2010). progressiveMauve: Multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 5, e11147.

Gill, G. (2006). Tomato psyllid detected in New Zealand. *Biosecurity*, 10–11. Available at: http://www.cabdirect.org/abstracts/20063158114.html;jsessionid=0A1FC96FA71EEE8F2D7E49F80A654D76 [Accessed December 1, 2014].

Hansen, A. K., Trumble, J. T., Stouthamer, R., and Paine, T. D. (2008). A new Huanglongbing species, "*Candidatus* Liberibacter psyllaurous", found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). *Appl Environ Microbiol* 74, 5862–5865.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., et al. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–1649.

Li, L., Stoeckert, C. J., and Roos, D. S. (2003). OrthoMCL: identification of ortholog groups for eukaryotic genomes. *Genome Res* 13, 2178–2189.

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

Liefting, L. W., Weir, B. S., Pennycook, S. R., and Clover, G. R. (2009). "*Candidatus* Liberibacter solanacearum", associated with plants in the family Solanaceae. *Int J Syst Evol Microbiol* 59, 2274–2276.

Lin, H., Lou, B., Glynn, J. M., Doddapaneni, H., Civerolo, E. L., Chen, C., Duan, Y., Zhou, L., and Vahling, C. M. (2011). The complete genome sequence of "*Candidatus* Liberibacter solanacearum", the bacterium associated with potato zebra chip disease. *PLoS One* 6, e19135.

Nelson, W. R., Fisher, T. W., and Munyaneza, J. E. (2011). Haplotypes of "*Candidatus* Liberibacter solanacearum" suggest long-standing separation. *Eur J Plant Pathol* 130, 5–12.

Nelson, W. R., Sengoda, V. G., Alfaro-Fernandez, A. O., Font, M. I., Crosslin, J. M., and Munyaneza, J. E. (2012). A new haplotype of "*Candidatus* Liberibacter solanacearum" identified in the Mediterranean region. *Eur J Plant Pathol* 135, 633–639.

Overbeek, R., Olson, R., Pusch, G. D., Olsen, G. J., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Parrello, B., Shukla, M., et al. (2013). The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res*, gkt1226–.

Pitman, A. R., Berry, N., Thompson, S., Taylor, N., Wright, P., Shah, F., Walker, M., Read, S., Beard, S., Jorgensen, N., et al. (2012). Yield and quality consequences of TPP and Lso: Year 2 of a New Zealand study. *Proc 12th Ann 2012 Zebra Chip Report Sess*, 26–30.

Pitman, A. R., Drayton, G. M., Kraberger, S. J., Genet, R. A., and Scott, I. A. W. (2010). Tuber transmission of "*Candidatus* Liberibacter solanacearum" and its association with zebra chip on potato in New Zealand. *Eur J Plant Pathol* 129, 389–398.

Smith, G. R., Thompson, S. M., Johnson, C. P., Frampton, R. A., Lu, A., Pitman, A. R., Wen, A., Duan, Y., and Gudmestad, N. C. (2014). A draft core genome of '*Candidatus* Liberibacter solanacearum' obtained by comparison of haplotype A and B metagenomic assembled sources. *Proc 13th Ann Zebra Chip Report Sess*, 59-63.

Teresani, G. R., Bertolini, E., Alfaro-Fernandez, A., Martínez, C., Tanaka, F. A. O., Kitajima, E., Rosello, M., Sanjuan, S., Ferrandiz, J. C., López, M. M., et al. (2014). Association of "*Candidatus* Liberibacter solanacearum" with a vegetative disorder of celery in Spain and development of a real-time PCR method for its detection. *Phytopathology* 104, 804–811.

Update on Zebra Chip Variety Screening Trial

[1]Munyaneza, J.E., [2]Wallis, C., [3]Novy, R., [4]Bester, G., [4]Ronis, D., [5]Nordgaard, J., [5]Buchman, J.L., [6]van Hest, P., and [7]Thompson, A.

[1]USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA 98951, USA
[2]USDA-ARS, Parlier, CA 93648, USA
[3]USDA-ARS, Aberdeen, ID 83210, USA
[4]Frito-Lay Inc., Rhinelander, WI 54501, USA
[5]Black Gold Farms, Grand Forks, ND 58201, USA
[6]Bejo Seeds Inc., Oceano, CA 93445, USA
[7]North Dakota State University, Fargo, ND 58108, USA

Abstract
Sustainable management of zebra chip (ZC) disease of potato requires the identification and development of tolerant or resistant potato varieties. For five years (2010-2014), over 280 potato varieties and advanced breeding lines were screened for ZC under controlled field cage conditions, by infecting plant material with the putative disease causal agent '*Candidatus* Liberibacter solanacearum' (Lso) with infectious potato psyllid vectors in no-choice tests and examining tubers for the development of both fresh and fried ZC symptom development. Twenty-nine lines exhibited no to light symptoms in freshly cut of fried potato tubers. From these, five tested lines were deemed ZC tolerant with little to no ZC symptoms over multiple years. These five selections were chosen for further screening to determine whether the lack of physiological responses to Lso infection was the cause of observed tolerance by examining differences in amino acid, sugar, and phenolic levels between Lso-infected and non-infected plants. Compared to the susceptible "Atlantic", which characteristically had great increases in most amino acid, sugar, and phenolic levels, these five putatively tolerant lines had less dramatic shifts in host physiology. This suggests the lack of a large-scale host response to Lso infection that results in changes in tuber biochemistry is a potential mechanism of ZC tolerance. This trait can be used to develop commercial potato varieties that are Lso-tolerant.

Introduction
Zebra chip (ZC) is a new and economically important disease of potato in the United States, Mexico, Central America and New Zealand. The disease is associated with the bacterium '*Candidatus* Liberibacter solanacearum' (Lso) vectored by the potato psyllid *Bactericera cockerelli*. Currently, the only means to manage ZC is by controlling its psyllid vector with insecticide applications. Development and identification of potato varieties and/or advanced breeding lines with resistance to or tolerance of ZC are essential to developing effective and sustainable management strategies for this disease. Unfortunately, all commercial potato varieties currently available are susceptible to ZC, underscoring the urgent need to identify and/or develop potatoes that are resistant to this important disease (Munyaneza et al. 2011, Munyaneza 2012). For the last five years (2010-2014), over 280 potato varieties and advanced breeding lines were screened for ZC at the USDA-ARS Wapato in WA. The plant material was screened in a non-choice test under field cage conditions by briefly exposing each plant to psyllids from an Lso-infective colony to quickly identify resistant material. Identified promising lines were further screened in replicated trials to assess yield, processing quality parameters, and biochemical changes in plant metabolites to identify source of resistance.

Materials and Methods

Over a five year period (2010-2014), 283 potato varieties and advance breeding lines were planted in the field at the USDA-ARS research farm at Moxee, WA. These potatoes were contained in 1.83 m by 4.57 m cages, with each cage enclosing six to eight plants each, as described by Munyaneza et al. (2008) and Buchman et al. (2011a,b). Plants were inoculated with Lso using Lso-positive potato psyllid adults (3 insects/plant) at tuber initiation stage for seven days, after which time the insects were eliminated through the use of pesticides and the plants were allowed to develop until harvest (Munyaneza et al. 2008; Buchman et al. 2011a,b). Upon harvest, the tubers were assessed for both fresh and fried ZC symptoms, as well as Lso infection confirmed by PCR (Munyaneza et al. 2008; Buchman et al. 2011a,b).

Based on findings from the first two years (2010 and 2011), a total of five promising lines were selected for further evaluation in 2012 and 2013. The promising lines were: ARS1 (also known as 00-3115-2) and ARS2 (also known as 00-3115-11) from USDA-ARS, Aberdeen, ID; BS2 (also known as T47022) from Bejo Seeds, Inc., Oceano, CA; and ZC73 and ZC74 from Frito-Lay, Inc., Rhinelander, WI. Each of these potato lines had six plants established in each of six cages. The susceptible cultivar Atlantic was established similarly for comparison. The promising lines and Atlantics had half of the cages exposed to Lso-infective psyllids at tuber initiation stage, and the remaining cages were left as controls. At harvest tubers were collected, ZC fresh and fried symptoms were assessed, and Lso infection confirmed by PCR as described by Munyaneza et al. (2008) and Buchman et al. (2011a,b). Subsets of non-infected and Lso-infected tubers for each entry were then shipped to USDA-ARS, Parlier, CA, for evaluation of tuber physiology, including changes in sugars, phenolic compounds and amino acids. Lso titers were also quantified.

Results and Discussion

During the five years of the field screening trial, all the Atlantic control plants in the cages produced tubers with very severe ZC foliar symptoms indicating that Lso inoculation by psyllids during each trial was successful. Furthermore, above ground plant symptoms were observed in all cages and plants generally died soon after ZC symptom development. Plant symptoms included leaf rolling of upper leaves and basal cupping of leaflets; foliar chlorosis; proliferation of axillary buds, shortened internodes, swollen nodes, and leaf scorching. Tuber symptoms from susceptible varieties following Lso infection included collapsed stolons, brown discoloration of the vascular ring, necrotic flecking of internal tissues, and streaking of the medullary ray tissues. A total of 254 potato entries had these tuber symptoms. The remaining 29 lines had ZC symptoms in fresh tubers ranging from light to almost none and this was consistent over multiple years. Lso infection was confirmed in tubers collected from inoculated plants of all 283 lines and the Atlantic controls. Tuber samples of the 29 entries with no to light symptoms were processed into chips and fried as described by Munyaneza et al. (2008). Of the 29 selections with no to light fresh ZC symptoms, only five breeding lines (ARS1, ARS2, BS2, ZC73, and ZC74) showed no to light ZC symptoms in fried chips. Therefore, tubers for each of the five promising lines, in addition to Atlantic for comparison, were sent to USDA-ARS Parlier in 2012 and 2013 for further symptom assessment and biochemical analyses.

PCR testing of tubers showed that Lso-infected Atlantic had much greater Lso titers than the promising selections. Biochemical analyses showed that, unlike Atlantic, there were no significant differences in phenolics, amino acids and sugars contents between Lso-infected tubers and controls for

each of the promising lines. Briefly, Lso infection increased sugar levels mainly in Atlantic tubers. Also, Lso infection increased amino acid levels only in Atlantic tubers. Furthermore, Lso infection increased levels of phenolic compounds (both chlorogenic acids and flavonoids) and these increases were far greater for Atlantic tubers, with increases of 100 fold or more compared to 50 fold or less for the tolerant selections. These results provide support that these Lso-tolerant potato lines respond less strongly to Lso infection, and therefore do not undergo significant changes in physiology associated with ZC symptom development. Increased levels of reducing sugars, phenolics, and amino acids in ZC-infected tubers are typical, so a lack of increase of these metabolites in Lso-infected tubers from the promising lines suggests a mechanism for disease resistance.

Identification of the source and mechanism of resistance in the identified promising lines and possible ways to incorporate the information into breeding programs will help affected potato growers and researchers to quickly minimize losses due to ZC by developing potato cultivars that are less vulnerable to this devastating disease.

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References
Buchman, J.L., Heilman, B.E. and Munyaneza, J.E. 2011a. Effects of *Bactericera cokerelli* (Hemipetra: Triozidae) density on zebra chip potato disease incidence, potato yield, and tuber processing quality. J. Econ. Entomol. 104: 1783-1792.

Buchman, J.L., V.G. Sengoda, V.G., and and Munyaneza, J.E. 2011b. Vector transmission efficiency of liberibacter by *Bactericera cokerelli* (Hemiptera: Triozidae) in zebra chip potato disease: effects of psyllid life stage and inoculation access period. J. Econ. Entomol. 104:1486-1495.

Munyaneza, J.E. 2012. Zebra chip disease of potato: biology, epidemiology, and management. Am. J. Pot. Res. 89: 329-350.

Munyaneza, J.E., Buchman, J.L., Upton, J.E., Goolsby, J.A., Crosslin, J.M., Bester, G., Miles, G.P., and Sengoda, V.G. 2008. Impact of different potato psyllid populations on zebra chip disease incidence, severity, and potato yield. Subtr. Plant Sci. 60:27-37.

Munyaneza, J.E., Buchman, J.L., Sengoda, V.G., Fisher, T.W., and Pearson, C.C. 2011. Susceptibility of selected potato varieties to zebra chip potato disease. Am. J. Pot. Res. 88:435-440.

Breeding for Host Plant Resistance to Zebra Chip in Texas

Scheuring, D.C[1], Koym, J.W. [2], Levy, J.[1], Henne, D.C.[3], Pierson, E.A.[1], Trumble, J.T.[4], Novy, R.G.[5], Charlton, B.A.[6], Higgins, C.[7], Jansky, S.H.[8], Brown, C.R.[9] and Miller, J.C., Jr.[1]

Department of Horticultural Sciences, Texas A&M University, College Station, TX[1], Texas A&M AgriLife Research, Lubbock TX[2], Texas A&M AgriLife Research, Weslaco TX[3], Department of Entomolgy, University of California, Riverside, CA[4], USDA-ARS, Small Grains and Potato Germplasm Research Unit, Aberdeen ID [5], Crop and Soil Science, Klamath Basin R&E Center, Klamath Falls, OR[6], National Potato Promotion Board, Denver, CO[7], USDA-ARS, University of Wisconsin, Madison, WI[8], USDA-ARS, Vegetable and Forage Crops Research Unit, Prosser, WA[9]

Abstract

Zebra chip screening for tolerance/resistance in potato was continued in 2013 and 2014. Screening studies were performed in hoop cages at two Texas locations – Weslaco and Springlake. Some 75 varieties/selections associated with specific traits were cage evaluated for ZC. Clones selected for evaluation were also obtained from other potato programs and the Potato Germplasm Collection, Sturgeon Bay, WI. An emphasis was placed on material with strong resistance to late blight (*Phytophthora infestans)* due to reports of associated resistances, as well as material with different species background. We continue to cooperate and facilitate the research of several other ZC-related programs throughout the state.

Introduction

Zebra chip (ZC) tolerant/resistant varieties are the optimal long-term solution to ZC. Over the past several years, our program has screened over 800 varieties/selections for ZC tolerance/resistance. Earlier screening included the most advanced material available to the program. There are multiple traits that make a variety commercially acceptable such as yield, tuber and chip appearance, etc. We have found limited variability in this advanced material and have proceeded to the screening of related *solanum* species. Most researchers working with ZC realize it is a complicated situation. We are dealing with multiple haplotypes of both the vector and pathogen plus the reaction of the different varieties,as well as year to year environmental differences.

While we have not found resistance, we have identified several selections which exhibit tolerance (still having symptoms which progress slowly and are less intense). These tolerant selections have been helpful to other investigators studying the associated insect and disease. Our program has worked with other researchers by providing material, planting and harvesting of trials, as well as evaluations.

Therefore, the overall objective of this project has been to evaluate a wide range of germplasm for tolerance/resistance to ZC in order to identify and/or develop varieties for the industry, which can be more successfully grown when/where conditions for expression of ZC are present.

Materials and Methods

2013 Weslaco, TX: Twenty-one cages were planted on January 17, 2013 at the Weslaco Center. Sixty-four different clones were planted, each replicated three times with one control. Emergence data was collected on February 11. Vigor data was recorded on February 28, March 14 and March 27. Three insects per plant were released at the base of each plant on March 1 (Fig. 1). Insecticide sprays were applied on March 8 and 14 to ensure that psyllid feeding was for only one week, and all of the psyllids within each cage were killed. ZC ratings were recorded on April 12. Cages were harvested on April 18,

(91 days after planting (DAP)), and seven weeks after insect infestation. Samples were chipped and evaluated on April 23.

2013 Springlake, TX: Ten cages containing 41 different clones replicated three times with one control, were planted on April 3, 2013 near Springlake. Three insects per plant were released at the base of each plant on July 2 (Fig. 1). Insecticide sprays were applied on July 9 and 16. Zebra chip ratings were recorded on August 14. Cages were harvested on August 20, (137 DAP), again, seven weeks after insect infestation. Samples were chipped and evaluated on August 21 (Scheuring et al., 2014)

Figure 1. Release of psyllids at base of plant, 2013

Figure 2. Psyllids in mesh bags on each plant, 2014

2014 Weslaco, TX: Twenty-three hoop cages were planted at the Weslaco Center on January 15, 2014. Fifty–eight different clones were planted in four treatment replications and included up to two controls. On March 18 (62 DAP), for each treatment plant, two male and two female insects were placed in a fine mesh bag which covered a complete leaf located in the upper third of the plant canopy (Fig. 2). This was in contrast to 2013, where psyllids were released at the base of each plant with the assumption that the psyllids would colonize the nearest plant and infect, or fly to any plant in the cage, thus providing some degree of plant choice. However, in the 2014 studies, there was no choice because the insects were caged on a fully expanded leaf near the top of each plant. Insects were left on the plant for one week and removed. Each mesh bag with the enclosed leaf was inspected for live adults and/or presence of eggs. If there were live adults and/or eggs on the leaf then feeding occurred during the week. Tubers were harvested April 28th (104 DAP). This was 42 days after infection (DAI). Tubers were counted, weighed, and a center slice was chipped within a week.

2014 Springlake, TX: Again, 50 clones were planted in 23 cages near Springlake on May 16, with three treatment replications and one check. On July 8 (53 DAP) three psyllids per plant were placed in a fine mesh bag on one leaf (Fig. 2). After one week bagged leaves were removed and live insects were noted. Tubers were harvested September 28 (134 DAP). This was 81 DAI, and as in Weslaco, tubers were counted, weighed, and single slices from each tuber were chipped for ZC evaluations. Zebra chip severity ratings were assigned using the scale shown in Figure 3.

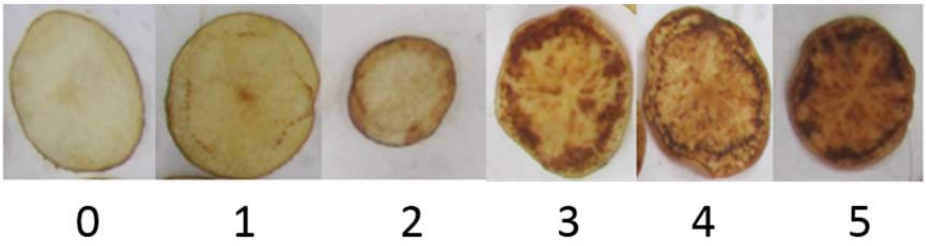

Figure 3. Rating of ZC severity based on chip symptoms 0-5 scale.

For all studies, psyllids were obtained from Don Henne's colony maintained at Weslaco - 70% of tested insects were positive for *Liberibacter* (Lso). The number of psyllids used in these studies was minimized to a) prevent over-whelming any plant resistance that might be present, and b) ensure that more than half of the psyllids used were positive for Lso.

Results and Discussion

Tested clones were assembled with an emphasis on late blight (LB), resistance, cold sweetening resistance (CSR), diploid species, clones from the US Potato Genebank, and testing and verification of chip clones currently in use, as well as clones previously identified in the Texas program. Late blight was of interest since colleagues from several disciplines reported that many other resistances are often associated with *phytopthera* resistance. These clones were obtained from Rich Novy. Cold sweetening resistant clones were screened to investigate if the mechanism for CSR might also reduce/prevent darkening symptoms with Lso infection (Brummell, 2011). These were obtained from Rich Novy and Shelly Jansky. Since past screening in our program had yielded little in the way of resistance, we decided to investigate enhanced germplasm from Jansky's Wisconsin program, as well as those from the Potato Genebank. A number of clones that have previously been identified as tolerant were also included. To screen for ZC tolerance, we harvested the tubers and chipped one slice per tuber. Those chips were assigned a ZC rating based on Fig. 1 above. Clones that received above a 2 chip rating were dropped after one season. Additional clones were included as new entries in 2014.

Twenty-one clones that have shown promise in these studies are listed in Table 1. The five clones listed at the bottom of Table 1 were comparison check chip varieties. Those deserving special mention include PA92A08-17 a PVY (Rysto) (Brigneti et al., 1997) and CSR resistant variety, as well as a parent to both AOR07783-1 and AOR07781-2. AOR7781-2 and AOR7783-1 performed very well last year (Scheuring et al., 2013) and again in 2014. 2013 involved insect choice, while 2014 was a non-choice study. However, they are russets and don't produce a high quality chip. A clone from CSR (00-3315-11) has performed well in our studies and was reported by Novy et al. (2014) to show some resistance last year. Scab 4-48, a diploid species clone from Wisconsin, will require further evaluation. Much of this species material was slow in emerging, weak, and very late maturing. W2717-5 (Lelah), a chip variety from Wisconsin, will be further investigated. Canasta and PI633600, clones received from the Potato Genebank, were tested only in Springlake in 2014. Higher quality seed will need to be obtained for retesting in 2015.

We continue to observe wide genetic variability among clones for ZC expression, however, no immunity has been found.

	Location and Trial Year				
Clone	Weslaco 13	Springlake 13	Weslaco 14	Springlake 14	Rationale
PA92A08-17			0* 0* 0 0 0	0* 1 2 0	Parent in both AOR07783-1 and AOR07781-2—the two AOR clones identified as having putative ZC resistance; PVY resistant (Rysto) and CSR.
scab 4-48			0* 0	2	Diploid, resistant to common scab { (US-W4 x 524-8)-1} x {(US-W4 x chc 524-8)-14}
00-3315-11	0* 1 2 2	0* 2 2 2	0* 0	0* 0	CSR parent line obtained from Shelly Jansky
AO6408-99LB		0* 1 2 2			Late blight resistant clone
B 1992-106			0* 0 0 0 0	0* 1 0	Beltsville chip clone
AOR7781-2	0* 1 1 2	0* 1 1 2	0* 0* 0 0 0	0* 0 1 2	Oregon clones that survived severe 2011 ZC field pressure
AOR7783-1	0* 1 1 1	0* 1 1 2	0* 0* 0 0 0	0* 1 1 1	
AOR7793-1	0* 1 2	0* 2 2	0* 0* 0 0 0	0 1 2	
NDTX081648CB-1W	0*	0* 1			2014 Texas NCPT chip entries
NDTX081648CB-4W	0* 1	0* 1			
COTX09022-5Ru/Y			0* 0 0 0 2		
NDTX102557-1W			0* 0 0 0 0		
NDTX059828-2W	0* 0 0 1				Texas advanced chip clones
TX05249-10W	0 * 0 2				
TX05249-11W	0 2 2		0* 0		
ZC-4	0* 2 2 2		0* 0 0	1 0 2	ZC clonal selection
W2324-1 Accumulator	0* 2 2 2				Wisconsin chip varieties
W2717-5 Lelah	0 0		0* 0 0 0	0* 0 2 2	
W8832-1Y	0* 2 0				Wisconsin advanced chip clones
Canasta				0* 1	Potato Genebank
PI633600				0*	Primitive cultivars. Tuber moth resistance.
NY 138 Waneta	0* 0* 0* 0* 3 4 3 3 0 2	0* 0* 0 1 1 1	0* 0* 0 0 0		Chip variety checks
Atlantic	0* 0* 0* 0* 4 4 5 4 4 3 3 5 3 5 3 0	0* 0* 2 3 3 3	0* 0* 0* 0 0 0 5 0 0 0	0* 0* 3 2 3 3 0 2	
Snowden	0* 3 3 2		0* 0 0 3		
Lamoka	5 4 2		0* 0 0 0	0* 0 4 4	
Nicolet	0* 3 4 4		0* 0 0 4		

* Control - no insect treatment

Table 1. Clone, ZC ratings and rationale for screening clone.

Acknowledgements
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References
Brigneti, G., Garcia-Mas, J., Baulcombe, D.C. 1997. Molecular mapping of the potato virus Y resistance gene Rysto in potato. Theor. Appl. Genet. (1997) 94: 198–203.

Brummell, D.A., Chen, R.K.Y, Harris, .J.C., Zhang, H., Hamiaux, C., Kralicek, A.V., and McKenzie, M.J. 2011. Induction of vacuolar invertase inhibitor mRNA in potato tubers contributes to cold-induced sweetening resistance and includes spliced hybrid mRNA variants. J. Exp. Bot. 2011: err043v1-err043.

Novy, R.G., Prager, S.M., Miller, J.C., Jr., Vindeola, B., and Trumble, J.T. 2014. Characterization of potato breeding clones to determine mechanisms conferring observed resistance/tolerance to zebra chip disease. pp. 39-43. In: F. Workneh and C.M. Rush (eds.), Proceedings of the 13th Annual Zebra Chip Reporting Session, San Antonio Crowne Plaza, San Antonio, TX, Nov. 3 – 6, 2013.

Rashed, A., Workneh, F., Grey, J., Paetzold, L., and Rush, C.M. 2012. Relationship between time of infestation, disease development, and tuber yield. pp. 22-26. In: F. Workneh and C.M. Rush (eds.), Proceedings of the 11th Annual Zebra Chip Reporting Session, San Antonio Crowne Plaza, San Antonio, TX, Nov. 6-9, 2011.

Scheuring, D.C., Levy, J.G., Koym, J.W., Henne, D.C., and Miller, J.C., Jr. 2013. ZC expression in several caged potato populations following infestation with the potato psyllid. pp. 34-38 In: F. Workneh and C.M. Rush (eds.), Proceedings of the 13th Annual Zebra Chip Reporting Session, San Antonio Crowne Plaza, San Antonio, TX, Nov. 3 – 6, 2013.

Breeding for Resistance/Tolerance to Zebra Chip Disease with the Use of Species-Derived Potato Germplasm

[1]Novy, R.G., [2]Prager, S.M., [2]Vindeola, B., [2]Trumble, J.T., and [3]Munyaneza, J.E.

[1]USDA-ARS, Small Grains and Potato Germplasm Research Unit, Aberdeen, ID 83210, USA; [2]Department of Entomology, University of California, Riverside, Riverside, CA 92521, USA; [3]USDA-ARS, Fruit and Vegetable Insect Research Unit, Wapato, WA 98951, USA.

Abstract

Five potato breeding clones derived from the hybridization of potato species with cultivated potato were evaluated in no-choice bioassays to identify antibiosis, and 2-choice bioassays to identify antixenosis (or nonpreference) to potato psyllid in 2014. Atlantic was also included in the bioassays as the potato psyllid-susceptible control. Species-derived breeding clones ETB 6-21-3, P2-4, PALB03016-3, and PALB03016-6 exhibited a reduced number of eggs in no-choice bioassays relative to Atlantic. In addition, ETB 6-21-3 also displayed reduced growth and development of nymphs relative to Atlantic. No significant differences for leaflet preference by adult psyllids were observed between Atlantic and the species-derived germplasm entries. Relatives of cultivated potato can provide resistance to potato psyllid, thereby potentially contributing to reductions in the transmission and incidence of zebra chip disease.

Introduction

Plant genetic resistance is an important component in many successful integrated pest management (IPM) programs and could also contribute to the control of zebra chip disease (ZC). However, an evaluation of nine potato varieties commonly grown in the U.S. and representing fresh-pack, chipping, and long processing market classes found none to have ZC resistance (Munyaneza et al., 2011). The authors stated that: *Identification and development of potato varieties with resistance to or tolerance of ZC are crucial to developing effective and sustainable management strategies for this important potato disease*.

A second evaluation of a breeding clone and 11 potato cultivars widely-grown in New Zealand for ZC incidence under full, reduced, and no insecticide treatments (for control of potato psyllid) identified the fresh market cultivar 'Nadine' as having reduced ZC symptoms in raw tubers (Anderson et al., 2013). However, 50% and 60% reductions in marketable yield were observed between full and no insecticide treatments for Nadine over the two years of evaluations. The authors concluded (with the exception of reduced ZC symptoms in raw tubers of Nadine) that: *There was no real indication that any of the other cultivars in the trial had any effective resistance to TPP (tomato-potato psyllid) or ZC.*

With no apparent resistance to ZC in U.S. and New Zealand potato cultivars, wild relatives of potato may provide resistance that has not been identified in cultivated potato. Unique germplasm derived from the *Solanum* species *S. etuberosum* and *S. berthaultii* has been shown to impact potato psyllid behavior (Butler et al., 2011), with antibiosis to potato psyllid being confirmed ((Diaz-Montano et al., 2014). Resistance to the insect vector of the Liberibacter associated with ZC, and possible resistance to the liberibacter as well (Butler et al., 2011) can be used by breeders in developing ZC resistant potato cultivars. It is likely that other sources of resistance, within the wild relatives of potato, also exist with further characterization warranted.

Materials and Methods

Potato Breeding Clones

Two breeding clones from the potato breeding program at Aberdeen, ID, previously identified by Butler et al. (2011) as displaying reduced infection by liberibacter in transmission assays (P2-4 and ETB 6-21-3) were evaluated in 2014. An additional clone, A05379-211, which displayed a biologically, but not statistically significant reduction in infection by liberibacter was also included to further characterize this promising long, russet-skinned breeding clone. A05379-11 and P2-4 were also shown to have very strong antibiotic effect on potato psyllid (Diaz-Montano et al., 2014).

PALB03016-3 originating from the breeding program of Dr. Chuck Brown, ARS, Prosser, WA, had indications of possible ZC resistance from field assessments in Hermiston, OR in 2011. This clone and its full sib, PALB03016-6 with its close relatedness to PALB03016-3 were also assayed. These two clones, were derived from the potato species *S. guerreroense* and were selected and seed maintained at Aberdeen, ID.

In total, five clones derived from species related to cultivated potato and the susceptible control Atlantic were evaluated in no-choice and 2-choice assays in 2014.

No-Choice Bioassay (antibiosis)

Two mesh cages, each containing three male: female pairs of post-teneral adult potato psyllids were placed on each entry plant, with a minimum of 5 replicates per entry. Psyllids used were maintained in colonies on tomato plants at UC-Riverside, with psyllids originally collected from potato fields in Weslaco, TX. Psyllid colonies were checked every two months for Liberibacter infection rate, using taqman based real time PCR. The average infection rate was 95%. Seventy-two hours following placement on plants, psyllids were removed and the egg numbers on each leaflet were counted and subsequent nymph development was evaluated.

2-Choice Bioassays (antixenosis)

2-choice bioassays were conducted to determine if potato psyllids showed preference in location/feeding when given the choice of a given species-derived entry relative to the susceptible cultivar Atlantic. Bioassays consisted of the placement of 5 pairs of post-teneral male: female adults (total of 10 adult psyllids) in a cage containing a leaflet of a species entry and a corresponding leaflet of Atlantic. Locations of the psyllids were then recorded over a 4 day period.

Results and Discussion

No-Choice Bioassay

Highly significant differences ($p<0.01$) were observed among entries for number of eggs laid as well as growth and development relative to Atlantic:

- Reduced number of eggs relative to Atlantic**: ETB 6-21-3, P2-4, PALB03016-3, and PALB03016-6**
- Reduction in nymph development relative to Atlantic: **ETB 6-21-3**
- **A05379-11** ($p=0.059$) also displayed reduced nymph growth and development near the 5% level of significance.

Choice Bioassay

- No statistically significant differences for leaflet preference between species-derived germplasm and Atlantic were observed within the sample size of this study.

In summary, species derived breeding clones **ETB 6-21-3, P2-4, PALB03016-3, and PALB03016-6** exhibited a reduced number of eggs in no-choice bioassays relative to the psyllid susceptible potato cultivar Atlantic. In addition, **ETB 6-21-3** also had reduced growth and development of nymphs relative to Atlantic. No significant differences for leaflet preference by adult psyllids were observed between Atlantic and the species-derived germplasm entries.

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References
Anderson, J.A.D., Walker, G.P., Alspach, P.A., Jeram, M., and Wright, P.J. 2013. Assessment of susceptibility to zebra chip and *Bactericera cockerelli* of selected potato cultivars under different insecticide regimes in New Zealand. *American Journal of Potato Research* 90, 58-65.

Butler, C.D., Gonzalez, B., Manjunath, K.L., Lee, R.F., Novy, R.G., Miller, J.C., and Trumble, J.T. 2011. Behavioral responses of adult potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozideae), to potato germplasm and transmission of Candidatus *Liberibacter psyllaurous*. *Crop Protection* 30:1233-1238.

Diaz-Montano, J., Vindiola, B.G., Drew, N., Novy, R.G., Miller, J. C., and Trumble. J.T. 2014. Resistance of selected potato genotypes to the potato psyllid (Hemiptera: Triozidae). *American Journal of Potato Research.* 91:363-367.

Liu, D. and Trumble, J.T. 2004. Tomato psyllid behavioral responses to tomato plant lines and interactions of plant lines with insecticides. *Journal of Economic Entomology* 97(3): 1078-1085.

Munyaneza, J.E., Buchman, J.L., Sengoda, V.G., Fisher, T.W. and Pearson C.C. 2011. Susceptibility of selected potato varieties to zebra chip potato disease. *American Journal of Potato Research* 88: 435-440.

Development of Multiplex Quantitative TaqMan Real-Time PCR for Haplotyping '*Candidatus* Liberibacter Solanacearum' in Potato and Psyllid

Wen, Aimin, Johnson, Chris, and Gudmestad, Neil C. Department of Plant Pathology, North Dakota State University, Fargo, ND 58108

Abstract

Multiplex quantitative TaqMan real-time PCR assays were developed in this study consisting of primers and probes of LsoA, LsoB and their respective internal control for psyllid and potato. PCR assays were optimized, and validated using field collected potato and psyllid samples. The assays successfully quantified Lso haplotypes in plant and psyllid samples with detection limit of 10 copies of Lso genome. This is the first qPCR assay that can quantify the two Lso haplotypes simultaneously in potato and psyllid.

Introduction

Currently there are five haplotypes of '*Candidatus* Liberibacter solanacearum' (Lso), namely A, B, C, D and E (Nelson et al., 2011, 2012; Teresani et al, 2014). Haplotypes A and B are associated with several diseases in solanaceous crops including the zebra chip (ZC) disease of potato reported in the USA, only haplotype A reported in New Zealand, whereas haplotype C, D and E have been reported to be associated with carrot and celery disorders in European countries (Glynn et al., 2012, Lin et al., 2011, 2012, Nelson et al., 2011, Wen et al., 2012, 2013a). Both haplotypes A (LsoA) and B (LsoB) are vectored by the tomato/potato psyllids (TPP). Biological difference between LsoA and LsoB has been observed in commercial potato field in Texas, where haplotype B showed to be more virulent based on the disease symptoms on the foliage and potato tubers (Gudmestad, 2012). Greenhouse transmission studies using the two haplotype infected psyllids in eggplant, tomato and potato also showed similar biological characteristics (Johnson et al., 2013, Levy et al., 2014, Wen et al, unpublished). Quantification of LsoA and LsoB in psyllid and plant samples is in demand in both transmission studies and psyllid surveys. Currently available haplotyping assays include a conventional PCR using SSR markers and SYBR green qPCR assays using Lso haplotype-specific primers (Wen et al., 2013a,b). However, due to the nature of SSR marker, single typing in co-infected samples was not successful and the ratio of the two Lso haplotypes cannot be determined. Relative quantification of Lso haplotypes was possible with SYBR green qPCR assays in two separate qPCR since there is no internal control. Multiplex quantitative TaqMan real-time PCR has many advantages, such as conserving precious samples, obtaining more data per sample volume, reducing numbers of false negatives when a control target is amplified within each sample, consistency of starting template for accuracy of haplotypes and reducing overall cost of qPCR assays. The objectives of this study was to develop multiplex TaqMan real-time PCR assays using LsoA, LsoB and respective internal control primers/probes in one reaction for Lso haplotype quantification in plant and in psyllid, respectively.

Materials and Methods

Potato and psyllid DNA samples. Archived psyllid DNA samples were extracted from 2011 and 2013 field collected psyllids, and 2012 samples were from psyllid survey provided by Dr. Jim Crosslin (USDA-ARS, Prosser, WA). Archived potato DNA samples were 2008, 2011, 2012 and 2014 as described previously (Wen et al. 2009).

Primers and probes. Primers for LsoA (HAfr) and LsoB (HBfr) were screened using SYBR green qPCR (Wen et al., 2013; Johnson et al., 2014), and the most sensitive/consistent primers of each

haplotype were chosen. Respective probes, HAp and HBp, were designed for each primer set using IDT PrimeTime. Primers and probes of internal control for potato (COX2fpr) and psyllid (CO1fpr) were designed and tested previously (Wen et al., 2013). Primers and probe were synthesized by IDT (Coralville, Iowa).

Multiplex TaqMan qPCR assays optimization and validation. Each set of primers/probe was tested and optimized, followed by multiplex of HAfpr+HBfpr+COX2fpr and HAfpr+HBfpr+CO1fpr for potato and psyllid samples. QPCR was conducted on a Stratagene Mx3005P using SsoAdvanced Universal Probes Supermix (BioRad, Hercules, CA) following manufacturer's instructions. Validation was conducted on fifty-three DNA samples extracted from field collected potato samples obtained from 2008 to 2014 for the assay LsoHAfpr+LsoHBfpr+COX2fpr, and on two-eight DNA samples extracted from field collected psyllids between 2011 and 2013.

Data analysis. Real-time PCR data were exported from the Mx3500P for calculation of mean crossing threshold (Ct) values and standard deviations (SDs). Standard linear regressions, PCR amplification efficiency, and data normalization were obtained as previously described (Li et al., 2009). Lso genome copy number was converted to logarithm. LsoA or LsoB Ct values of were first normalized against their respective internal control Ct values, and then used to calculate Lso genome logarithm copy number based their respective regression.

Results and Discussion

The multiplex TaqMan real-time PCR assay using primers/probes set, HAfpr+HBfpr+CO1fpr, for detection in psyllid were successfully developed with detection limit of 10 genome copies of LsoA and LsoB per reaction (Fig.1). The efficiencies of the new PCR assay are 117.35% for HA primers and 104.89% for HB primers. This assay improved sensitivity to 10 genome copies compared to the existing SYBR green qPCR (Wen et al, 2013b). Quantification of field psyllid samples showed that three types of psyllid populations were in natural psyllid colonies, which are LsoA-infected, Lso-B infected and co-infected (Fig. 2). The proportions of each psyllid type varied among the psyllid samples collected in the three years. In 2011 and 2012, more LsoB-infected psyllids were detected, 60.0% and 75.0% respectively, whereas only 22.2% in 2013 collected psyllid samples. The high frequency of LsoB-infected psyllid population in 2012 can explain why the highest field ZC incidence was observed (Workneh, et al., 2012). LsoA and B titres ranged from $10^{1.29}$ to $10^{7.17}$ genome copies in the total samples tested.

The multiplex TaqMan real-time PCR assay using primers/probes set, HAfpr+HBfpr+COX2fpr, for detection in potato were successfully developed with detection limit of 10 genome copies of LsoA and LsoB per reaction (Fig.3). Efficiency of the new PCR assay was satisfactory: 102.21% for HA primers and 98.11% for HB primers. Tests on field collected potato samples showed that 2008 did not have co-infected, and about 1/3 of the samples collected in 2011, 2012 and 2014 were detected having both mixed LsoA and LsoB infections (Fig. 4). Concentrations of LsoA and B ranged from $10^{1.03}$ to $10^{5.44}$ genome copies in the samples tested, which were much lower than that in psyllid samples.

Lso detection and typing plays critical role in ZC disease management. Previously Lso haplotyping takes several round of PCR operations to get the results which include first Lso detection, followed by two reactions of Lso typing. The newly developed TaqMan qPCR assays can quantify the two Lso haplotypes in plant and psyllids in one reaction. These assays are more sensitive, accurate, user-friendly, and cost-effective, especially with large amount of samples. They will facilitate epidemiological studies and ecological studies as well as field surveys.

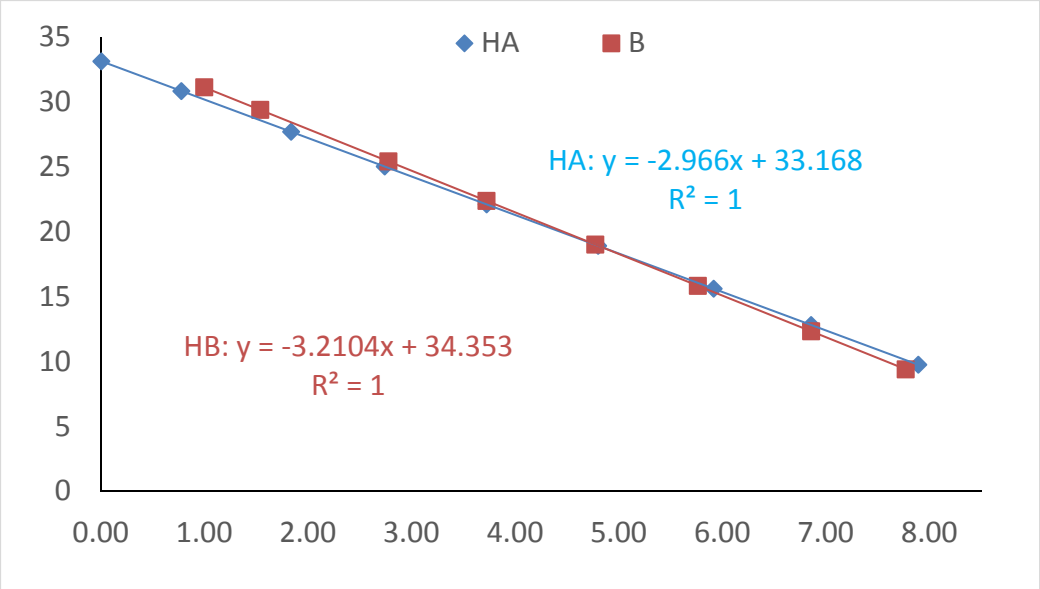

Figure 1. Sensitivity and efficiency of multiplex qPCR assay HAfpr+HBfpr+CO1fpr

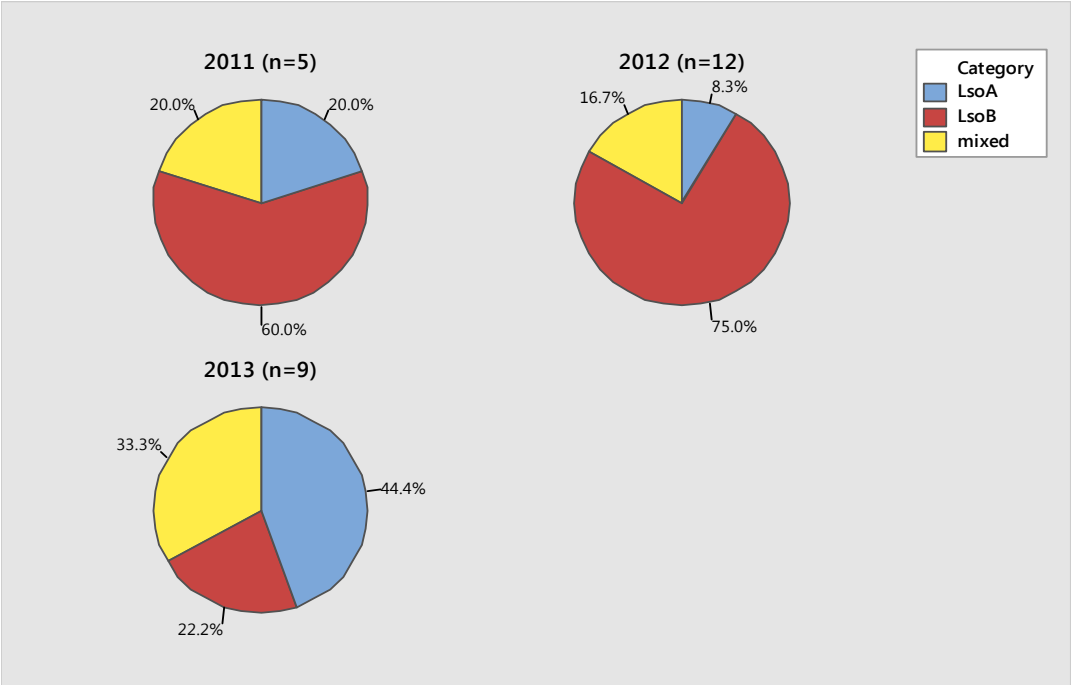

Figure 2. LsoA and LsoB population in field collected psyllid samples in the USA

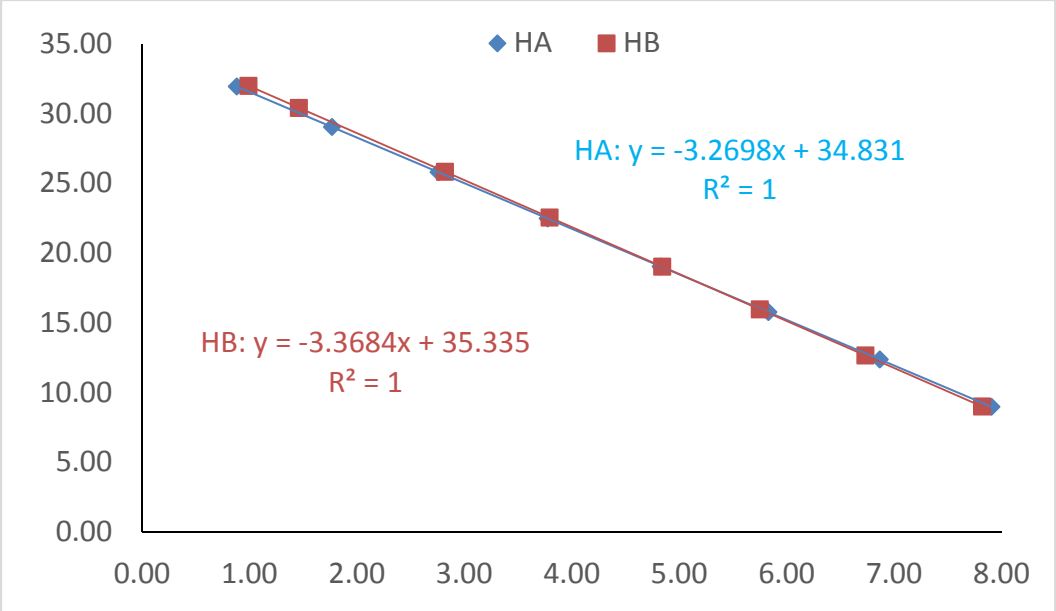

Figure 3. Sensitivity and efficiency of multiplex qPCR assay HAfpr+HBfpr+COX2fpr

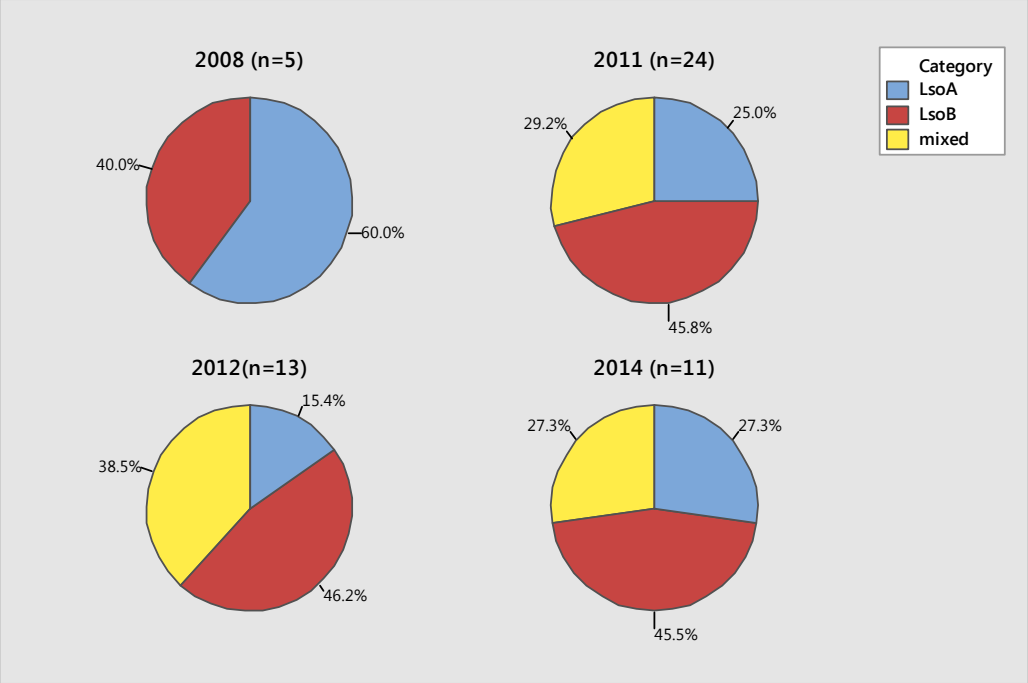

Figure 4. LsoA and LsoB population in field collected potato samples in the USA

References

Glynn, J.M., Islam, M.S., Bai, Y., Lan, S., Wen, A., Gudmestad, N.C., Civerolo, E.L., and Lin, H. 2012. Multilocus sequence typing of '*Candidatus* Liberibacter Solanacearum' strains from North America and New Zealand. Journal of Plant Pathology. 94 (1), 223-228.

Johnson, C., Wen, A., and Gudmestad, N. C. 2013. Variation in Zebra Chip Disease Symptomatology Related to Lso Haplotype. Proceedings of the 13th Annual SCRI Zebra Chip Reporting Session. Pp 94.

Johnson, C., Thompson, S., Wen, A., and Gudmestad, N.C. 2014. Conformity of genome variation among isolates of 'Candidatus Liberibacter solanacearum' from North America. Proceedings of the 14th Annual SCRI Zebra Chip Reporting Session.

Levy, J., Yao, J., Mendoza, A., Huot, O., Ibanez, F., and Tamborindeguy, C. 2014. Investigating the effect of Lso haplotype on plant infection and symptoms development. Proceedings of the 14th Annual SCRI Zebra Chip Reporting Session.

Li, W., J.A. Abad, R.D. French-Monar, J. Rascoe, A. Wen, N.C. Gudmestad, G.A. Secor, I.M. Lee, Y. Duan, and L. Levy. 2009. Multiplex real-time PCR for detection, identification and quantification of '*Candidatus* Liberibacter solanacearum' in potato plants with zebra chip. J. Microbiol. Methods 78: 59-65.

Lin, H., Wen, A., and Gudmestad, N. C. 2011. Multilocus sequencing typing Markers of genotyping and population genetic analyses of '*Candidatus* Liberibacter solanacearum'. Proceedings of the 11th Annual SCRI Zebra Chip Reporting Session. Pp32-36.

Lin, H, Islam, M. S., Bai, Y., Wen, A., Lan, S., Gudmestad, N. C., and Civerolo, E. L. 2012. Genetic diversity of '*Candidatus* Liberibacter solanacearum' strains in the United States and Mexico revealed by simple sequence repeat markers. Eur J Plant Pathol. 132:297–308.

Nelson, W. R., Fisher, T. W., and Munyaneza, J. E. 2011. Haplotypes of '*Candidatus* Liberibacter solanacearum' suggest long-standing separation. Eur. J. Plant Pathol. 130:5-12.

Nelson, W. R., Sengoda, V. G., Alfaro-Fernández, A., Font, M. I., Crosslin, J. M., and Munyaneza, J. E. 2012. A new haplotype of '*Candidatus* Liberibacter solanacearum' identified in the Mediterranean region. Eur. J. Plant Pathol. 135:633-639.

Gudmestad, N. C. 2012. Psyllids and Zebra Chip. World Potato Congress Workshop 3- Championing Plant Health.

Teresani, G. R., Bertolini, E., Alfaro-Fernández, A., Martínez, C., Tanaka, F. A. O., Kitajima, E. W., Roselló, M., Sanjuán, S., Ferrándiz, J. C., López, M. M., Cambra, M., and Font, M. I. 2014. Association of '*Candidatus* Liberibacter solanacearum' with a vegetative disorder of celery in Spain and development of a real-time PCR method for its detection. Phytopathology 104:804-811.

Wen, A., Johnson, C., and Gudmestad, N. C. 2012. Spatial-Temporal Distribution of '*Candidatus* Liberibacter solanacearum' biotypes in the United States. Proceedings of the 12th Annual SCRI Zebra Chip Reporting Session. Pp 79-83.

Wen, A., Johnson, C., and Gudmestad, N.C. 2013a. Development of a PCR assay for the rapid detection and differentiation of '*Candidatus* Liberibacter solanacearum' haplotypes and their spatiotemporal distribution in the United States. American Journal of Potato Research. Am. J. Potato Res. 90:229-236.

Wen, A., C. Johnson, and N.C. Gudmestad. 2013b. Development of Multiplex Conventional and Real-Time PCR for Detection and Genotyping of '*Candidatus* Liberibacter solanacearum'. Proceedings of the 13th Annual SCRI Zebra Chip Reporting Session. pp 68-72.

Workneh, F., Henne, D. C., Goolsby, J., Crosslin, J. M., Whipple, S, Bradshaw, J.D., Rashed, A., Paetzold, L., R. Harveson, R., and Rush, C.M. 2012. Assessing the Associations of Management and Environmental Factors with Regional Psyllid Abundance and Zebra Chip Intensity. Proceedings of the 12th Annual SCRI Zebra Chip Reporting Session. Pp 65-69.

Improved PCR-based Detection of '*Candidatus* Liberibacter solanacearum'

Lin, Hong
USDA-ARS
CDPG Research Unit, San Joaquin Valley Agricultural Sciences Center
Parlier, CA 93648

Abstract
An improved diagnostic system combined with nested PCR and Taq-Man® PCR in a single tube was developed for sensitive and specific detection of '*Candidatus* Liberibacter solanacearum' (Lso), bacterium associated with potato zebra chip (ZC) disease. The procedure involves two PCR steps using the species-specific outer and inner primer pairs. Different annealing temperatures allow both the first and the second rounds of PCR to be performed sequentially in a single tube. The first round PCR (outer primers) is performed at the annealing temperature higher than that of the secondary round PCR (inner primers) to prevent inner-outer primer interference. The specificity of the dual primer Taq-Man® is high because the fluorescent signal can only be generated from the TaqMan® probes that match to the product sequentially amplified by the outer and inner primers. This new detection system can reliably detect down to single copies of target DNA. The sensitivity of the dual primer system is comparable to the conventional two-tube nested PCR, but it eliminates the potential risk of cross contaminations commonly associated with conventional nested PCR and significantly improves detection sensitivity while maintaining specificity and reliability. The system is suitable for high through-put screening plant tissue and insect samples for ZC disease.

Introduction
Sensitive, accurate and reliable pathogen detection is essential for efficient management and regulatory responses to prevent the introduction into, and spread of ZC-associated '*Candidatus* Liberibacter solanacearum' (Lso) in unaffected areas. Various molecular diagnostic methods have been developed for detection of Lso since the pathogen was identified to be responsible for ZC. PCR-based detection methods are widely used for detection of Liberibacter species because they are sensitive and specific. These include conventional PCR (Crosslin et al, 2011), a LAMP-based method (Ravindran et al., 2012), nested PCR (Deng et al, 2007) and Real-Time PCR (Li et al., 2009). While these methods generally work well with symptomatic samples, it has often been observed that the methods are not reliable for consistently detecting Lso-infected, but asymptomatic field samples with low titers in plant hosts and in psyllids. This limitation is particularly critical in areas where psyllid vectors have been introduced but ZC has not yet been reported. Consequently, by the time ZC was confirmed the critical time for early disease detection and eradication of infected plants had passed. In addition, most Lso detections methods were designed in 16S ribosomal region. Sequence variation in this region has been found in different haplotypes. Thus, sequence variation could reduce detection sensitivity if such variations happen to cause mismatch to primer sequences. Sensitive detection is critical for promptly eradicating ZC and preventing its spread at an early stage. To address the current situation and urgent need for potato industry, in this study, we have developed a novel ultra-sensitive diagnostic system. This new detection method is conceptually analogous to conventional nested PCR, but incorporates quantitation and automation features of Real-Time PCR. Specifically, this ultra-sensitive and quantitative diagnostic system combines nested PCR and TaqMan® PCR in a single tube reaction for Lso detection. The procedure involves two PCR steps using the species-specific inner and outer primer pairs. Different annealing temperatures for inner and outer primer pairs allow each pair of primers operating in the first and the second rounds of PCR sequentially in a single closed tube. The first PCR will be run with an

annealing temperature that is suitable for outer primers but is too high for inner primers to prevent interference with the inner primers. This system integrates the features of conventional nested PCR and Real-Time PCR providing significantly increased sensitivity, improved reliability and high through-put capability suitable for routine, large scale diagnoses of clinical plant tissue and insect samples. Unlike conventional nested PCR which requires two steps of PCR in separate tube reactions making it high risk of potential false positives due to possible cross-contamination. This single tube nestd TaqMan (STN TaqMan) PCR operates in one closed tube PCR rendering this system easy to operate and eliminate the possible cross contamination while maintaining the nested PCR sensitivity. A similar diagnostic system had been designed for the detection of citrus huanglongbing associated Liberibacter pathogen and Pineapple mealybug wilt virus which showed ~10-50 times more sensitive than that of standard TaqMan PCR and is comparable with nested PCR sensitivity (Lin et al, 2010, Dey et al., 2012).
We have developed this robust diagnostic system that improved the sensitivity and specificity for Lso diagnosis. To evaluate practical application of this diagnostic system, we have validated sensitivity and specificity of the detection system using field Lso-infected potatoes and potato psyllids.

Materials and Methods

A genome wide sequence search was performed to identify Lso-specific sequence loci suitable for designing and developing a diagnostic system for Lso detection. The optimum TaqMan® probes and primers were designed using Beacon Design Software v7.0 (Premier Biosoft International, CA, USA) with the following criteria: GC% ≥ 40-50, Tm = 55 ^{o}C ± 2, primer length = 18-22 bp with amplicon size ranging from 120-200 bp. The melt temperature (Tm) for the TaqMan® probe was set 10^{o}C higher than the Tm for inner primer. To ensure amplification efficiency, the primers and probes were designed in a region where no secondary structures were observed. Among the designed primers and probes, only those having the least possibility of forming a hairpin, self/cross dimer structures were selected for further validation. For designing the outer primers, the same criteria were applied, except that a higher Tm of 65 ^{o}C (10^{o}C higher than inner primer Tm) and longer amplicons size (i.e., 300-500 bp) flanking upstream and downstream of forward and reverse inner primers were selected. A computational algorithm was then performed to conduct pair-wise comparisons of all primer/primer and primer/probe and to select the best primers/probe set that had the least stability of forming self/cross dimers between inner and outer primers and between primer and probe ($\Delta G \geq -2$ kcal/mol). The fluorescent reporter dye, 6-carboxyl-fluorescein (FAM) was labeled at the 5'end of the TaqMan® probe. A non-fluorescent quencher, minor groove binder (MGB) was labeled on the 3' end of probe. The probe was synthesized by Applied Biosystems Inc. (ABI, Foster City, CA).

To evaluate the sensitivity of STN TaqMan® PCR, the cloned plasmid DNA containing a target DNA amplicon was used as molecular standards. A serial 10x dilution of molecular standards was then used as templates to generate standard curves. Results were analyzed for its sensitivity and specificity in comparison with other published detection protocols using field samples including symptomatic and asymptomatic potatoes and psyllid collected in various growing seasons.

Results and Discussion

In this study, we designed and implemented STN TaqMan® PCR system for sensitive detection of Lso responsible for ZC. Five sets of primers/probes were initially designed and selected for experimental evaluation. Results from primer annealing temperature

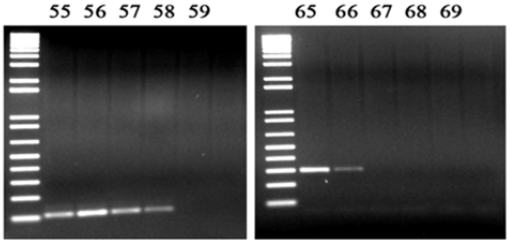

Figure1. Optimization of inner primer (left) with annealing temperatures ranged 55-59 °C and outer primer (right) with annealing temperatures ranged 65-69 °C.

tests indicated that optimization temperature for the inner primers is 56 ^{o}C and for the outer primers is 65 ^{o}C, respectively (Figure1). Primer sets that produced strong and clean amplified products with expected ranges of annealing temperatures were selected for further assessment. To evaluate the sensitivity, a series of dilutions of known copies of plasmid DNA cloned with Lso target sequences were mixed with healthy potato DNA to mimic infected ZC samples and used as molecular standards to generate standard curves. All tests were repeated three times. Amplification efficiency is 99.87 indicating there is unlikely any primer-primer interruptions (Figure 2). The system can reliably detect as low as 1-10 copies of target DNA. To further evaluation of utility of this system, PCR experiments were carried out using ZC-infected field samples, we compared this STN TaqMan PCR system with previously published Lso detection method (Li et al., 200X). For example, data showed that three samples that were detected positive with Ct values ~28 by a standard TaqMan method were reported in Ct values ~21 by this new system (Figure 3). Furthermore, three samples that were undetectable by standard TaqMan PCR were detected by this system with Ct value around 32-33, suggesting that the new system has significantly improved detection sensitivity.

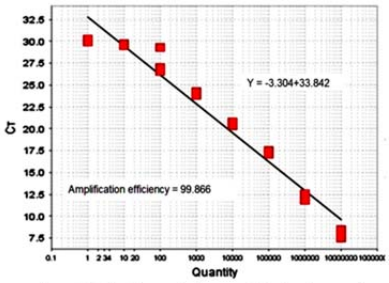

Figure2. Molecular standard curve of single tube nested TaqMan® PCR.

This one-tube PCR method provides gel free, reduced hands-on time and is more cost effective while detection system has significantly improved sensitivity, reliability and high throughput capability suitable for routine use in large scale year around epidemiological studies, post-harvested tuber screening and for quarantine surveys of ZC including symptomless samples. The system is also ideal for pooled potato psyllid samples in area wide survey where frequency of Lso-affected psyllid in populations is usually low in new introduction areas or during early growing season. Thus, this system will fill in gap we need for ZC disease management. This versatile system can also be used for standard TaqMan® quantitative PCR if only the inner primer pairs and TaqMan® probe are used, standard SYBR realtime PCR if only the inner primers and SYBR Green-I dye are included. This system can also be used for conventional gel-based PCR if the only outer primers are used. The high sensitivity and specificity of the system make this detection particularly useful in the areas where the potato psyllid populations have been established and yet disease has not yet been reported. This method could also be used all year round to obtain information about the population dynamics of Lso and the epidemiology of ZC.

Standard TaqMan				STN TaqMan			
Ct value				Ct value			
141	33	N/A		A04	FAM	Unkn	141	28	N/A
142	34	N/A		B04	FAM	Unkn	142	26	N/A
143	35	N/A		C04	FAM	Unkn	143	27	N/A
144	36	N/A		D04	FAM	Unkn	144	28	N/A
145	37	N/A		E04	FAM	Unkn	145	29	N/A
146	38	N/A		F04	FAM	Unkn	146	30	N/A
147	39	N/A		G04	FAM	Unkn	147	31	N/A
148	40	28.89		H04	FAM	Unkn	148	32	21.54
149	41	29.18		A05	FAM	Unkn	149	33	21.29
150	42	28.46		B05	FAM	Unkn	150	34	21.66
151	43	N/A		C05	FAM	Unkn	151	35	N/A
152	44	N/A		D05	FAM	Unkn	152	36	N/A
153	45	N/A		E05	FAM	Unkn	153	37	N/A
154	46	N/A		F05	FAM	Unkn	154	38	32.13
155	47	N/A		G05	FAM	Unkn	155	39	N/A
156	48	N/A		H05	FAM	Unkn	156	40	N/A
245	49	N/A		A06	FAM	Unkn	245	41	N/A
246	50	N/A		B06	FAM	Unkn	246	42	N/A
247	51	N/A		C06	FAM	Unkn	247	43	N/A
248	52	N/A		D06	FAM	Unkn	248	44	33.55
249	53	N/A		E06	FAM	Unkn	249	45	31.58
250	54	N/A		F06	FAM	Unkn	250	46	N/A
251	55	N/A		G06	FAM	Unkn	251	47	N/A
252	56	N/A		H06	FAM	Unkn	252	48	N/A

Figure3. Comparative detection of "*Candidatus* Liberibacter solanacearum" with standard Lso TaqMan PCR (Li et al., 2009), single tube nested TaqMan PCR (this study). Yellow highlight indicated ZC positive detected by both systems. Green highlight indicated the detected positive only by STN TaqMan,

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References

Crosslin, J.M., Lin, H., and Munyaneza, J. E. 2011. Detection of '*Candidatus* Liberibacter Solanacearum' in the potato Psyllid, *Bactericera cockerelli* (Sulc), by conventional and Real-Time PCR. Southwestern Entomol. 36:125-135.

Deng, X., Zhou, G., Li, H., Chen, J., and Civerolo, E. L. 2007. Nested-PCR Detection and sequence confirmation of '*Candidatus* Liberibacter asiaticus' from murraya paniculata in Guangdong, China. Dis. Notes 91:1051.

Dey, K.K., Lin, H., Borth, W.B., Melzer M.J., and Hu, J. 2012. A highly sensitive single-tube nested PCR assay for the detection of *Pineapple mealybug wilt associated virus*-2 (PMWaV-2). J. Virol. Met. 183:215–218.

Li, W., Abad, J.A., French-Monar, R.D., Rascoe, J., Wen, A., Gudmestad, N. C., Secor, G.A., Lee, I.M., Duan, Y., and Levy, L. 2009. "Multiplex real-time PCR for detection, identification and quantification of 'Candidatus Liberibacter solanacearum' in potato plants with zebra chip". J. Microbiol. Met. 78:59–65.

Lin, H., Chen, C., Doddapaneni, H., Duan, Y.P., Civerolo, E. L., Bai, X.J., and Zhao, X.L. 2010. A New diagnostic system for ultra sensitive and specific detection and quantitation of '*Candidatus* Liberibacter asiaticus', the bacterium associated with citrus huanglongbing. J. Microbiol. Met. 81, 17–25. 2010.

Ravindran, A., Levy, J., Pierson, E., and Gross, D.C. 2012. Development of a loop-mediated isothermal amplification procedure as a sensitive and rapid method for detection of 'candidatus Liberibacter solanacearum' in potatoes and psyllids. Phytopath. 102:899-907.

Diagnostics Targeting Haplotype Specific Regions in '*Candidatus* Liberibacter solanacearum' Genomes

Thompson, Sarah M.[1,2], Johnson, Chris P.[3], Lu, Ashley Y.[1,2], Frampton, Rebekah A.[1,2], Sullivan, Kerry L.[1,2], Wen, Aimin[3], Pitman, Andrew R.[1,2], Crowhurst, Ross N.[1,4], Scott, Ian A. W.[1,2], Gudmestad, Neil C.[3] and Smith, Grant R.[1,2]

[1]The New Zealand Institute for Plant & Food Research Limited, Lincoln, New Zealand; [2]Plant Biosecurity Cooperative Research Centre, Canberra, Australia; [3]North Dakota State University, Fargo, USA; [4]The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand

Abstract

Two '*Candidatus* Liberibacter solanacearum' (CLso) haplotype A genomes from New Zealand (NZ1) and the USA (HenneA), assembled to near completion, were analysed and compared with the haplotype B CLso-ZC1 genome using a reciprocal BLAST analysis revealing 44 loci which were only present in haplotype A. Most of these unique loci were located in phage remnant regions and annotated as hypothetical proteins. A qPCR assay was developed to initially validate the 44 putative haplotype A differential loci in a mini-panel screen and twelve loci were selected for further analysis against a larger sample collection.

Introduction

Zebra chip (ZC) disease, an important disease of potato, has emerged over the last 20 or so years in Central and North America. In 2008 the causal agent, '*Candidatus* Liberibacter solanacearum' (CLso), was discovered in New Zealand in potato (Liefting et al. 2008). In the same year the USA, Hansen et al. (2008) reported '*Ca.* L. psyllaurous' in the USA, which is now generally regarded as a synonym of CLso, in tomato. CLso is an unculturable alpha-proteobacterium vectored by phloem-feeding psyllids. *Bactericera cockerelli* (Hemiptera: Triozidae) and can also be spread in infected plant propagules. Five haplotypes (A to E) of CLso have been described based on insertion-deletions (indels) and single nucleotide polymorphisms (SNPs) in the 16S rRNA, 16S/23S intergenic spacer region (ISR) and 5S rRNA regions (Nelson et al. 2011, Nelson et al. 2012, Teresani et al. 2014). Haplotypes A and B are found in solanaceous crops in New Zealand (A) and Central and North America (A and B) and are vectored by *Bactericera cockerelli*, the tomato/potato psyllid (TPP). Haplotypes C, D and E are found in the apiaceous plants carrot (C in Scandinavia, D in Europe) and celery (E). Haplotype C is vectored by *B. apicalis*, D by *B. trigonica*, whilst E has no known insect vector.

ZC disease was first reported in New Zealand in 2008 (Liefting et al. 2008), two years after the discovery of TPP in the country (Gill 2006). The symptoms of ZC in New Zealand are often less severe than the symptoms in the USA (Berry et al., 2011, Pitman et al., 2011, Pitman et al., 2012). Potato cultivar, environmental conditions, vector behaviour and differences between CLso haplotypes may contribute to the differences in pathology and symptomology between the USA and New Zealand. Variability in symptoms between haplotypes A and B has been recently reported in the USA by Wen et al. (2013) who noted that '*...field observations suggest haplotype B ...produces a more severe and destructive disease symptom than haplotype A...*'. The SNPs and indels present in the rRNA regions are highly unlikely to contribute to the differences in ZC pathology between haplotypes A and B. Previous studies identified non-rRNA loci that were different between the two solanaceous-infecting haplotypes. Glynn at al. (2012) developed a MLST PCR assay targeting ten house-keeping genes to type CLso sources, defining two sequence typing (ST) profiles, ST-1 and ST-2 which corresponded with

haplotypes B and A respectively. They also identified a range of CLso sources that contained mixed ST profiles and suggested this was due to recombination. Wen et al. (2013) developed a PCR assay based on an SSR marker first described by Lin et al. (2012). This assay had limitations with the authors recommending the genome sequencing of haplotype A and development of more specific primers particularly to improve the sensitivity of the assay. Whole genome comparisons have been used previously to identify *Ca*. Liberibacter species loci for diagnostics: for example Kogenaru et al. (2014) identified 34 unique loci in *Ca.* Liberibacter asiaticus using this approach. The CLso ZC1 genome, a haplotype B source from northern Texas, is the only completely assembled CLso genome to date and one of six *Ca.* Liberibacter genomes that have been completely assembled. Here, we describe the identification, via bioinformatic analysis of two near complete haplotype A genomes, of dispersed loci that distinguish CLso haplotype A from CLso haplotype B and the initial validation by qPCR of these loci as differential makers between the two haplotypes.

Materials and Methods

The two CLso haplotype genomes were assembled to near completion using the materials and methods described for NZ-HapA-tpp (NZ1) and US-HapA-tpp (HenneA) by Smith et al. (2013). A reciprocal BLAST analysis (E-value >e^{-5} and ≥70% coverage of both sequences) was used to identify sequences unique to haplotype A (NZ1, JMTK00000000.2 and HenneA, JQIG00000000.1) by comparison to ZC1 (NC_014774.1), followed by a second reciprocal BLAST analysis (E-value >e^{-5} and ≥70% coverage of both sequences) to indentify the sequences from the first analysis that were not present in any known (*Ca*.) Liberibacter species. The remaining sequences were then analysed by BLASTn against all Genbank sequences and sequences with an E-value >e^{-10} retained. Primers were selected from the sequence comprising the putative differential loci using the Primer3 (Untergasser et al., 2012) module in Geneious v 6.1.7 (Biomatters, http://www.geneious.com) with product size between 70 and 250 bp and a GC content of 50%. qPCR reactions were undertaken on a StepOne Plus (Applied Biosystems) using a 70 to 62°C touchdown protocol (Larsen et al. 2002). Amplicons were analysed by both melt curve (60 to 95°C) and visualisation via electrophoresis in a 1.5% agarose gel. All 44 primer sets were assessed against a mini-panel of six reference DNA sources using the touchdown qPCR protocol.

Results and Discussion

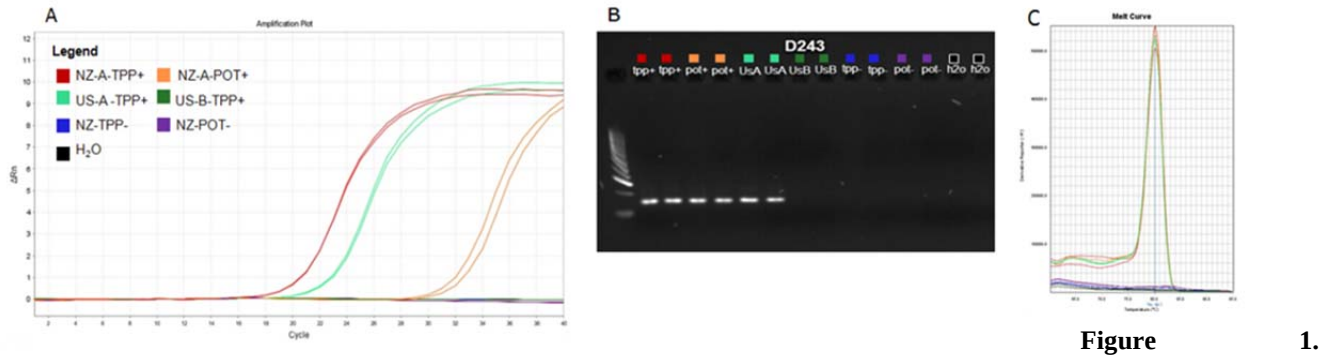

Figure 1. Amplification plot (A), amplicon electrophoresis profile (B) and melt curve (C) for the qPCR assay targeting the hypothetical protein D243 in the *Ca.* Liberibacter solanacearum haplotype A genome. Legend abbreviations: NZ/US country of sample origin; A/B haplotype; TPP/ POT psyllid/ potato; +/- CLso presence/ absence.

An initial set of 280 differential loci (145 coding and 135 non-coding) were obtained by reciprocal BLAST analysis of both near complete genomes against the ZC1 and other (*Ca.*) Liberibacter genomes. This set was reduced to 44 loci (30 coding and 14 non-coding) after comparison against all DNA

sequences in Genbank. Most of these 44 loci were found in regions that appeared to be phage remnants (regions of the bacterial genome, which may be the result of previous lysogenic/ lytic phage activity). Primers were selected for these loci and initially screened.

The Ct plots (Figure 1A), amplicon gel profiles (Figure 1B) and melt curves (Figure 1C) of D243 are an example of the loci which successfully differentiated CLso haplotype A from B by presence/ absence. Most (37 of 44) of the qPCR assays targeting the selected haplotype A putative unique loci successfully differentiated CLso haplotype A from haplotype B by presence/ absence of amplicon. These 37 loci produced amplicons of the expected size, a consistent melt curve profile of the amplicons from the different samples, and no amplification of a product from CLso haplotype B sources, nor from the negative controls. Further, no amplicons were produced from the plant (potato), insect (psyllid) or microbial (associated with plant or insect) DNA present in the samples. Twelve loci from the 37 which successfully differentiated haplotype A from B were proposed for evaluation against the large CLso source reference collection at the Plant Pathology Department, North Dakota State University (Johnson et al. 2014). The Ct values from the touchdown qPCR assay and target of these 12 loci are described in Table 1.

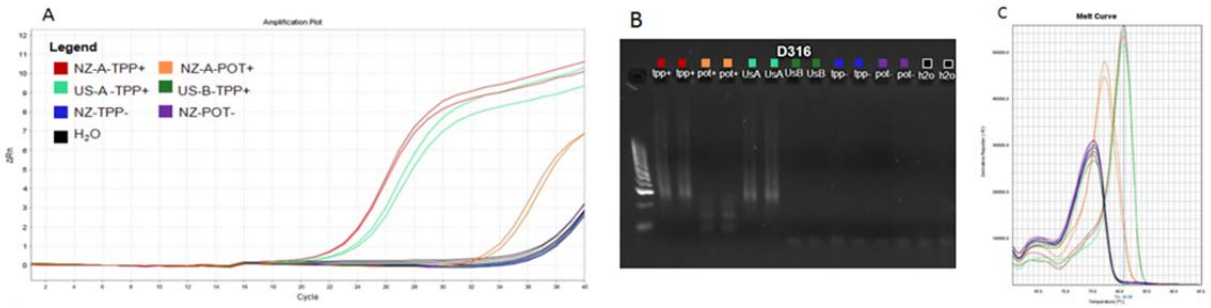

Figure 2. Amplification plot (A), amplicon electrophoresis profile (B) and melt curve (C) for the qPCR assay targeting the hypothetical protein D316 in the *Ca.* Liberibacter solanacearum haplotype A genome. Legend abbreviations:NZ/US country of sample origin; A/B haplotype; TPP/ POT psyllid/ potato; +/- CLso presence/ absence.

Seven of the loci (16%) did not yield the expected amplicons (size and/ or melt curve) during the initial screening phase. For example D316, targeting a hypothetical protein, had a Ct value of 21.3 from the NZ-A-TPP+ sample (Figure 2A): however the amplicons from the samples were of variable size (Figure 2B) and also showed variable melt curves (Figure 2C). This may have been due to different genomic sequences being amplified rather than expected loci, or that the loci were variable in the different CLso sources being evaluated.

The touch-down qPCR protocol was not optimised for each qPCR assay targeting the identified loci, which may have also contributed to these results. Further, the primer selection parameter for primers with a 50% GC selected from a genome with an overall GC of 35% also restricted the selection of optimal target sequences within the target loci. Genomic diversity has previously been described in both solanaceous plant-infecting haplotypes of CLso. Glynn et al (2012) noted genetic diversity and possible recombination between haplotypes when analysing two possible alleles of each of ten house-keeping genes via MLST profiles in 59 CLso sources for the Mexico, the USDA and New Zealand, whilst Lin et al. (2012) concluded that on average, there were 2.5 effective alleles at the eight SSR loci analysed in 62 CLso sources from different locations in the USA and Mexico.

Using bioinformatic analysis of three genomes, we have identified 37 loci that putatively differentiate CLso haplotype A from haplotype B via presence/ absence of loci rather than allelic variation at a locus. Twelve of these assays have been selected for further evaluation against a larger sample collection to test this conclusion. The genomic location of these differential loci in regions that appear to be phage remnant is consistent with the suggestion by Lin et al. (2012) that integration of phage plays a role in genome rearrangements in *Ca.* Liberibacter. These loci, whilst still requiring more testing and validation, may provide a significant additional resource for both investigating and differentiating the differences in biology and pathogenicity across all CLso haplotypes.

Table 1. Mini-panel screen Ct values of 12 of 44 putative diagnostic loci targets against haplotype *Ca*. Liberibacter solanacearum A and B samples. NZ/US country of sample origin; A/B haplotype; TPP/ POT psyllid/ potato; +/- CLso presence/ absence; UD Undetected.

Name	Target	NZ-A-TPP+	NZ-A-POT+	US-A-POT+	US-B-POT+	NZ-TPP-	NZ-POT-
D205	Hypothetical protein	17.3	28.7	18.8	UD	UD	UD
D209	Hypothetical protein	22.7	34.8	23.4	UD	UD	UD
D228	Hypothetical protein	18.2	29.1	19.6	UD	UD	UD
D243	Hypothetical protein	19.6	31.4	21.8	UD	UD	UD
D244	Hypothetical protein	18.1	31.1	20.3	UD	UD	UD
D1166	Phage terminase	17.0	28.7	19.2	UD	UD	UD
D1169	Hypothetical protein	19.1	31.1	21.3	UD	UD	UD
D1191	Hypothetical protein	19.4	31.1	21.2	UD	UD	UD
D1204	Hypothetical protein	18.3	30.1	21.1	UD	UD	UD
D1205	Hypothetical protein	20.8	32.2	22.7	UD	UD	UD
DNC167	Non-coding	20.6	32.0	22.4	UD	UD	UD
DNC412	Non-coding	22.1	36.8	23.7	UD	UD	UD

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References

Berry, N., Thompson, S., Taylor, N., Wright, P., Shah, F., Walker, M., Beard, S., Jorgensen, N., Butler, R., Scott, I., and Pitman, A. R. 2011. The impact of *Ca.* Liberibacter infected seed tubers on potato production in New Zealand. Proc 11th Ann 2011 Zebra Chip Reporting Session:178-182.

Hansen, A. K., Trumble, J. T., Stouthamer, R., and Paine, T. D. 2008. A new Huanglongbing Species, "*Candidatus* Liberibacter psyllaurous", found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). Appl Environ Microbiol 74:5862-5865.

Gill, G. 2006. Tomato psyllid detected in New Zealand. Biosecurity 69:10-11.

Glynn, J., Islam, M., Bai, Y., Lan, S., Wen, A., Gudmestad, N., Civerolo, E., and Lin, H. 2012. Multilocus sequence typing of '*Candidatus* Liberibacter solanacearum' isolates from North America and New Zealand. J Plant Pathol 94:223-228.

Johnson, C., Thompson, S., Wen, A., Lu, A., Sullivan, K., X Frampton, R., Pitman, A., Crowhurst, R., Smith, G. and Gudmestad, N. 2014. Conformity of genome variation among isolates of 'Candidatus

Liberibacter solanacearum' from North America. Proceedings of the 14th Annual SCRI Zebra Chip Reporting Session.
Kogenaru, S., Yan, Q., Riera, N., Roper, M. C., Deng, X., Ebert, T. A., Rogers, M., Irey, M. E., Pietersen, G., Rush, C. M., and Wang, N. 2014. Repertoire of novel sequence signatures for the detection of *Candidatus* Liberibacter asiaticus by quantitative real-time PCR. BMC Microbiol 14:39.
Larsen, H. H., Masur, H., Kovacs, J. A., Gill, V. J., Silcott, V. A., Kogulan, P., Maenza, J., Smith, M., Lucey, D. R., and Fischer, S. H. 2002. Development and evaluation of a quantitative, touch-down, real-time PCR assay for diagnosing *Pneumocystis carinii* pneumonia. J Clin Microbiol 40:490-494.
Li, L., Stoeckert, C. J., and Roos, D. S. 2003. OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res 13:2178-2189.
Liefting, L. W., Perez-Egusquiza, Z. C., Clover, G. R. G., and Anderson, J. A. D. 2008. A new '*Candidatus* Liberibacter' species in *Solanum tuberosum* in New Zealand. Plant Dis 92:1474-1474.
Lin, H., Islam, M. S., Bai, Y., Wen, A., Lan, S., Gudmestad, N. C., and Civerolo, E. L. 2012. Genetic diversity of '*Candidatus* Liberibacter solanacearum' strains in the United States and Mexico revealed by simple sequence repeat markers. Eur J Plant Pathol 132:297-308.
Lin, H., Lou, B., Glynn, J. M., Doddapaneni, H., Civerolo, E., Chen, C., Duan, Y., Zhou, L., and Vahling, C. M. 2011. The Complete Genome Sequence of 'Candidatus Liberibacter solanacearum', the Bacterium Associated with Potato Zebra Chip Disease. PLoS ONE 6(4): e19135.
Nelson, W. R., Fisher, T. W., and Munyaneza, J. E. 2011. Haplotypes of "*Candidatus* Liberibacter solanacearum" suggest long-standing separation. Eur J Plant Pathol 130:5-12.
Nelson, W. R., Sengoda, V. G., Alfaro-Fernandez, A. O., Font, M. I., Crosslin, J. M., and Munyaneza, J. E. 2012. A new haplotype of "*Candidatus* Liberibacter solanacearum" identified in the Mediterranean region. Eur J Plant Pathol 135:633-639.
Pitman, A. R., Berry, N., Thompson, S., Taylor, N., Wright, P., Shah, F., Walker, M., Read, S., Beard, S., Jorgensen, N., Butler, R., and Thompson, S. 2012. Yield and quality consequences of TPP and Lso: Year 2 of a New Zealand study. Proc 12th Ann 2012 Zebra Chip Reporting Session 26-30.
Pitman, A. R., Drayton, G. M., Kraberger, S. J., Genet, R. A., and Scott, I. A. 2011. Tuber transmission of "*Candidatus* Liberibacter solanacearum" and its association with zebra chip on potato in New Zealand. Eur J Plant Pathol:389-398.
Smith, G. R., Thompson, S. M., Johnson, C. P., Frampton, R. A., Lu, A., Pitman, A.R. Wen, A., Duan, Y. and Gudmestad, N.C. (2014). A draft core genome of '*Candidatus* Liberibacter solanacearum' obtained by comparison of haplotype A and B metagenomic assembled sources. Proceedings of the 13th Annual SCRI Zebra Chip Reporting Session pp59-63.
Teresani, G. R., Bertolini, E., Alfaro-Fernandez, A., Martínez, C., Tanaka, F. A. O., Kitajima, E., Rosello, M., Sanjuan, S., Ferrandiz, J. C., López, M. M., Cambra, M., and Font-San-Ambrosio, M. I. 2014. Association of '*Candidatus* Liberibacter solanacearum' with a vegetative disorder of celery in Spain and development of a real-time PCR method for its detection. Phytopathology 104:804-811.
Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., and Rozen, S. G. 2012. Primer3--new capabilities and interfaces. Nucleic Acids Res 40:e115.
Wen, A., Johnson, C., and Gudmestad, N. C. 2013. Development of a PCR assay for the rapid detection and differentiation of '*Candidatus* Liberibacter solanacearum' haplotypes and their spatiotemporal distribution in the United States. Am J Potato Res 90:229-236.

Improved '*Candidatus Liberibacter* solanacearum' Detection in Plants and Insects

[1]Bextine, B. R., [1]Hail, D., [1]Powell, C. M., and [2]Hansen, J. [1]University of Texas At Tyler, Tyler, TX 75701, USA [2] Research and Testing Laboratory, 4321 Marsha Sharp Fwy #2, Lubbock, TX, 79407, USA

Abstract
The potato psyllid (*Bactericera cockerelli*) is the primary insect vector of the bacterial pathogen '*Candidatus Liberibacter* solanacearum' (CLso), the causal agent of Zebra chip disease in solenaceious crops. The biology of phloem feeding insects is heavily influenced by their bacterial communities, which are largely determined by their food sources. Horizontal transmission of bacteria between individuals of the same species and insects of different species, with their host plants as reservoir has been demonstrated in several plant insect systems. CLso has a symbiotic role in potato psyllid and can colonize plants before being passed to its next insect host. Interestingly, thrips (*Frankliniella tritici*) that co-colonize plants have tested positive for the presence of CLso, though vector status is unknown. In this study, the total bacterial community of thrips co-colonizing CLso plants was analyzed and compared to potato psyllids maintained on the same plants. Samples were then subjected to bacterial community sequencing via the Illumina miSEQ platform and analyzed with the macQIIME.

Introduction
The potato psyllid (*Bactericera cockerelli*) is the primary insect vector of the bacterial pathogen '*Candidatus Liberibacter* solanacearum' (CLso), the causal agent of Zebra chip disease in solenaceious crops (Hansen et al., 2008, Liefting et al., 2009, Secor et al., 2009). The biology of phloem feeding insects is heavily influenced by their bacterial communities (Baumann, 2005), which are largely determined by their food sources and vertical transmission (Nachappa et al., 2011). '*Candidatus* Carsonella ruddii' is the primary endosymbiant of the potato psyllid, and is encased in the bacteriocyte. *Wolbachia pipientis* and CLso are the main secondary symbionts, and make up the vast majority of the bacterial content of *B. cockerelli* (Arp et al., 2014). Horizontal transmission of bacteria between individuals of the same species and insects of different species, with their host plants as reservoir has been demonstrated in several plant insect systems.

Thrips (*Frankliniella tritici*) commonly co-colonize plants fed on by psyllids, and can be a serious pest in laboratory colonies. These phytophagus insects have been shown to transmit plant virus, little investigation into bacterial communities of thrips has been conducted using culture independent techniques. Some studies have shown that the primary symbiont for thrips appears to be a species of Erwinia (de Vries et al., 2008). CLso has a symbiotic role in potato psyllid and can colonize plants before being passed to its next insect host. Interestingly, thrips (*Frankliniella tritici*) that co-colonize plants have tested positive for the presence of CLso, though their vector status is unknown.

In this study, the total bacterial community of thrips co-colonizing CLso infected plants was analyzed and compared to potato psyllids maintained on the same plants. Samples were then subjected to bacterial community sequencing via the Illumina miSEQ platform and analyzed with the macQIIME.

Materials and Method
PCR CLso *Screening:* Bacterial DNA was isolated from 17 individual *B. cockerelli* and 13 pools of 5 *F. tritici* collected from hot insect colonies using Qiagen DNEasy DNA Isolation Kit (Valencia, CA) as per the manufacturer's protocol. Samples were then subjected to PCR screening using OI2c (Liefting et al.,

2008, Crosslin et al., 2011) using protocol described in Arp et al. (2013). PCR product was then visualized on a 1% agarose gel.

Illumina MiSeq 16s Sequencing: Bacterial DNA was isolated from 3 individual *B. cockerelli* and 3 pools of 5 *F. tritici* collected from hot insect colonies using Qiagen DNEasy DNA Isolation Kit (Valencia, CA) as per the manufacturer's protocol. Extracted DNA was then processed and Illumina MiSeq 16s Sequencing was preformed at Research and Testing Laboratory, Lubbock, TX.

Analysis: Forward and reverse reads were recorded in FASTQ format and merged using the PEAR Illumina paired-end read merger, these files were then converted to FASTA and quality files. The read quality was assessed in order to remove those with low quality scores. Sequences were arranged by length (longest to shortest) and identical sequences were clustered using the USEARCH algorithm. These clusters were then arranged by length and 99% similar clusters were merged, this was repeated for 95% similarity and any un-used reads were considered base calling errors and discarded. The UCHIIME de novo method was then employed to detect and remove chimeras; all remaining sequences greater than 250bp in length are placed into a single FNA file with corresponding map and quality files (Research and Testing, Lubbock, TX).

Data was analyzed using the macQIIME pipeline v1.8 (Caporaso *et al.*, 2010), in order to preserve chimera detection, base call error detection, and paired end read assembly provided by Research and Testing FNA and quality files are converted to a single FASTQ file, the required format for processing Illumina data with macQIIME; a barcode file is then generated, using the hamming 8 barcode system. Multiplex reads are assigned to samples using the split libraries FASTQ command with the following parameters, Phred quality score of 1 (default 3), minimum number of consecutive high quality base calls as a fraction of the input read length was 0.50 (default 0.75), maximum number of N characters allowed to retain it was 5 (default 0). Closed reference OTU picking was then carried out against the August 2013 green genes training set, and an OTU table was generated. From the OTU table the rep set and rep set tree were generated. Multiple rarefactions were calculated from 5000 to 10000 random samples at 250 sample intervals and replicated 10 times; from these rarefactions alpha diversity (intra-habitat diversity) metrics, observed species and chao1 were calculated and a sample size of 8000 random samples was chosen to display maximum diversity. This allows samples to be normalized for different depths of coverage that are inherent in massively parallel sequencing; samples that produced higher numbers of reads can misrepresent the distribution of taxa, by selecting a standard rarefaction for all samples this bias is mitigated. Beta diversity (inter-habitat diversity) was then calculated, providing weighted (abundance included in calculation) and unweighted (abundance not included in calculation), and then compared using unifrac measures. To test the strength of the comparison jackknifing was used to calculate the bootstrap values for the phylogenetic comparisons. Bootstrapped phylogenetic trees were generated from the comparisons.

Results and Discussion

PCR CLso Screening: Of the potato psyllids screened 76% of individuals showed a band, and 30% of thrips samples showed a band (Figure 1). This suggests that thrips can acquire CLso from feeding on CLso plants, but does not indicate if they are capable of transmitting the pathogen. Dr. Henny (Yang et al., 2014) has also showed this, though the pathogen loads of field samples have appeared consistently lower than those of lab colonies.

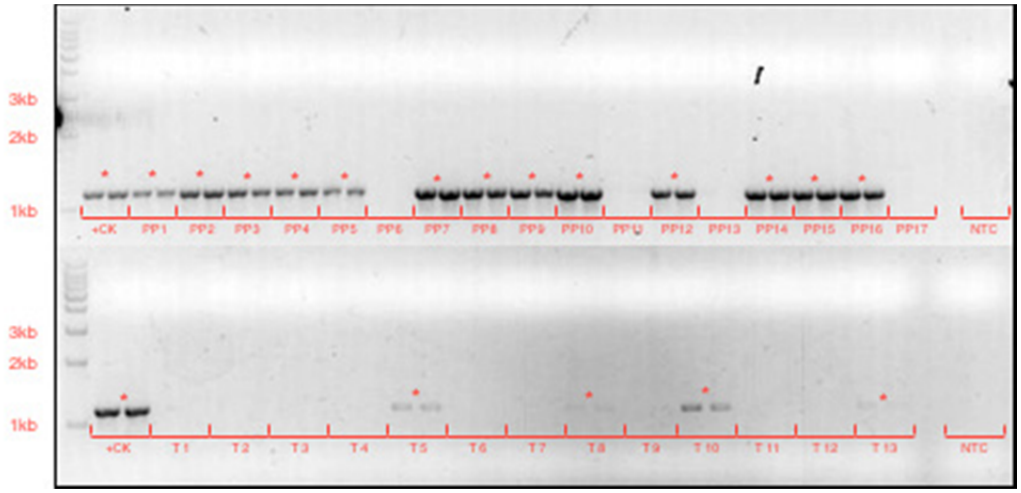

Figure 1. Electrophoresis gel (1% agrose) showing amplification of CLso using OI2c diagnostic protocol, the top row shows potato psyllid samples, the bottom row shows thrips samples. Approximately 76% of potato psyllid and approximately 30% of thrips samples show amplification. +CK denotes positive control and NTC denotes no template control.

Illumina MiSeq 16s Sequencing: Potato psyllid samples were heavily loaded with their thee main symbionts; *Wolbachia*, CLso, and *C. ruddii* accounted for a large portion of the recovered sequences. The other most common bacteria present are generally associated with environmental sources. This data agrees with previous survey work on the potato psyllid bacterial community (Arp et al., 2014). The 10 most common OTUs are shown in Table 1.

The thrips samples had much more evenly distributed communities, though in two out of the three samples *Wolbachia* was the dominant constituent. *Acinetobacter*, *Erwinia*, and Enterobacteriaceae are all commonly associated with insects; *Pseudomonas* and *Staphylococcus* are both commonly associated with environmental sources, and the soil dwelling life stage of thrips may contribute. Corynebacterium has been previously reported in 16s study of plant feeding insects (Rodrigues et al., 2003), though it is not commonly associated with insects. The high volume of this bacterium in thrips may be future point of study. The 10 most common OTUs are shown in Table 2. It is also interesting that Erwinia is not the dominate taxa in thrips, as it has been reported to be their primary symbiont.

There were three bacterial taxa common between the 10 most populous OTUs of potato psyllids and thrips. The same strain of *Wolbachia* was the most populous OTU in five of the six samples; this is interesting but not surprising. Horizontal transmission within communities of insects feeding on the same plants has been demonstrated (Sintupachee et al., 2006). The presence of CLso corresponds to the OI2c results, though the number of sequences is significantly lower in thrips than potato psyllids. One interesting note is that the thrips contained no *C. ruddii*, with the amount of shared bacterium this is somewhat surprising (Table 3).

Table 1. Top 10 populous OTUs present in potato psyllid samples, classification is to most specific level available.

GreenGenes OTU ID	Potato Psyllid 2	Potato Psyllid 1	Potato Psyllid 3	Potato Psyllid Total	Classification
835499	34116	10784	26304	71204	*Wolbachia*
2867020	*211*	8993	*7177*	16381	*Candidatus Liberibacter*
4372621	957	418	645	2020	Carsonella ruddii
6092	551	48	476	1075	*Wolbachia*
687185	15	559	488	1062	*Staphylococcus*
6118	29	6	23	58	*Wolbachia*
164612	1	28	13	42	*Staphylococcus*
4001495	0	0	13	13	Streptophyta
792863	3	1	8	12	*Pseudomonas*
759378	8	0	0	8	*Alkaliphilus*

Table 2. Top 10 populous OTUs present in thrips samples, classification is to most specific level available.

GreenGenes OTU ID	Thrip 2	Thrip 1	Thrip 3	Thrip Total	Classification
835499	278	4518	6787	11583	*Wolbachia*
687185	*1583*	744	*3184*	5511	*Staphylococcus*
1004445	2438	740	467	3645	*Corynebacterium*
277094	856	245	2399	3500	*Pseudomonas*
4334049	1756	664	100	2520	*Acinetobacter*
817254	1626	102	66	1794	*Erwinia dispersa*
2867020	837	122	558	1517	*Candidatus Liberibacter*
200021	203	38	650	891	Pseudomonas
4416562	68	363	391	822	*Enterobacteriaceae*
268101	390	88	47	525	*Enterobacteriaceae*

Table 3. Comparison of the top 10 populous OTUs present in potato psyllid and thrips samples, highlighted OTUs are common between species.

Potato Psyllid		Thrips	
GreenGenes OTU ID	Classification	GreenGenes OTU ID	Classification
835499	*Wolbachia*	835499	*Wolbachia*
2867020	*Candidatus Liberibacter*	687185	*Staphylococcus*
4372621	Carsonella ruddii	1004445	*Corynebacterium*
6092	*Wolbachia*	277094	*Pseudomonas*
687185	*Staphylococcus*	4334049	*Acinetobacter*
6118	*Wolbachia*	817254	*Erwinia dispersa*
164612	*Staphylococcus*	2867020	*Candidatus Liberibacter*
4001495	Streptophyta	200021	*Pseudomonas*
792863	*Pseudomonas*	4416562	Enterobacteriaceae
759378	*Alkaliphilus*	268101	Enterobacteriaceae

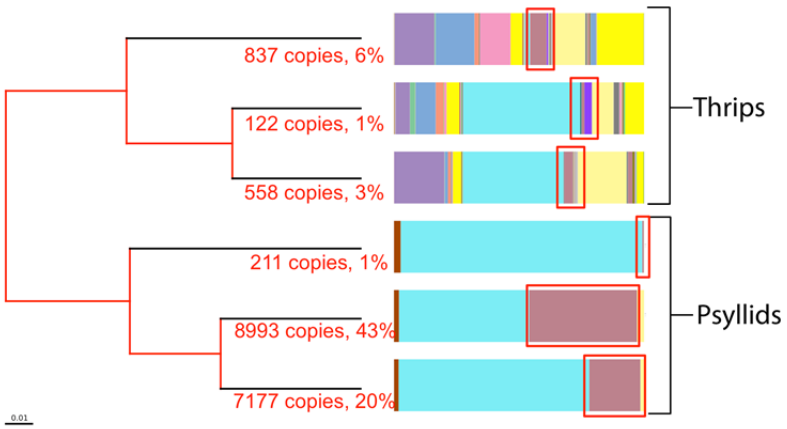

Figure 2. Weighted phylogenetic tree showing separation of potato psyllids and thrips, red text and boxes correspond to the relative content of CLso in each sample.

Acknowledgements
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References
Arp, A.P., Chapman, R., Crosslin, J.M., and Bextine, B.R. 2013. 'Low-Level Detection of *Candidatus* Liberibacter Solanacearum in *Bactericera cockerelli* (Hemiptera: Triozidae) by 16s rRNA Pyrosequencing' Envrion. Ent. 42:868-873

Arp, A.P., Munyaneza, J.E., Crosslin, J.M., Trumble, J., and Bextine, B.R. 2014. 'A global comparison of *Bactericera cockerelli* (Hemiptera: Triozidae) microbial communities' Envrion. Ent. 43:344-352

Crosslin, J. M., H. Lin, and J. E. Munyaneza. 2011. Detection of *Candidatus* Liberibacter solanacearum in the potato psyllid, *Bactericera cockerelli* Sulc, by conventional and real-time PCR. Southwest. Entomol. 36: 125Ð135.

de Vries, E.J., van der Wurff, A.W., Jacobs, G., Breeuwer, J.A., 2008. Onion thrips, Thrips tabaci, have gut bacteria that are closely related to the symbionts of the western flower thrips, *Frankliniella occidentalis*. J Insect Sci. 8:1-11

Liefting, L. W., Z. C. Perez–Egusquiza, G.R.G. Clover, and J.A.D. Anderson. 2008. A new Candidatus Liberbacter species in Solanum tuberosum in New Zealand. Plant Dis. 92: 1474-1474.

Rodrigues, J.L., Silva-Stenico, M.E., Gomez, J.E., Lopes, J.R.S., Tsai, S.M. 2003. "Detection and Diversity Assessment of *Xylella fastidiosa* in Field-Collected Plant and Insect Samples by Using 16S rRNA and *gyrB* Sequences' Appl. Envrion. Microbiol. 69:4249-4255

Sintupachee, S., Milne, J.R., Poonchaisri, S., Baimai, V., Kittayapong, P. 2006. 'Closely Related Wolbachia Strains within the Pumpkin Arthropod Community and the Potential for Horizontal Transmission via the Plant' Microbiol. Eco. 51:294-301

Yang, X., Campos, M., Munyaneza, J., and Henne, D. 2014. Transmission potential of the zebra chip disease pathogen, Candidatus Liberibacter solanacearum, by whitefly and thrips on potato. Southwestern Branch, Entomological Soceity of America, Poster.

Assessment of preference of *Bactericera cockerelli* for '*Candidatus* Liberibacter solanacearum'-infected and pathogen-free solanaceous hosts

Thinakaran, J[1,4], Pierson, E[2], Tamborindeguy, C[3], and Henne, D[1,5]
[1]Texas A&M AgriLife Research, Weslaco, TX 78596, USA, [2] Department of Horticulture, Texas A&M University, College Station, TX 77843, USA, [3] Department of Entomology, Texas A&M University, College Station, TX 77843, USA, [4] USDA-ARS, Yakima Agricultural Research Laboratory, 5230, Konnowac Pass Road, Wapato, WA 98951, USA, [5] Monsanto Company, 2111 Piilani Highway, Kihei, HI 96753, USA

Abstract
The potato psyllid, *Bactericera cockerelli* is a major pest of potato and a vector of the bacterial pathogen, '*Candidatus* Liberibacter solanacearum' (Lso) responsible for causing zebra chip (ZC) disease of potato. Experiments were conducted in the laboratory to demonstrate preference of Lso-infected and Lso-free *B. cockerelli* adults for Lso-infected and Lso-free potato, tomato, eggplant, pepper and silverleaf nightshade plants. It was observed that adults either preferred Lso-free or settled equally on Lso-infected and Lso–free hosts indicating there was no preference for Lso-infected plants although previous studies indicate the importance of olfactory cues to guide *B. cockerelli* orientation to Lso-infected host plants. It is possible that volatiles specific to solanaceous plants may serve as attractant for migrating *B. cockerelli* adults, and further visual, tactile or gustatory stimuli may serve as short range cues that determine acceptability of the plant species for settling and subsequent feeding and/or oviposition.

Introduction
The potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is the only known vector of the bacterial pathogen, '*Candidatus* Liberibacter solanacearum' (Lso), responsible for causing zebra chip (ZC) disease. Lso has been reported on cultivated and wild solanaceous hosts (Murphy et al. 2014, Thinakaran et al. 2015) and the combined presence of vector and pathogen is becoming a limiting factor in potato production in the North and Central America and New Zealand. In the LRGV, both vector and ZC symptomatic potato plants can be present in abundance underscoring the role of alternative hosts in the disease transmission process. If resident *B. cockerelli* populations already harbor Lso, Lso-infected plants may not necessarily contribute to further disease spread. Thus, Lso-infected plants and resident *B. cockerelli* adults play a crucial role in the epidemiology and management of ZC. The objective of this study was to evaluate preference of *B. cockerelli* for Lso-infected and Lso-free solanaceous hosts as indicated by their settling behavior and symptoms expressed by Lso-infected plants.

Materials and Methods
*Source of plants***.** Potato (cultivar 'Atlantic'), tomato (*Solanum lycopersicum*) (cultivar 'Lance'), bellpepper (*Capsicum annuum*) (cultivar 'Capistrano'), eggplant (*Solanum melongena*) (cultivar 'Italian') and the common weed silverleaf nightshade were used in the study. Disease-free potato seed pieces were obtained from J. W. Farms (Edinburg, TX) and seeds of tomato, eggplant, pepper, and SLN were obtained from locally propagated stock. Individual seeds of each of the host plants examined (except potato) were planted in foam trays containing cone-shaped pots measuring 3 x 3 x 4 cm filled with Metro-Mix 360 growth medium (SunGro Horticultural Distribution, Bellevue, WA). Potato tubers were cut in half and allowed to suberize before planting in 10 cm diameter plastic pots with potting mix added. One week-old tomato, pepper, and eggplant seedlings were transferred to 10 cm diameter plastic pots filled with the same potting mix. All plants were fertilized with Miracle-Gro (Scotts Miracle-Gro Products, Inc. Marysville, OH) once a week and watered twice every week or as needed. Four to five week-old potato, tomato, eggplant, pepper and SLN plants of uniform size were used in experiments.

Source of insects. Lso-infected B. cockerelli. Bactericera cockerelli adults were originally collected from a potato field at the Texas A&M AgriLife Research Center at Weslaco, TX in May 2006 and maintained in an insectary on potato. Beginning December 2011, separate *B. cockerelli* colonies were established on all five hosts, including SLN in 60 x 60 x 60 cm BugDorm insect cages (catalog # BD2120F, MegaView Science Co, Taichung, Taiwan) for several generations in an insectary maintained at 25-27˚C, 65-70% RH, and a photoperiod of 16:8 (L:D) h. *B. cockerelli* adults were tested once every three months for Lso using conventional PCR. DNA extractions and PCR were performed according to Buchman et al. (2011) with modifications. Presence of Lso was detected using the primer pair OA2/OI2C and DNA quality was confirmed using BC 28S F/R primers. During the study, Lso infection rate in adult psyllids was 90-100% and the sex ratio was ~0.42 females to 0.58 males.

Lso-free B. cockerelli. Bactericera cockerelli adults originally collected and isolated from Texas fields were provided courtesy of Dr. Joseph Munyaneza (USDA-ARS, Wapato, WA) and were confirmed to be Lso-free via PCR testing. The Lso-free adults were maintained on potato inside BugDorm cages under conditions identical to the Lso-infected colony, but in an insectary separated from the Lso-infected colony room. Lso-free *B. cockerelli c*olonies were periodically tested for Lso using conventional PCR and found to be 100% free of Lso.

Lso transmission to host plants. 3-4 week-old potato, tomato, eggplant, pepper, and SLN plants were selected for the study. Seven *B. cockerelli* adults from Lso-infected colony were restrained within white organza pouches (3" x 4" organza draw string pouches, handmade supplied from various sources) and tied to a lower tier leaf on 12 plants of each of the five host species (Fig. 1). One pouch containing seven psyllids was tied to each plant. One week after release the entire leaf and bag containing adults were removed. Three weeks later the plants were tested for the presence of Lso via PCR using DNA extracted from leaf midrib samples F/R primer pair was indicative of plant samples being Lso-positive and were used in the study (Fig. 2).

Experimental setup

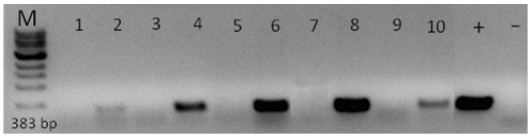
Settling behavior of B. cockerelli on Lso-infected and Lso-free solanaceous hosts. Lso-infected and Lso-free potato (1, 2, and 3 weeks after Lso-inoculation), tomato (3 and 6 weeks after Lso- inoculation), pepper (3 and 6 weeks after Lso- inoculation), eggplant (3 and 6 weeks after Lso- inoculation), and SLN plants (4 weeks after Lso- inoculation) of uniform size were arranged into the following pairs.

Pair 1: neither plant infected (Lso-free);
Pair 2: reciprocal pairings of one infected and one Lso-free plant;
Pair 3: both plants Lso-infected.

All three pairs were replicated four times for each experiment. A set of ten experiments were conducted with Lso-infected *B. cockerelli* and ten with Lso-free adults. Each plant pair was placed in individual BugDorm insect rearing cage (cage size 30 x 30 x 30 cm, BioQuip Products, Rancho Dominguez, CA). Thirty Lso-positive *B. cockerelli* adults were aspirated into pipette tips, the opening plugged with cotton, and were starved overnight prior to release in cages. The tips were then placed inside each cage from a mid-point facing upwards, and the cotton plug was removed to allow adults to disperse. Settling response of *B. cockerelli* was assessed at 1, 4, 8, 24, 48 and 72 h after release. All experiments were conducted at 26-28 ˚C, 65-70 % RH, and a photoperiod 16:8 (L:D) h. Each pair of plants within a cage constituted an experimental unit. Differences in number of psyllids that settled on each of the two hosts in a pair were analyzed based on repeated measures ANOVA for the six time points using SAS PROC MIXED procedure with replication and cage nested within replication as random factors. The Pr>|t| values obtained were unadjusted and Bonferroni correction was applied. Accordingly, they were compared against P=0.0167 (0.05/3) for the 15 comparisons tested.

Figure 1. (Left) Inoculation of plants with Lso using Lso-infected *B. cockerelli* adults restrained within white organza pouches.

Figure 2. (Below) Plant DNA tested for Lso using TX 1623F/R primers.
1)Lso-free potato 2)Lso-infected potato 3) Lso-free tomato 4)Lso-infected tomato 5) Lso-free eggplant 6) Lso-infected eggplant 7) Lso-free pepper 8) Lso-infected pepper 9)Lso-free SLN 10) Lso-infected SLN, "+" indicates known Lso-infected plant sample, "- " indicates no-template DNA (water sample). M is a 1kb DN.A ladder



Results

Settling behavior of B. cockerelli on Lso-infected vs. Lso-free hosts and host plant symptomatology. Results of the 20 experiments are summarized in Table 1.

A. Potato- one, two and three weeks after Lso-inoculation. Lso-infected potato plants exhibited symptoms of wilting three to four weeks after Lso-inoculation. There was no settling preference of Lso-infected and Lso-free *B. cockerelli* adults for Lso-infected or Lso-free hosts but settled uniformly.

B. Tomato- three and six weeks after Lso-inoculation. Lso-infected tomato plants did not show any visible symptoms until four weeks after inoculation. Starting fifth week onwards leaves started to disfigure. Six to seven weeks later, symptoms of yellowing and intermittent browning were evident with gradual wilting of plants. Lso-infected *B. cockerelli* preferred Lso-free tomato, with significant differences at 1, 48 and 72 h (P=0.0062, 0.0001, 0.0004, respectively). Lso-free *B. cockerelli* did not exhibit preference for Lso-infected or –free tomato.

C. Pepper- three and six weeks after Lso-inoculation. Lso-infected pepper plants did not exhibit visible symptoms up to five weeks beyond which leaves started to disfigure. Symptoms of intermittent yellowing and wilting were observed two weeks later. *Bactericera cockerelli* did not make a choice but settled uniformly, and no significant differences were observed using Lso-infected and -free adults.

D. Eggplant- three and six weeks after Lso-inoculation. There was no symptom on eggplants up to six weeks after Lso inoculation beyond which plants started to shrink in size. Lso-infected *B. cockerelli* adults settled uniformly on similar host pairs (infected vs. infected, Lso-free vs. Lso-free) and were not significantly different from each other. However, six weeks after Lso-infection, Lso-free eggplant in the infected vs. Lso-free pair had significantly more Lso-infected *B. cockerelli* at 4, 24, and 72 h (P= 0.0104, 0.0076, and 0.0166 respectively). No significant differences were observed in any of the comparisons using Lso-free *B. cockerelli*, and they settled equally on both plants in all three pairs.

E. SLN-four weeks after Lso-inoculation. SLN did not exhibit visual symptoms four to five weeks after Lso inoculation. Beyond five weeks plants dropped their leaves but sprouted later. There was no preference for Lso-infected or Lso-free SLN by Lso-infected or Lso-free *B. cockerelli* during any of the time points tested as indicated by non-significant P-values (P>0.0167) (Table 1).

Discussion

Elucidating interactions between insect vectors, plant pathogens, and host plants is important to fully understand the epidemiology of vector-transmitted plant diseases. Settling behavior studies designed to evaluate preference in the laboratory revealed that *B. cockerelli* adults moved to Lso-free plants and in most cases preferred both Lso-infected and Lso-free hosts. According to Thinakaran (2014), plant size significantly contributed to a shift in *B. cockerelli* preference when selecting host plants for settling and subsequent feeding and/or oviposition. Although it is unknown at this time if *B. cockerelli* adults use visual and/or olfactory cues to select their preferred hosts it appears that these cues could vary with the length of time a plant has been infected. Mann et al. (2012) showed that citrus plants infected by *Candidatus* Liberibacter asiaticus', developed yellow shoots and lost their turgor and were no longer preferred by Asian citrus psyllid (ACP), *Diaphorina citri.* Thus color and volatile compounds emitted by young shoots played an important role in guiding ACP to locate the host plants. Even a visual stimulus such as a yellow sticky card can be equally attractive to *B. cockerelli* in the presence or absence of olfactory cues. According to Davis et al. (2012) *B. cockerelli* were attracted to Lso-infected potato seven days after being challenged by the pathogen in response to volatiles from the infected plant but moved to Lso-free potato within a week. Although this behavior of *B. cockerelli* adults may positively influence ZC disease progression under field conditions, chemical reception in insects may be governed by several other ecological factors such as arrival (long distance) and movement (short distance) of adults, crop infectivity status, Lso-infection of arriving adults etc. Contrary to our hypothesis and reports in the literature, *B. cockerelli* settled uniformly on Lso-free and -infected hosts and at certain time points they preferred Lso-free plants. The disagreement between published reports and the present study suggests that several factors other than plant volatiles may guide *B. cockerelli* to select their preferred hosts. Y-tube olfactometer studies and experiments under dark conditions will help tease out and elucidate important differences between olfactory and visual responses.

References

Buchman, J. L., Sengoda, V. G., and Munyaneza, J. E. 2011. Vector transmission efficiency of Liberibacter by *Bactericera cockerelli* (Hemiptera: Triozidae) in zebra chip potato disease: effects of psyllid life stage and inoculation access period. J. Econ. Entomol. 104: 1486-1495.

Davis, T. S., Horton, D. R., Munyaneza, J. E., and Landolt, P. J. 2012. Experimental infection of plants with an herbivore-associated bacterial endosymbiont influences herbivore host selection behavior. PLoS ONE 7: 1-8.

Mann, R. S., Ali, J. G., Hermann, S. L., Tiwari, S., Pelz-Stelinski, K. S., Alborn, H. T., Stelinski, L.T. 2012. Induced release of a plant-defense volatile 'deceptively' attracts insect vectors to plants infected with a bacterial pathogen. PLoS ONE 8: 1-13.

Murphy, A. F., Cating, R. A., Goyer, A. Hamm, P.B., and Rondon, S. I. 2014. First report of natural infection by '*Candidatus* Liberibacter solanacearum' in bittersweet nightshade (*Solanum dulcamara*) in the Columbia basin of Eastern Oregon. Pl. Dis. 98: 1425.

Thinakaran, J. 2014. Evaluation of potato psyllid, *Bactericera Cockerelli* (Šulc) (Hemiptera: Triozidae), host preferences, adaptation, behavior, and transmission of '*Candidatus* Liberibacter solanacearum' among wild and cultivated solanaceous hosts in the Lower Rio Grande Valley of Texas. Ph.D. Dissertation submitted to Texas A&M University, College Station, TX 77843, USA.

Thinakaran, J., Pierson, E., Kunta, M., Munyaneza, J. E., Rush, C., and Henne, D. 2015. Silverleaf nightshade (*Solanum elaeagnifolium*), a reservoir host for '*Candidatus* Liberibacter solanacearum', the putative causal agent of zebra chip disease of potato. Pl. Dis. (in press).

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Table 1. Settling response of Lso-infected and Lso-free *B. cockerelli* adults on Lso-infected and Lso-free plants. The 'estimate' column is the actual difference in the mean number of psyllids that settled on the two hosts in each paired comparison. A positive value indicates a greater number of psyllids on host 1 compared to host 2 and vice versa. The presented P-values were pooled across six time points and mean of four replications. The P-values are unadjusted and compared against P=0.0167 (0.05/3) to account for Bonferroni correction.

	Lso-infected *B. cockerelli*				Lso-free *B. cockerelli*		
Experiment	**Estimate**	**t Value**	**Pr > |t|**	**Experiment**	**Estimate**	**t Value**	**Pr > |t|**
1. Potato 1week after Lso inoculation				11. Potato 1week after Lso inoculation			
Lso-free vs. infected	8.2500	2.64	0.0298	Lso-free vs. infected	-5.3750	-1.68	0.1246
2. Potato 2weeks after Lso inoculation				12. Potato 2weeks after Lso inoculation			
Lso-free vs. infected	4.4583	1.22	0.2516	Lso-free vs. infected	9.6667	2.37	0.0422
3. Potato 3weeks after Lso inoculation				13. Potato 3weeks after Lso inoculation			
Lso-free vs. infected	15.6250	2.55	0.0291	Lso-free vs. infected	-1.2500	-0.53	0.6077
4. Tomato 3weeks after Lso inoculation				14. Tomato 3weeks after Lso inoculation			
Lso-free vs. infected	4.6250	2.73	0.0185	Lso-free vs. infected	-6.5000	-2.29	0.0576
5. Tomato 6weeks after Lso inoculation				15. Tomato 6weeks after Lso inoculation			
Lso-free vs. infected	2.5000	0.85	0.4117	Lso-free vs. infected	0.5417	0.27	0.7954
6. Pepper 3weeks after Lso inoculation				16. Pepper 3weeks after Lso inoculation			
Lso-free vs. infected	-1.0417	-0.52	0.6150	Lso-free vs. infected	-5.5833	-1.60	0.1548
7. Pepper 6weeks after Lso inoculation				17. Pepper 6weeks after Lso inoculation			
Lso-free vs. infected	7.1250	2.03	0.0692	Lso-free vs. infected	5.0833	2.28	0.0487
8. Eggplant 3weeks after Lso inoculation				18. Eggplant 3weeks after Lso inoculation			
Lso-free vs. infected	0.2917	0.09	0.9320	Lso-free vs. infected	-0.7917	-0.25	0.8091
9. Eggplant 6weeks after Lso inoculation				19. Eggplant 6weeks after Lso inoculation			
Lso-free vs. infected	7.7917	2.80	0.0206	Lso-free vs. infected	-2.5417	-1.06	0.3178
10. SLN 4weeks after Lso inoculation				20. SLN 4weeks after Lso inoculation			
Lso-free vs. infected	1.0417	0.32	0.7588	Lso-free vs. infected	-0.1667	-0.06	0.9507

Transcriptomic Analysis of Two Potato Cultivars in Response to '*Candidatus* Liberibacter Solanacerum' Infection

Levy, Julien [1], Mendoza, Azucena [3], Ravindran, Aravind [2], Miller, J. Creighton [1], Scheuring, Douglas [1], Gross, Dennis [2], Tamborindeguy, Cecilia [3] and Pierson, Elizabeth [1]
[1]Department of Horticultural Sciences, [2]Department Plant Pathology and Microbiology, and
[3]Department of Entomology, Texas A&M University, College Station, TX77843, USA

Abstract
In our previous studies we showed that both in the field and in the laboratory NY138 and Atlantic responded differently to '*Candidatus* Liberibacter solanacearum' (Lso) infection [1]. To understand the molecular mechanism involved in both varieties during this interaction we initiated a transcriptomic analysis of both varieties infested with psyllids harboring Lso or without Lso.

Introduction
Zebra chip (ZC) is an economically important disease of potato that first appeared in Texas in 2000 and has become a major threat throughout potato producing regions of the US and other countries [2]. The best long-term solution for the management of ZC is the development of disease resistant cultivars. However, *breeding for disease resistance has been hampered by a lack of understanding of the molecular dialog established between the plant and the microbe during the infection process.* Nevertheless, over the past seven years more than 1000 potato cultivars have been screened for ZC resistance in Texas [3] and [4] and presently ~10 genotypes have been selected as being ZC tolerant based on repeated field performance [[3, 4] and ongoing]. Furthermore, data from laboratory trials controlling field variables (including insect pressure) demonstrate that certain potato selections/cultivars are ZC-tolerant (such as NY138), i.e., develop disease symptoms leading to plant death at a slower rate than susceptible cultivars (such as Atlantic or Russet Norkotah) [1]. The availability of these genotypes selected for ZC tolerance based on field performance provides an excellent opportunity to move the breeding program to the next level: discovering the molecular mechanisms involved during the Lso infection and colonization.

Stress resistant and susceptible genotypes respond to pathogen infection and other stresses by changes in gene expression of specific genes or specific pathways [5]. Transcriptomic approaches such as RNAseq are powerful tools for identifying genes or gene networks activated in response to specific stresses [5, 6]. For example, RNA-seq analyses of Lso populations residing within its vector *Bactericera cockerelli* [7], as well as populations of *B. cockerelli* with and without Lso have been used to investigate the molecular dialog between Lso and its vector [8]. To date, no such studies have investigated gene expression patterns of Lso within potato, or potato response to Lso infection. However, previous transcriptomic analysis of plant response to infection by '*Ca*. Liberibacter asiaticus' revealed differential regulation of several pathways such as carbohydrate metabolism and cell wall biogenesis and several genes involved in resistance and responses to pathogens, which constitute ideal targets for disease resistance [9]. Of interest, one of the most highly upregulated genes in citrus has high sequence homology to potato genes involved in defense against other important pathogens. The long-term goal is to use improved understanding of the genetic mechanisms of interaction between Lso and potato to guide breeding strategies. The goal of this study is to fulfill the two following objectives:

Objective 1: Identify genes specifically induced by Lso by comparing plant gene expression patterns in Lso-infected and uninfected plants.

Objective 2: Characterize gene expression patterns in the susceptible cultivar Atlantic compared to a tolerant cultivar Waneta (NY138).

Material and Methods

Growth chamber experiments

We utilized a transcriptomic approach to analyze plant response to Lso at the genomic level focusing on early time points after infection. We employed a 2-factorial design approach that included susceptible (Atlantic) and tolerant (NY138) potato selections, with and without Lso infection. Insects used for Lso infection were obtained from a colony harboring both the LsoA and LsoB haplotypes (Lso^+) [10]. Insects obtained from a colony that does not harbor Lso were used for the no-Lso treatment (Lso^-) [10]. Prior to using the Lso^- insects and Lso^+ insects, the populations were tested for the presence of Lso using polymerase chain reaction (PCR).

Briefly, eight seed pieces from cultivars Atlantic and NY138 were planted individually in pots containing sterilized potting soil and grown in a growth chamber with constant temperature (24 °C) and light cycle (16:8 hour day:night) for four weeks to bud development. Then, four of the eight plants from each cultivar were infested each with three Lso^+ insects. The remaining four plants from each cultivar were infested each with three Lso^-. Insects were maintained in clip cages placed on a single leaf located near the top of the plant for an insect acquisition period of 7days and then removed with the leaf. Additional leaves (one leaf from among the upper most leaves) were removed for sampling 3 and 7 weeks post infestation (wpi). These dates correspond with the times symptoms are typically first observed and the Lso is detectable in the plant, and when there is the greatest difference in symptomology between tolerant and susceptible cultivars [11]. All leaf samples collected at each time point were flash frozen in liquid nitrogen and stored immediately at -80 °C until the end of the experiment. Plants were examined throughout the experiment to assess ZC symptoms development. At the end of the experiment (7-8 weeks wpi) leaf and tuber DNA was extracted from all plants and tested for the presence of Lso using PCR. Tuber slices were fried to assess tuber symptom development [4].

Lso detection using PCR

DNA extraction and PCR detection were performed following methods currently used in our laboratory and previously published, for plant tissue [12, 13] and for insect tissue [14].

Transcriptomic Analyses

Total RNA from 100mg of plant tissue leaf tissue (including the central vein) from twelve plants samples was isolated: e.g. 3 biological replicates from each cultivars (Atlantic vs NY138) x Lso treatment (Lso^+ vs Lso^-). To date we have only isolated RNA from samples collected three weeks post infestation; the sample collected at 7 wpi may be analyzed at a later date, if appropriate. Total RNA was isolated using a Qiagen RNeasy Plant kit. Samples were treated with DNase to remove genomic DNA contamination, and RNA quantification and quality assurance was performed using a Bioanalyzer (Agilent Technologies). The construction of the cDNA libraries and RNA sequencing were performed by the Texas A&M AgriLife Genomics & Bioinformatics Services. One lane of the Illumina Solexa Hiseq 2000 was used for the 12 samples with the sequencing format of 130 bp single read and eight fold barcoding (for multiplexing).

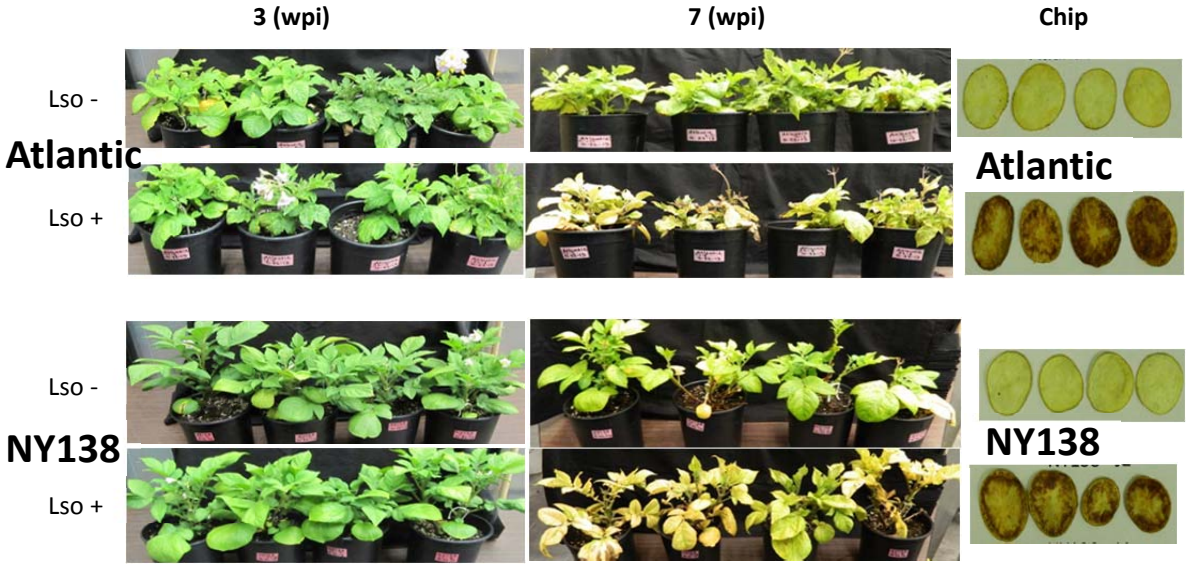
Assembly of the RNA sequences was performed using programs in the iplantcollaborative.org RNAseq workflow to create a unigene set and then compared to the potato reference genome: http://solgenomics .net/organism/Solanum_tuberosum/genome. The data analysis pipeline includes the following steps and software: 1) quality analysis and removal of barcode tags using FastQC; 2) alignment of transcripts to the potato reference genome using TopHat; 3) creation of the final transcriptome assembly using Cufflinks. All analyses will be performed independently for each sample and time point [8]. To identify transcripts that are differentially expressed in Lso-infected and Lso-uninfected libraries of each cultivar, each library will be mapped to the created unigene set, and the expression score of each gene transcript will be represented in FPKM (Fragments Per Kilobase Of Exon

Per Million Fragments Mapped) using the Cufflinks and Cuffdiff softwares. Gene clusters exhibiting differential expression between samples will be subject to further pathway analysis, Functional annotation of the unigene set will be performed using blast2go based on GO annotation.

Result and Discussion

Growth Chamber Experiments

Symptoms of Zebra chip disease were apparent in all plants (both cultivars) at 7 weeks post infestation (wpi) following infestation by insects harboring LsoA and LsoB (Lso^+ treatment) (Fig. 1). Symptoms included chlorosis and reduced growth as well as darkening of the tubers upon frying [11]. In contrast, no Zebra chip symptoms were observed following infestation by insects not harboring Lso (Lso^- treatment). PCR analysis of leaf tissue 7 weeks post infestation revealed Lso was present in all Atlantic plants infested with the Lso^+ insects, confirming that all plants were suitable for the transcriptomic analysis. However Lso was detected only in the leaf samples of plants #5 and #6 and only in the tuber samples of plants #6 and #7, indicating that only these three replicates were suitable for the transcriptomic analyses. Based on these data, three Atlantic samples were randomly selected and only the three Lso positive NY138 samples were selected for subsequent analysis. As expected, Lso was not detected in any of the plants infested with the Lso^- insects and so three samples from each cultivar were randomly selected for subsequent transcriptomic analysis.



<u>Figure 1:</u> Leaf & tuber symptoms 3 and 7 weeks post infection. Symptom development at 3 and 7 weeks post infection (wpi) for Atlantic (top) and NY138 (bottom) plants are shown. Lso^- and Lso^+ indicate that plants were infested with psyllids harboring no detectable Lso or harboring both LsoA and LsoB, respectively. Tuber slices obtained at 7 wpi were fried and observed for darkening of the medullary rays associated with Lso infection.

Transcriptomic Analyses

The RNA sequencing yielded approximately 17 million reads per sample. This number of reads represents almost 3 times the coverage of the potato genome (844 Mb). Currently all data analyses of all comparisons (cultivars Atlantic vs NY138 / Lso^+ vs Lso^-) are in progress although the first alignments have been completed. The preliminary analyses have revealed a number of interesting findings.

A majority of the sequences match the published potato reference genome

A pie chart summarizing results of alignment of Illumina RNA-seq reads to the potato reference genome sequence (topHat2) using the Atlantic – Lso^- sample (total read = 16856327) is shown in figure 4. The purple and blues portion of the chart represents RNA-seq reads that failed quality checks and were filtered out of our data set (13.8% of all RNA-seq reads). The remaining 86.2% of RNA-seq reads passed quality checks. The red portion of the chart represents reads that mapped to the potato genome sequence (60% of all RNA-seq reads). The green portion of the chart represents reads that failed to map to the potato genome sequence (26%).

Differential Gene Regulation

Although our analysis is preliminary, in Atlantic we identified 463 transcripts that were significantly differentially regulated in $Lso^+$and Lso^- treatments. In NY138, we identified 1004 that were significantly differentially regulated in Lso^+ and Lso^- treatments. Among those transcripts 146 that are differentially regulated in both varieties, 14 are up regulated when treated with Lso^+ insects, 63 are down regulated. Therefore 77 genes respond in the same way to Lso in both varieties. There are 69 more genes that are differentially regulated in both varieties, but these do not show the same pattern of response. Interestingly, there is no commonality in the majority of genes regulated by Lso in NY138 or Atlantic; suggesting large differences in plant response to Lso between cultivars.

First look at plant functions that are differentially regulated in Lso^+ and Lso^- treatments:

Among all of the genes that are differentially regulated, some have interesting functions in plant physiology or plant defenses. For example:

- A large number of genes differentially regulated when infected with Lso, are linked to *chlorophyll synthesis* (13 genes in NY138) or *cytochrome P450* (8 genes in NY138). There are 18 genes encoding *ATP binding protein* that are down-regulated in NY138 Lso^+. This is an expected finding given that the plant is under stress and performing poorly. The plant is using more resources for the defense than for producing energy and harvesting light. This also is consistent with increasing chlorosis as the disease progresses. Although at 3 weeks post infection none of the aerial symptoms can be observed.
- Another set of differentially regulated genes is involved in responses to plant hormones. The regulation of those genes is probably due to the advancement of the disease which jeopardizes the plant basic function, or to the induction or deregulation of plants defense. Some examples are:
  - 3 genes coding for *auxin regulated protein* were down-regulated in NY138 and of the 6 *SAUR* (*SMALL AUXIN UP RNA):* 4 were up-regulated in NY138 and 2 were down-regulated in NY138. The SAUR family is the largest family of auxin-responsive genes. Auxin is an important plant development hormone that has been shown to be involved in plant defenses.
  - 5 genes coding for *Brassinosteroid insensitive 1-receptor kinase 1* were down-regulated in NY138. This group is known to control the expression of genes associated with innate immunity.
  - 10 genes coding for *ethylene response protein* were down-regulated in NY138. Ethylene is a plant hormone involved in development (in particular ripening or senescence) that has also been characterized as a stress hormone. It functions as a signal for plants to activate defense mechanisms against invading pathogens, in this case against Lso.
- Interestingly, some genes involved in plant defense such as the *Avr9/Cf-9 rapidly elicited proteins* (19 genes from this family in NY138) were regulated by Lso infection. These proteins function in the initial development of the defense response. In NY138 most of the genes of this family were down regulated (17/18) suggesting that the Lso might be able to hijack plant defense mechanisms.

- Similarly, a couple of genes involved in cellular signaling such as *calmodulin* (13 genes in NY138) and *calcium regulated protein* (20 genes in NY138) were identified. This category of genes can play a role in the early detection of the pathogens. Because both of these groups play key roles in the development of the ZC disease they are good candidates for further characterization of the plant response. Focus on these signaling pathways will help us narrow the focus to genes that differentially control the development of the disease symptoms in each cultivar.

Future work: To connect the relationships between the potentially interesting candidate genes, future work will look at the regulation of entire functional pathways using software such as Blast2Go (BioBam Bioinformatics) or The Ontologizer (Institute for Medical Genetics and Human Genetics), tools for statistical analysis and visualization of data using gene ontology. These analyses will allow us to identify better targets from whole pathways that are altered in response to Lso infection. Our next steps also will include the verification of these transcriptome results using real-time quantitative PCR as an independent measure of transcript abundance.

Acknowledgements

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References

Aritua, V., et al., Transcriptional and Microscopic Analyses of Citrus Stem and Root Responses to Candidatus Liberibacter asiaticus Infection. PLoS One, 2013. 8(9): p. e73742.

Crosslin, J.M., et al., Potato zebra chip disease: A phytopathological tale. Plant Health Progress, 2010: p. doi:10.1094/PHP-2010-0317-01-RV.

Gyetvai, G., et al., The Transcriptome of Compatible and Incompatible Interactions of Potato Solanum tuberosum) with Phytophthora infestansRevealed by DeepSAGE Analysis. PLoS ONE, 2012. 7(2)

Ibanez, F., J. Levy, and C. Tamborindeguy, Transcriptome analysis of 'Candidatus Liberibacter solanacearum' in its psyllid vector, Bactericera cockerelli. PLoS One, 2014. 9(7): p. e100955.

Levy, J.G., et al., 'Candidatus Liberibacter solanacearum' translocation and quantification in potato and tomato. Phytopathology, 2011. 102(11): p. 4-5.

Levy, J., et al., Investigations on Putative ZC Tolerant Potato Selections. Proceedings of the Zebra Chip Conference, San Antonio, TX, , 2012. October 30 - November 2, 2012.

Levy, J., et al., Translocation of 'Candidatus Liberibacter solanacearum', the Zebra Chip pathogen, in potato and tomato. Phytopathology, 2011. 101(11): p. 1285-91.

Levy, J., et al., Methods for rapid and effective PCR-based detection of 'Candidatus Liberibacter solanacearum' from the insect vector Bactericera cockerelli: streamlining the DNA extraction/purification process. J Econ Entomol, 2013. 106(3): p. 1440-5.

Massa, A., et al., The Transcriptome of the Reference Potato Genome Solanum tuberosum Group Phureja Clone DM1-3 516R44. PLoS ONE, 2011. 6(10).

Nachappa, P., J. Levy, and C. Tamborindeguy, Transcriptome analyses of Bactericera cockerelli adults following 'Candidatus Liberibacter solanacearum' infection. MGG, 2012. 287: p. 803-817.

Nachappa, P., et al., Correlation between 'Candidatus Liberibacter solanacearum' infection levels and fecundity in its psyllid vector. J Invertebr Pathol, 2014. 115: p. 55-61.

Ravindran, A.R., Aravind)[1] ; Levy, J (Levy, Julien)[2] ; Pierson, E (Pierson, Elizabeth)[2] ; Gross, DC (Gross, Dennis C.)[1], Development of Primers for Improved PCR Detection of the Potato Zebra Chip Pathogen, 'Candidatus Liberibacter solanacearum'. PLANT DISEASE, 2011. 95(12): p. 5.

Ravindran, A., et al., Development of Primers for Improved PCR Detection of the Potato Zebra Chip Pathogen, 'Candidatus Liberibacter solanacearum'. PLANT DISEASE, 2011. 95(12): p. 5.

Scheuring, D., et al., ZC Expression in Several Caged Potato Populations Following Infestation with the Potato Psyllid Proceedings of the 13th annual Zebra Chip Reporting Session, San Antonio, TX, 2013.

RNA Interference: Potato/Tomato Psyllid, *Bactericera cockerelli,* Oral Delivery of Double – Stranded RNAi Constructs

Sharma, Bijaya K. [1], Hail, Daymon [1], Hunter, Wayne [2] and Bextine, Blake [1]
1 The University of Texas at Tyler, Department of Biology, 3900 University Blvd., Tyler TX 757992
USDA ARS, Fort Peirce**,** Fl

Abstract
Potato psyllids, *Bactericera cockerelli*, are economically important pests of the potato, tomato and other solanaceous crops. This insect is the primary vector of the phytopathogenic bacterium '*Candidatus* Liberibacter solanacearum' which causes zebra chip of potato. This disease has caused millions of dollars in losses to the potato industry. *B. cockerelli* is primarily managed by the application of chemical insecticides but even with chemical pesticides it is difficult to manage. Additionally, the risk of insects developing resistance to certain insecticides is a real concern. Management of this pest by down-regulation of endogenous mRNA using RNAi technology is an environmentally friendly and species specific approach to pest management. In this study, a feeding assay has been developed for the oral delivery of dsRNA (RNAi constructs). Down-regulation of α-tubulin was attempted by introducing novel double stranded RNAi constructs (dsRNA). The introduction of dsRNA caused significant mortality of the psyllids relative to control psyllids and the down - regulation of targeted gene was monitored by RTPCR.

Introduction
Potato psyllids, *Bactericera cockerelli*, are responsible for the transmission of a proteobacterium, '*Candidatus* Liberibacter solanacearum' (CLs) to various solanaceous crops (Senogoda et al., 2010). The potato psyllid is associated with psyllid yellows and zebra chip disease, the later becoming a major threat to US potato production. Current management of this pest by application of chemical pesticides has various limitations. Specific insecticides should be used for management of psyllids based on the life stage being targeted. Some insecticides cause significant mortality in adults might not necessarily be as affective on nymphs or eggs (Goolsby et al. 2007; Zens et al. 2009). Another risk of insects developing resistance to chemical insecticides is still a thoughtful question. Liu and Trumble (2007) found the populations of psyllids from California were resistance to imidacloprid and spinosad compared to the psyllids from central USA.

Sequence specific gene silencing in insects can be achieved by feeding double stranded RNA through RNA interference (RNAi) technology (Baum et al., 2007). This strategy of controlling insect pests and plant pathogens is gaining attention in the scientific community (Price and Gatehouse 2008). In a 2010 survey of adult and late instar potato psyllid transcriptomes, Hail et. al. (2010) utilized 454 pyrosequencing (Roche) to identify several potential targets for RNA intereference. Wuriyanghan et al. (2011) delivered gene specific dsRNAs or siRNAs targeting to Actin, ATPase, Hsp70 and CLIC through microinjection and/or oral delivery system in teneral adult *B.cockerelli.* Wuriyanghan et al. (2013) also induced gene silencing in *B.cockerelli* that fed on the plants infected with recombinant *Tobacco mosaic virus*.
In the present study, a feeding assay has been developed for the oral delivery of dsRNA (RNAi constructs). Ten adult psyllids were offered sachet that contains artificial diets amended with dsRNA (RNAi constructs), targeting for α-Tubulin and monitored for five days. Down-regulation of targeted genes were achieved over time as monitored through quantitative real time PCR. Several other potential

targets will be chosen for down-regulation and percentage of down-regulation will be quantified using qRT-PCR for the next phase of the experiment.

Materials and Methods

Initially potato psyllid was provided by Texas A&M University. The psyllid colonies were maintained in separate insect cages (36in. X 24in. X 18in.) within insectarium at temperature of 25 C and 70% humidity under 14:10 (light:dark) photoperiod. Four different fluorescent chemicals (Curcumin [1M], Riboflavin [1M], Uranine [1M] and Quinone [1:10/V:V]) and green food coloring [1:10 V:V] were used in feeding assay. Fluorescence microscope (NIKON ECLIPSE Ti series) was used to visualize the psyllids after feeding with fluorescent compounds. The exposure time for all images was auto exposure (AE) 6S (+1.0 EV). Blue, red and green filters were used for imaging.

The target sequence for different genes including three sites in α –tubulin (α–tubulin F2R1, α-tubulin F3R1 and α–tubulin F3R2), β–tubulin, Yellow B, Integrin, Proteasome β4, Disulphide Isomerase, and various ribosomal proteins (ribosomal protein L27Ae, ribosomal protein S7e, ribosomal protein L34, ribosomal protein L7, ribosomal protein L7A and ribosomal protein S8) were identified using the EST sequence information from NCBI database. Geneious software was used to design the primer for in-vitro dsRNA synthesis. The dsRNA was synthesized and purified by using HiScribe T7 *In Vitro* Transcription Kit (New England BioLabs, Ipswich, MA).
Ten adult psyllids were offered sachet that contains artificial diets (15% W: V sucrose solution) amended with dsRNA (RNAi constructs, concentration, 750 ng/µL), targeting for the above mentioned targets and monitored for five days.

Total RNA was isolated using TRIZOL extraction method and run for qRT-PCR using specific primer. All the experimental data after feeding assay and psyllid mortality was visualized by using the statistical software Graph Pad Prism. T-Test was done to test the significance in mortality.

Results and Discussions

Psyllids that fed on artificial diet with fluorescent compounds added showed fluorescence while those fed with water, sucrose and GFC did not, which shows that sachet feeding was successful in delivering dsRNA/RNAi constructs orally.

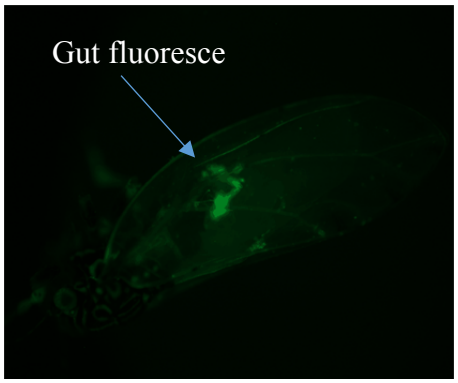


Figure 1. Psyllid feds with curcumin shows fluorescence

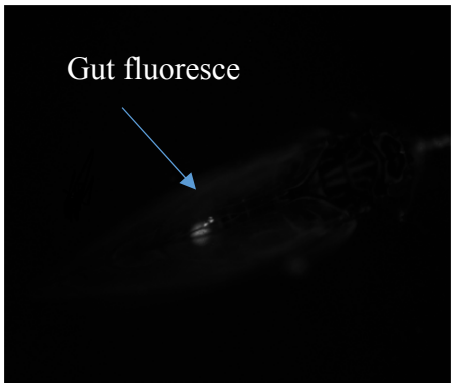


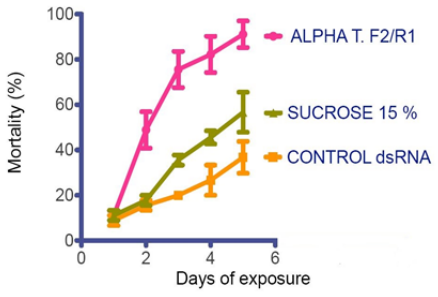
Figure 2. Psyllid feds with quinone shows fluorescence

Figure 3. Psyllid feds with sucrose shows no fluorescence

Figure 4. Sachet apparatus for the oral delivery of dsRNA (RNAi construct)

Among all of the above mentioned targets, the targets for α - tubulin could able to cause the mortality of the psyllid in comparison with the control dsRNA. The increase in Ct value (except that in day 1) for each replicates showed the possible down regulation of the target genes over time. The *P*-value from the t - test (Figure 5B) also shows the significant number of death with dsRNA treatment.

A



B

ALPHA T. F2R1	0.1514515	0.04587	0.520254	0.60953	1
ALPHA T. F3R1	0.249352075	0.047828	0.885985	1	0.609535
ALPHA T. F3R2	0.285081611	0.046384	1	0.88599	0.520254
Control dsRNA	0.237768467	1	0.046384	0.04783	0.04587
Sucrose 15% (W/V)	1	0.237768	0.285082	0.24935	0.151452
	Sucrose 15%	C. dsRNA	A. T. F3R2	A. T. F3R1	A. T. F2R1

Figure 5. (A) Potato psyllid mortality percentage and time response after feeding with dsRNA targets for Alpha-Tubulin, (B) Comparison of *P* values obtained after t-test from the potato psyllid mortality data for all the three different targets of α – tubulin with control dsRNA and artificial diet (Sucrose 15%) at P < 0.05. (Note: the *p* values in red color greater than 0.05 shows the significant mortality due to the application of 3 different sites of α – tubulin).

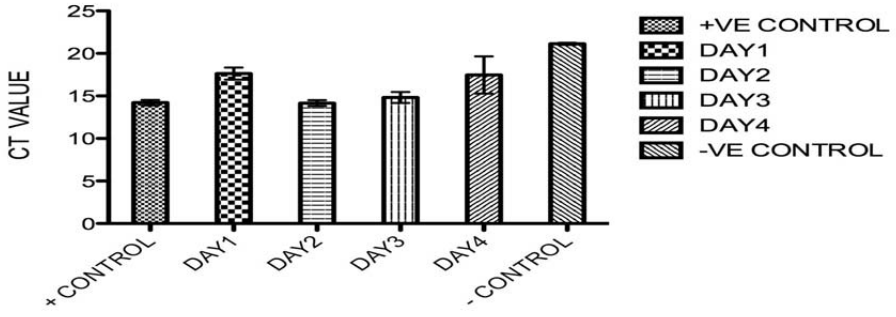


Figure 6. Ct Values obtained after qRT - PCR using specific primer to quantify the down - regulation of target (α – tubulin) over time, with respect to positive control (total RNA from no dsRNA treated psyllids) and negative control (no sample treatment).

For the next phase of the experiment fluorescently labeled dsRNA will be used to document the entry of dsRNA in the hemolymph and to the different organs. Various potential genes will be targeted to down-regulate the specific mRNA by oral delivery of dsRNAs.

Acknowledgements
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References

Baum J.A., T. Bogaert, W. Clinton, G. R. Heck, P. Feldmann. 2007. Control of coleopteran insect pests through RNA interference. *Nat Biotechnol* 25: 1322-1326.

Goolsby, J.A., J. Adamczyk, B. Bextine, D. Lin, J.E. Munyaneza, and G. Bester. 2007. Development of an IPM program for management of the potato psyllid to reduce incidence of zebra chip disorder in potatoes. *Subtropical Plant Science* 59: 85–94.

Hail, D.A., S. Dowd, W. H. Hunter, and B. Bextine. 2010. Investigating the transcriptome of the potato psyllid (*Bactericera cockerelli*) toward an RNAi based management strategy, pp. 183-186. In *Proceedings of the 10th Annual Zebra Chip Reporting Session*, ed. F. Workneh and C.M. Rush. Dallas, TX (November 7 – 10, 2010).

Liu, D., and J. T. Trumble. 2007. Comparative fitness of invasive and native populations of the potato psyllid (*Bactericera cockerelli*). Entomologia Experimentalis et Applicata 123:35-42.

MunyAaneza JE (2012) Zebra Chip Disease of Potato: Biology, Epidemiology and Management. Am. J. Pot Res (2012) 89:329–350. DOI 10.1007/s12230-012-9262-3.

Price, D.R.G., and J.A. Gatehouse. 2008. RNAi-mediated crop protection against insects. *Trends in Biotechnology* 26: 393–400.

Sengoda V, Munyaneza J, Crosslin J, Buchman J, Pappu H (2010) Phenotypic and etiological differences between psyllid yellows and zebra chip diseases of potato. Am J Potato Res 87:41–49.

Wuriyanghan H, Falk BW (2013) RNA Interference towards the Potato Psyllid, *Bactericera cockerelli*, is induced in Plants Infected with Recombinant Tobacco mosaic virus (TMV). PLoS ONE 8(6): e66050.doi: 10.1371/journal.pone.0066050.

Wuriyanghan H, Rosa C, Falk BW (2011) Oral Delivery of Double – Stranded RNAs and siRNAs induces RNAi Effects in the Potato/Tomato Psyllid, *Bactericera cockerelli*. *PLoS ONE* 6(11): e27736. doi:10.1371/journal.pone.0027736.

Wuriyanghan H, Rosa C, Falk BW (2011) Oral Delivery of Double – Stranded RNAs and siRNAs induces RNAi Effects in the Potato/Tomato Psyllid, *Bactericera cockerelli*. *PLoS ONE* 6(11): e27736. doi:10.1371/journal.pone.0027736.

Zens, B., C.M. Rush, D.C. Henne, F. Workneh, E. Bynum, C. Nansen, and N.C. Gudmestad. 2010. Efficacy of seven chemical programs to control potato psyllids in the Texas Panhandle, pp. 83–87. In *Proceedings of the 10th Annual Zebra Chip reporting Session*, ed. F. Workneh and C.M. Rush. Dallas, TX (November 7–10, 2010).

Characterization of the Serralysin Gene Cluster of '*Ca.* Liberibacter solanacearum' and its Relationship to Zebra Chip Development

Ravindran, A[1]., Saenkham, P[2]., Levy, J[2]., Pierson, E[2]., Lin, H[3]., and Gross, D.C[1]. [1]Department of Plant Pathology and Microbiology, and [2]Department of Horticulture, Texas A&M University, College Station, TX, 77843, USA; [3]Department of Agriculture-Agricultural Research Service, CDPG, San Joaquin Valley Agricultural Sciences Center, Parlier, CA, 93648.

Abstract

Zebra chip disease is caused by a non-culturable bacterium '*Candidatus* Liberibacter solanacearum' (Lso). The production of antimicrobial proteins and peptides is one of the major defense strategies utilized by a plant in response to infection by pathogenic organisms. The serralysin protein is hypothesized to modify the plant defense, possibly by degrading host antimicrobial peptides. By computational analysis of the Lso whole genome, we found the presence of a putative serralysin gene sequence that is located next to and divergently transcribed from a type I secretion system (T1SS). These data suggest that the putative serralysin may be secreted to the extracellular space directly through a T1SS where it may act as a virulence factor in the host. Our goal is to characterize the functional activity of the Lso serralysin-like gene, which could be a potential target to control the Lso pathogen in potato. Because Lso is unculturable, we need to use a culturable (surrogate) bacterium for these analyses. To do this, we used serralysin-deficient mutants of *Serratia liquefaciens* FK01, which is an endophytic bacterium associated with insects. The hypothesis to be tested is that the Lso serralysin-like gene encodes an active metalloprotease that can be expressed and secreted by the T1SS of the serralysin-deficient mutants of *S. liquefaciens*.

Introduction

Zebra chip (ZC) is a major disease of potato caused by '*Candidatus* Liberibacter solanacearum' (Lso) and vectored by the potato/tomato psyllid (Munyaneza et al., 2007). The ZC disease affects all market classes of potatoes by reducing yield and quality, and all cultivated potato varieties are susceptible to the disease. Insecticides for control of the Lso psyllid vector are expensive and often ineffective, and factors which contribute to virulence are largely unknown. Thus, successful ZC management is best achieved by identifying the disease related genes from the Lso pathogen. Genomic analyses revealed that the genome size of Lso is ~1.26 Mbp with approximately 1,157 protein-coding genes (Lin et al., 2011). A putative serralysin protein is encoded by CKC_02265 and is located next to a type I secretion system (T1SS) in the Lso genome. Based on sequence similarity to characterized proteins, a serralysin (*ser*) gene is hypothesized to encode a protease that contributes to ZC pathogenesis.

We are studying pathogenicity factors associated with ZC disease of potatoes focusing on analyzing a potential serralysin effector-like protease that could affect plant immune responses (Wang and Trivedi, 2013). Serralysins are bacterial extracellular metalloproteases that show complete conservation of a zinc binding motif (HEXXHXUGUXH), a Met-turn (SXMXY) sequence, and glycine rich repeats (GGXGXD). Maeda and Morihara (1995) described proteases assigned to the serralysin family in Gram-negative bacteria associated with plants, humans, and animals. Serralysin-like proteins typically serve as bacterial virulence factors and are produced by *Serratia* spp., *Pseudomonas* spp., *Dickeya chrysanthemi*, and *Proteus mirabilis*. Serralysins are ~50-kDa proteins that are secreted using glycine rich Gram-negative type secretion signals. Protein translocation occurs through a Type I secretion channel spanning across the entire Gram-negative bacterial cell envelope. Secretion depends

on three specific proteins, namely a polytopic inner membrane protein with a cytoplasmic ATPase domain operating as an ABC exporter, a membrane fusion protein, and an outer membrane protein.

Our aim was to functionally characterize the putative Lso serralysin gene with the goal of determining whether it serves as virulence factor to result in ZC disease symptoms in potato.

Materials and Methods

Analysis of the putative serralysin gene in Lso genome: The Lso genome sequence for the ZC1 strain available in the National Center for Biotechnology Information (NCBI) database was downloaded as a GenBank file for analysis. The comparison of putative serralysin genes in '*Candidatus* Liberibacter' species was done using IMG gene content analysis (Mavromatis et al., 2009) (https://img.jgi.doe.gov/cgi-bin/w/main.cgi). To compare the serralysin gene of *Serratia* spp. with the putative serralysin gene of '*Ca*. Liberibacter' species, the CLUSTAL X Multiple Sequence Alignment program was used. Protein sequences available in the NCBI database from *Serratia marcescens* [GenBank accession WP_004934318], *Serratia liquefaciens ser1* [GenBank accession BAK39731], *Serratia liquefaciens ser2* [GenBank accession BAK39733], '*Ca*. L. solanacearum' (GenBank accession WP_013461860) and '*Ca*. L. asiaticus' [GenBank accession WP_015452346] were downloaded and the sequences aligned with CLUSTAL X.

Expression of putative serralysin gene by qPCR assays: Quantitative real time reverse-transcription PCR (qRT-PCR) analysis was used to determine if the expression of serralysin gene occurred in Lso-infected tomato plants. Total RNA was extracted using an RNeasy® Mini Kit (Qiagen) from leaves at weeks 1 and 3 of two Lso-infected tomato plants; visible symptoms were not yet expressed in the plant. RNA samples were treated with TURBO™ DNase (Ambion®) using the manufacturer's protocol. Total RNA samples were quantified using spectrophotometry (Nano-Drop Technologies, Inc.). Total RNA was converted to double stranded cDNA. The double stranded cDNA was quantified using spectrophotometry and samples were diluted to 10 ng μl^{-1}. qRT-PCR primers were designed for the serralysin-like gene and the *recA* housekeeping genes of Lso. The primers were evaluated using conventional PCR. The following was used for qRT-PCR analysis of each 20 µl reaction mixture: 10 ml SYBR® GreenER™ qPCR SuperMix Universal, 8.16 µl nuclease free water, 0.04 µl ROX reference dye, 0.4 µl forward primer (200 nM final), 0.4 µl reverse primer (200 nM final), and 1 µl template DNA (10 ng μl^{-1}). The reactions were performed using an Applied Biosystems 7500 Fast Real-Time PCR System.

Cloning of the putative Lso serralysin and S. liquefaciens *serralysin genes*: The '*Ca*. L. solanacearum' primer set was designed based on the putative serralysin gene sequence (CKC_02265). The sequences of the forward (Lso-CKC_2265 F NcoI) and reverse (Lso-CKC_2265 R XhoI) primers were amplified for the putative serralysin gene in Lso. Using the primer pair, the Lso *ser* gene was amplified from the Lso infected genomic DNA from psyllid and the PCR product was directly sequenced. The fragments containing complete sequences of *ser2* gene were amplified from genomic DNA of *S. liquefaciens* FK01 by PCR using primer pairs, *ser2*FNcoI/*ser2*RXhoI, respectively (Kaibara et al., 2012). Both the amplified fragments were cloned in pET14b (Novagen [EMD Millipore]) and moved into *E. coli* DH5α. Colony PCR was performed to confirm presence of the insert (gene of interest). The plasmids (pET14b*ser*-Lso & pET14b*ser*-SI) were moved into *E. coli* DE3 and the serralysin-deficient mutant FK04 of *S. liquefaciens*.

Screening for protease activity: Plate assays for *Serratia* protease production were used to detect proteolytic activity on skim milk agar plates, which were incubated at 28°C for five days. Skim milk agar contained 10% skim milk powder (Oxoid), 0.5% peptone and 1.5% agar (autoclaved at 121°C for 5 min). Microorganisms with proteolytic activity were detected by the formation of clear zones around

colonies. Zymography assays used 20 µl of cell culture mixed with a 2x zymogram sample buffer (Bio-Rad), and then incubated at 20°C for 1 h. A 20-µl portion of the denatured samples was then separated on a 10% polyacrylamide gel containing 0.05% casein. Samples were separated by electrophoresis at a 10 mA constant current (maximum voltage was 100 V) at room temperature until the blue tracking dye reached the bottom of the gel. The gel was incubated in a renaturing buffer (2.5% Triton X-100, v/v) for 30 min, repeated a second time, and then incubated in a 10x zymogram developing buffer for 16 h with gentle agitation at 37°C. The gel was stained with Coomassie Brilliant Blue R-250 and then destained.

Results and Discussion

The Lso serralysin gene sequence was identified in the NCBI database and the closest match was with '*Candidatus* Liberibacter asiaticus' (Las) strains gxpsy and psy62. The serralysin genes in Lso strain ZC1 and the Las strain gxpsy are predicted to have 493 and 497 amino acids, respectively. In contrast, the Las strain psy62 serralysin gene is predicted to be much larger with 665 amino acids. In both '*Ca*. Liberibacter' species the Type I secretion genes are located upstream of the serralysin gene. Similarly, the Type I secretion system for all three strains encodes homologs for the ATPase, PrtD, and DctA genes (Fig. 1). The computational analysis of '*Ca*. L. asiaticus' also evidenced a putative serralysin, encoded by CLIBASIA_01345, which is located next to the T1SS locus in the genome (Cong et al., 2012).

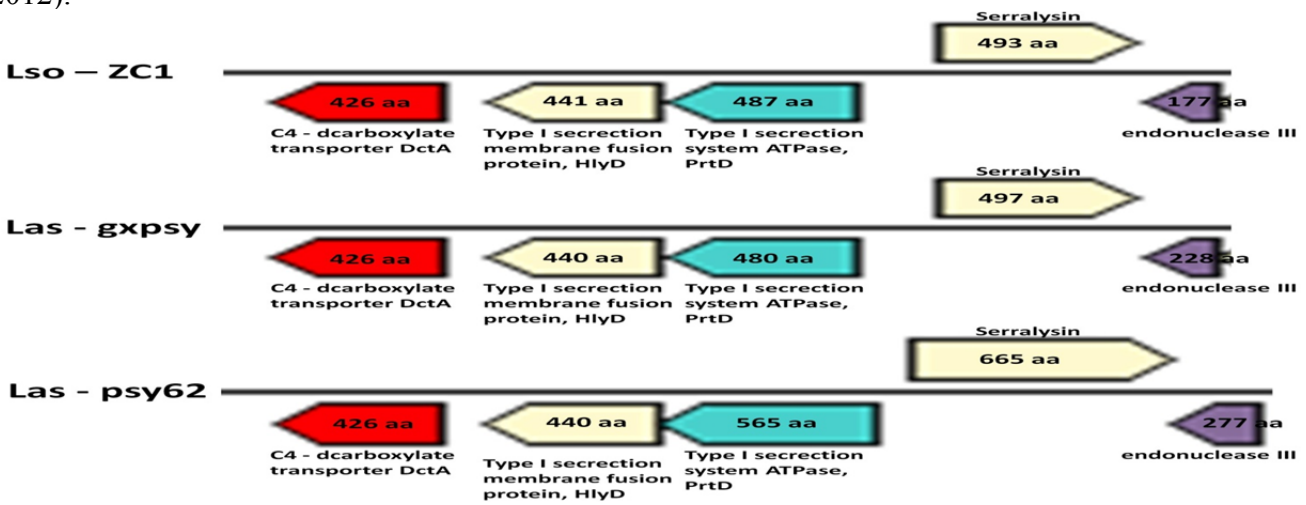

Fig. 1. Serralysin gene cluster of Lso strain ZC1, Las strain gxpsy, and Las strain psy62 were checked to match a particular ortholog neighborhood. Colors indicate ortholog groups and the size of the bar approximately corresponds to gene size. This comparison was done using JGI-IMG/ER (https://img.jgi.doe.gov/cgi-bin/w/main.cgi).

The putative serralysin proteins of '*Ca*. Liberibacter' species were compared to the serralysin sequences in *Serratia* spp. (Kaibara et al., 2012) and the conserved motifs (zinc binding, glycine rich repeats, and ABC exporter) were identified in the predicted serralysin-like metalloproteases. Results indicated the putative serralysin gene of Lso encoded a serralysin-like metalloprotease (Fig. 2).
Studies of the expression of a putative Lso serralysin gene by QPCR: Tomato leaves infected with Lso were collected at week 1 and week 3 and did not exhibit visible disease symptoms. The C_t values were normalized to the *recA* housekeeping gene. The Lso serralysin-like gene expression was 2.5-fold more in leaf samples at week 3 as compared to week 1. Yan et al., (2013) also suggested that the serralysin biosynthesis gene of '*Ca*. L. asiaticus' was 5.5-fold up-regulated in an infected citrus host.

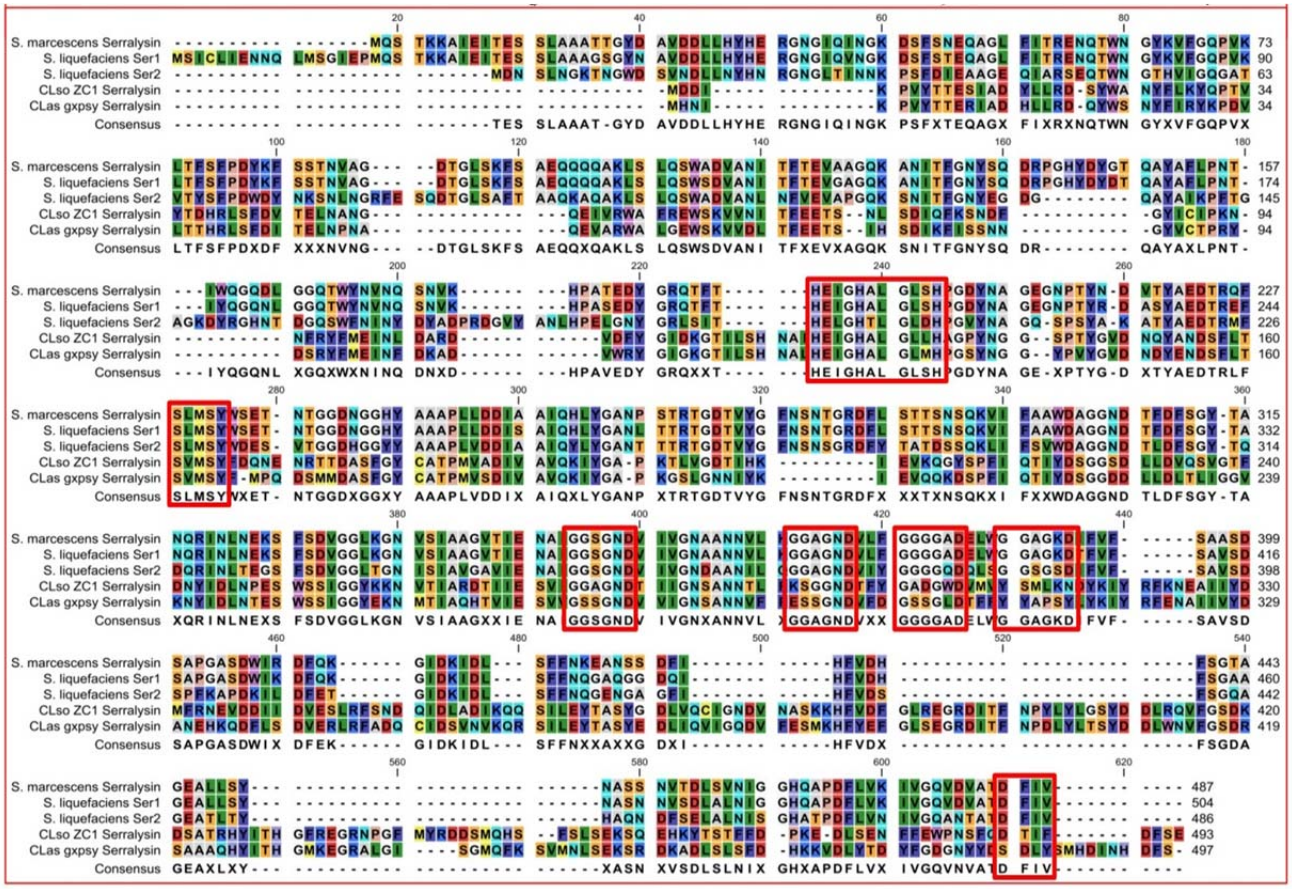

Fig. 2. Multiple alignments of serralysin protein sequences from *Serratia* species and putative serralysin protein sequences from '*Ca*. Liberibacter' species. Serralysins show complete conservation of a zinc binding motif (HEXXHXUGUXH), Met-turn (SXMXY), glycine rich repeats (GGXGXD) and ABC exporter motif (DXXX). Conserved motifs in serralysin-like metalloproteases are shown in the red box.

Studies of the of putative serralysin protein activity in Lso: We used wild type *S. liquefaciens* FK01, and the serralysin-deficient mutant FK04 to study the Lso serralysin-like metalloprotease function (Kaibara et al., 2012). The complete sequences of the *S. liquefaciens* serralysin gene (1979 bp) and the Lso serralysin-like gene (1479 bp) were cloned separately into a broad host range expression vector (pET14b) and moved into the serralysin-deficient mutant FK04 to test for protease activity.

A proteolytic enzyme assay was used to determine if the Lso serralysin-like protein expressed in *S. liquefaciens* exhibited enzymatic activity (Fig. 3). Initial screening was on a skim milk agar medium where protease activity is visible due to formation of a clearing zone around the colonies. The protease activities of *S. liquefaciens* strains also were assayed by casein zymography. The cell culture supernatants were analyzed by electrophoresis on 10% polyacrylamide gels containing 0.05% casein under non-reducing conditions. The proteolysis of the substrate was observed as a clear band against a dark blue background. Kaibara et al. (2012) reported the wild type strain FK01 produced serralysin with proteolytic activity (produces a halo zone on plates and a clear band on gels) whereas the serralysin-deficient mutant FK04 had no proteolytic activity (does not produce a halo zone on plates and no clear band on gels) (Fig. 4). The *S. liquefaciens* serralysin gene completely restored protease activity in the serralysin deficient mutant (FK04/pET14b*ser*-Sl). In contrast, the Lso serralysin-like gene did not restore protease activity to the serralysin-deficient mutant (FK04/pET14b*ser*-Lso) (Fig. 4).

We predict the Lso serralysin metalloprotease cannot be secreted from *S. liquefaciens* due to an incompatible TISS. This hypothesis is based on the observation that the *S. liquefaciens* serralysin cannot be secreted by *E. coli* (DE3/pET14b*ser*-Sl), which has a TISS different from *S. liquefaciens*. Studies are in progress to improve the ability of *E. coli* DE3 and *S. liquefaciens* FK04 to secrete the Lso serralysin and then assay for proteolytic activity.

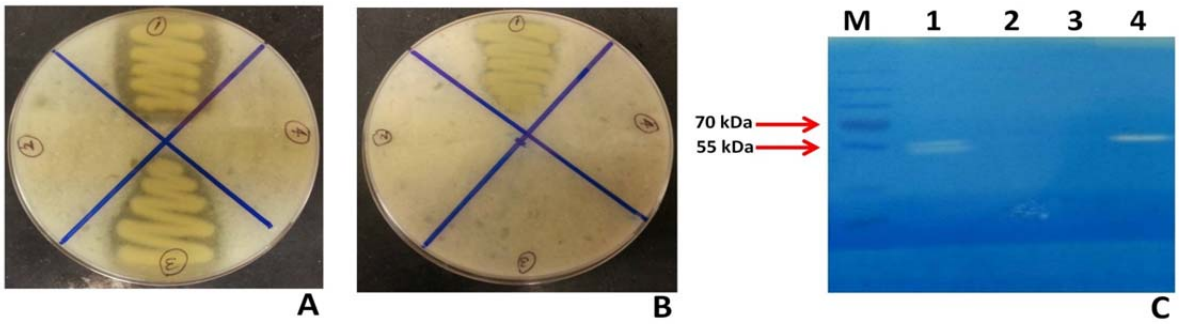

Fig. 3. Proteolytic activity. (A) Skim milk agar plate was incubated at 28°C. 1, FK01; 2, FK04; 3, FK04/pET14b*ser*-Sl; 4, FK04/pET14b*ser*-Lso, and (B) skim milk agar plate was incubated at 37°C. 1, FK01; 2, FK04; 3, DE3/pET14b*ser*-Sl; 4, DE3/pET14b*ser*-Lso. (C) Casein zymography: M, Protein marker; 1, FK01; 2, FK04, 3, FK04/pET14b*ser*-Lso; and 4, FK04/pET14b*ser*-Sl.

Acknowledgements

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References

Cong, Q., Kinch, L.N., Kim, B.H., and Grishin, N.V. 2012. Predictive sequence analysis of the '*Candidatus* Liberibacter asiaticus' proteome. PlosOne 7:e41071.

Lin, H., Lou, B., Glynn, J.M., Doddapaneni, H., Civerolo, E. L., Chen, C., Duan, Y., Zhou, L., and Vahling. C.M. 2011. The complete genome sequence of '*Candidatus* Liberibacter solanacearum', the bacterium associated with potato zebra chip disease. PlosOne 6:e19135.

Kaibara, F., Iiyama, K., Chieda, Y., Lee, J.M., Kusakabe, T., Yasunaga-Aoki, C. and Shimizu, S. 2012. Construction of serralysin-like metalloprotease-deficient mutants of *Serratia liquefaciens* and their virulence in the silkworm, *Bombyx mori*. J. Insect Biotechnol. Sericology 81:55-61.

Maeda, H., and Morihara, K. 1995. Serralysin and related bacterial proteases. Methods Enzymol. 248: 395-413.

Markowitz, V.M., Mavromatis, K., Ivanova, N.N., Chen, I.M., Chu, K., and Kyrpides, N.C. 2009. IMG ER: A system for microbial genome annotation expert review and curation. Bioinformatics 25: 2271-2278.

Munyaneza, J.E., Goolsby, J.A., Crosslin, J.M., and Upton, J.E. 2007. Further evidence that zebra chip potato disease in the Lower Rio Grande Valley of Texas is associated with *Bactericera cockerelli*. Subtropical Plant Sci. 59:30-37.

Wang, N., and Trivedi, P. 2013. Citrus huanglongbing: A newly relevant disease presents unprecedented challenges. Phytopathology 103:652-665.

Yan, Q., Sreedharan, A., Wei, S., Wang, J., Pelz-Stelinski, K., Folimonova, S., and Wang, N. 2013. Global gene expression changes in '*Candidatus* Liberibacter asiaticus' during the transmission in distinct hosts between plant and insect. Mol. Plant Pathol. 14:391-404.

In Planta Maintenance, Enrichment, and Whole Genome Sequence Analyses of '*Candidatus* Liberibacter solanacearum' Strain R1 from California

Frigulti, T.[1], Zheng, Z.[2], Clark, N.[1], Wallis, C. M.[3], Lee, R.[4], Keremane, M.[4], Bushoven, J.[1], and Chen, J.[3]

[1]Department of Plant Sciences, California State University, Fresno, CA; [2]Department of Plant Pathology, South China Agricultural University, Guangzhou, P. R. China; [3]San Joaquin Valley Agricultural Sciences Center, United States Department of Agriculture-Agricultural Research Service, Parlier, CA; [4]National Clonal Germplasm Repository for Citrus and Dates, United States Department of Agriculture-Agricultural Research Service, Riverside, CA

Abstract

'*Candidatus* Liberibacter solanacearum' (CLso) infects solanaceous plants such as potato and tomato. CLso is currently unable to be cultured in artificial media which makes characterization of the bacterium challenging. One solution developed to circumvent the *in vitro* cultivation problem was the use of tomato plants as hosts to maintain CLso strains through grafting. This is named as the Tomato *in planta* Culture System (TIPCS). This study fine-tuned the CLso TIPCS system by testing several tomato cultivars for CLso sensitivity through grafting and monitoring bacterial population dynamics. The CLso strain R1, of California origin, was used in this study. Among the four tomato cultivars (Ace, Bush, Heinz, and Rio) evaluated, Heinz and Rio had a greater number of plants that became infected. Heinz and Rio also were infected significantly ($p<0.05$) quicker. From infected tomato tissue, DNA of CLso Strain R1 was extracted along with host plant DNA and used to determine its whole genome sequence through the next generation sequencing technology. The R1 strain has the genome size of 1,204,257 bp, G+C content of 35.3%, 1,101 predicted open reading frames, and 57 RNA genes. Whole genome sequence comparison indicated that the California R1 strain was significantly different from the other Texas psyllid-based CLso genome, suggesting that a minimum of subspecies level variation existed within the known CLso population. In conclusion, this study investigated and fine-tuned the CLso TIPCS. TIPCS served to provide DNA source for whole genome sequencing of CLso strain R1.

Materials and Methods

In Planta culture

CLso Strain R1, which originated from California, was maintained in tomato (cv. Big Boy) in a greenhouse and used as inoculum source. Seeds from four cultivars, Ace, Bush, Heinz, and Rio, were germinated and grown in potted soil under greenhouse conditions. Young tomato shoots from CLso-infected plants were used for grafting. Healthy tomato shoots were used as control. The shoots were grafted onto healthy tomato plants at the four-to-six leaf stage using the cleft graft technique. Two reps (six plants each) were set up for each cultivar. Graft scions that shriveled and dried up within two weeks were considered dead. Grafted scions that had at least some turgidity after two weeks were considered successful grafts.

CLso Population Dynamics

Beginning two weeks after inoculation, a petiole sample (70-100mg) from the lowest most mature leaf of each plant was taken weekly for eight (for experiment one) and six weeks (for experiment two). DNA was extracted using the GeneJET Plant Genomic DNA Purification Mini Kit (Thermo-Scientific, Pittsburg, PA) by following the recommended protocol. DNA extractions were stored at 4° C.

Polymerase Chain Reaction (PCR) was performed using LsoTX 16-23 forward and reverse primers (Ravindran et al., 2011) and OA2/OI2c (Liefting, et al., 2009) for CLso detection. PCR amplification was performed in 25-µl, using TaKaRa Hot Start Taq system (Clontech Laboratories, Inc., Madison, WI) protocol. EZ-Vision Two dye and loading buffer (Amresco Inc., Solon, OH) were added to 5-µl of PCR reactions and run on a 1.5% agarose gel in 0.5X TBE buffer at 120 volts. All Purpose Hi-Lo DNA marker (Bionexus Inc., Oakland, CA) was also used to evaluate band-size. DNA bands were visualized using an ultraviolet light.

Whole genome sequencing of Strain R1

CLso DNA was extracted from infected tomato tissues. Bacterial DNA was enriched, amplified, and sequenced both with the 454 GS-FLX using Titanium chemistry (Roche, Bradford, CT) and Illumina MiSeq systems (Illumina, Inc., San Diego, CA). CLso sequence reads were extracted based on available Liberibacter DNA sequences and assembled using Velvet 1/2/10 (https://www.ebi.ac.uk/~zerbino/velvet/) and CLC Workbench 7.0 software. The CGView Comparison Tool (CCT) package was used to visualize similarity between genomes.

Results and Discussion

CLso Detection

The number of plants that tested positive for CLso (n =12 plants per cultivar) over the seven weeks is shown in Figure 1. A total of 29 out of the 48 grafted plants were CLso positive. By six weeks, CLso was detected in 23 of the 33 plants that were successfully grafted. Two additional plants from the Ace cultivar tested positive after the six week period. Unsuccessful grafting did not always lead to failure of CLso transmission, although inoculation success was low (only four of fifteen plants).

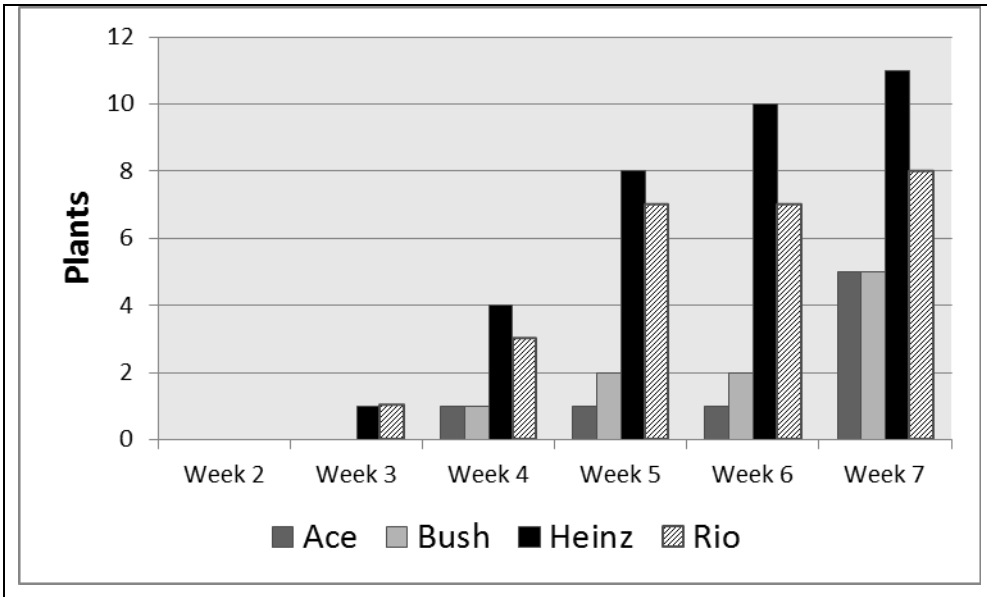

Figure 1. Cultivar and time distribution of successful inoculated tomato plants with '*Candidatus* Liberibacter solanacearum' Strain R1.

Using the number of CLso positive plants from both experiments, the average time (in weeks) of CLso infection for each cultivar was calculated. An Analysis of Variance test using SPSS 20.0 (IBM, Armonk, NY) detected significant differences ($P < 0.05$) due to cultivar between the mean times for infections to be detected. A Tukey means separation test showed that the Heinz and Rio cultivars required significant less time for CLso to be detected than the Ace or Bush cultivars (Figure 2).

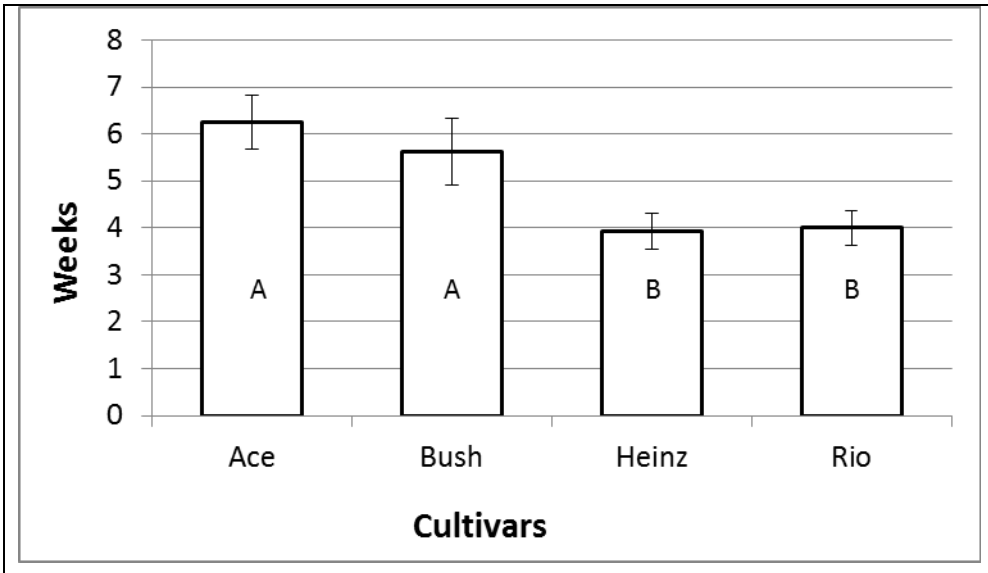

Figure 2. Differences in average time of successful '*Candidatus* Liberibacter solanacearum' infection among four tomato cultivars.

Previous observations have shown that infection of CLso was detrimental to potato plant but could be maintained in tomato plants for a long period of time under greenhouse condition (Chen et al., 2010, 2011). However, such a tomato *in planta* culture system (TIPCS) was mainly built on a single cultivar (BigBoy). There are many commercially available tomato cultivars of different genetic background. Information on tomato cultivar sensitivity to CLso infection is in general limited because economic damage of CLso has mainly been in potato and therefore most research funding supports potato research. Results from this study demonstrate that there exist significant differences of CLso sensitivity among various tomato cultivars. With cultivars Heinz and Rio, CLso infection could be well established in three weeks (Fig. 1). Further evaluation of CLso titer and greenhouse maintainability of different tomato cultivars is underway.

Whole Genome sequencing of CLso Strain R1

The 454 sequencing of the California CLso strain R1 yielded 100,867 sequence reads with an average size of 511 bp. The Illumina sequencing yielded 3.9×10^7 reads with an average size of 251 bp. While most of the sequence reads were of tomato origin, our TIPCS did increase the titer of CLso to the point that a significant amount of CLso reads were generated. The collection of these reads led to the successful assembling of the whole CLso R1 genome with the size of 1,204,257 bp. Whole genome sequence comparison indicated the California R1 strain was significantly different from the ZC1 strain from Texas (Lin et al., 2011), suggesting the existence of at least a subspecies lineage. It should also be noted that the CLso Strain R1 was maintained in TIPCS that can continuously provide source DNA for future analyses. In contrast, ZC strain was from a psyllid and source DNA could be depleted.

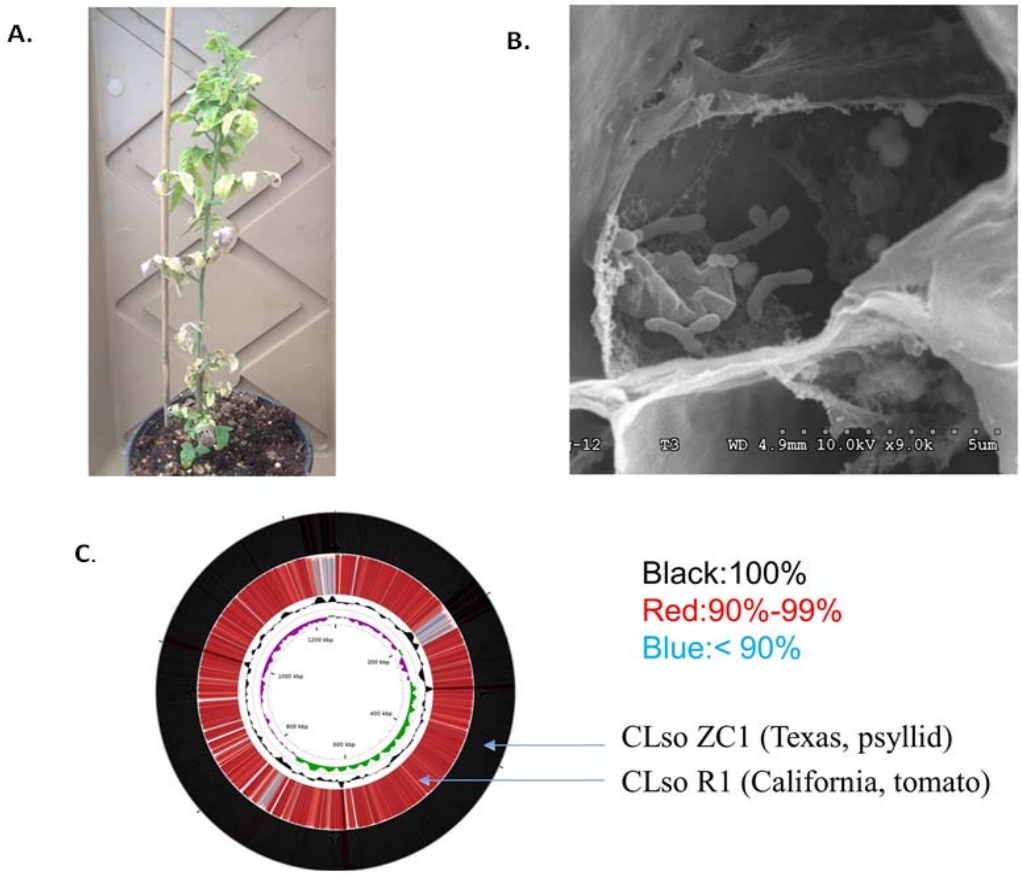

Figure 3. A. '*Candidatus* Liberibacter solanacearum' Strain R1 maintained in tomato in plant culture system (TIPCS). B. Scanning electron microscopy of phleom tissue from TIPCS showing possible '*Ca*. L. solanacearum'. C. Whole genome sequence comparison of '*Ca*. L. solanacearum' Strain R1 from California and Strain ZC1 from Texas.

Acknowledgements

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References

Chen, J., van Hest, P. A. and Civerolo, E. L. 2010. Establishing and fine-tuning an in planta system for potato Zebra chip disease research in California. Proceedings of the 10th Annual Zebra Chip Reporting Session, page 115-119.

Chen, J. 2011. Enrichment and characterization of "*Candidatus* Liberibacter solanacearum" in tomato host. Proceedings of the 11th Annual Zebra Chip Reporting Session, page 154-158.

Liefting, L. W., Sutherland, P. W., Ward, L. I., Paice, K. L., Weir, B. S., and Clover, G. R. G. 2009. A new "Candidatus Liberibacter" species associated with diseases of solanaceous crops. Plant Disease 93:208-214.

Lin, H., Lou, B., Glynn, J. M., Doddapaneni, H., Civerolo, E. L., Chen, C., Duan, Y., Zhou, L. and Vahling, C. M. 2011. The complete genome sequence of "*Candidatus* Liberibacter solanacearum," the bacterium associated with potato zebra chip disease. PLoS One 28:e19135.

Ravindran, A., Levy, J., Pierson, E., and Gross, D. C. 2011. Development of primers for improved PCR detection of the potato zebra chip pathogen, "*Candidatus* Liberibacter solanacearum". Plant Disease 95:1542-1546.

Zheng, Z., Clark, N., Keremane, M., Lee, R., Wallis, C., Deng, X., and Chen, J. 2014. Whole-genome sequence of "*Candidatus* Liberibacter solanacearum" strain R1 from California. Genome Announc. 2(6):e01353-14.

The Dynamic Role of Pulse Water Stress in Plant Affects Psyllid Infestation

Ordom Brian Huot and Cecilia Tamborindeguy
Department of Entomology, Texas A&M University, College Station, Texas 77843

Abstract
The ability of plants to resist insect herbivory and pathogen infection depends on environmental factors that affect plant susceptibility. Water stress resulting from the scarcity of water can elicit plant susceptibility to insects and pathogens by changing plant physiological responses. Thus, studying plant susceptibility to insects transmitting pathogens is crucial to enhance the understanding of pest outbreaks in the context of the world's global climate changes. This study was conducted to evaluate the susceptibility of *Solanum lycopersicum* to *Bactericera cockerelli* under water stress conditions. The results suggested that water stress increased plant susceptibility to psyllid infestation by increasing the number of psyllid adults and offspring on host plant. In non-choice assays, psyllid population was significantly higher on water-stressed plants than on well-watered plants. In choice assays, the mean number of offspring between well-watered and water-stressed plants was not significantly different while the mean number of adults was significantly higher on water-stressed plants. Psyllids attracted to plants with reduced relative water content and water pressure may be explained by the increase of nitrogen rich amino acids in plants as a consequence of water stress.

Introduction
Plant physiological responses to insect herbivory and pathogen infection can be influenced by abiotic conditions such as water stress (Adie et al., 2007; Huberty and Denno, 2004; Mattson and Haack, 1987). Under scarcity of water, plants may experience water stress resulting from a reduction of plant water potential and turgor pressure (Pardossi et al., 1991). As a result, there is an increase of abscisic acid that signals the closing of stomata and a surge of reactive oxygen species (ROS) (Jiang and Zhang, 2002; Adie et al., 2007).

ROS, such as hydrogen peroxide, trigger plant hypersensitive response to insect feeding and pathogen infection. Hence, stressed plants may be less susceptible to herbivory and infection. However, overproduction of ROS can result in cell death by causing irreversible damage to the cell DNA and membranes. ROS can reduce plant growth by signaling the closing of stomata to prevent water loss and reduce gas exchange (Jiang and Zhang, 2002), resulting in reduced photosynthesis under water-stress. Therefore, water-stressed plants have limited resources to allocate for defenses against insects and pathogens. Consequentially, insects may prefer plants that experienced scarcity of water to exploit the plant limited ability to mount defenses against herbivory. On the contrary, insects may prefer well-watered plants to avoid ROS-mediated plant defenses if the insects are susceptible to plant hypersensitive response. Understanding plant responses to psyllid infestation under water stress is important for predicting the consequences for future pest outbreaks when crops experience drought stress (Mattson and Haack, 1987). In this study, we used *Solanum lycopersicum* (tomato) to examine the role of water pulse stress on plant susceptibility to *Bactericera cockerelli* (potato psyllid) infestation carrying '*Candidatus* Liberibacter solanacearum' (Lso).

Materials and Methods
Plant source and water treatments

Solanum lycorpesicum cv. Money Maker (Thompson & Morgan) were grown from seeds in Sun Gro® Metro-Mix 900 soil. Plants received weekly water and Miracle-Gro® Water Soluble Tomato Plant Food (24-8-16 NPK). Plants were grown under 16:8 (L:D) light cycle throughout their development. After four weeks, tomato plants were individually transplanted into 4-inch square pots and

received either a weekly (pulse) water regimen of 50 ml or 200 ml for two weeks. Plant water pressure and plant relative water content (RWC) were then evaluated for each plant prior to conducting the biological assays.

Plant water pressure assay

The six-weeks old tomato plants of relative similar size were selected to test for water pressure after the two weeks water regimen. The bottom-most and fully-expanded tomato leaf was precisely excised and immediately used in the calibrated pressure chamber (Model 615, PMS Instrument Company) to test for the chamber pressure (CP). The pressured measured by the pressure chamber is the force required to push sap out of the cut end of the leaf, which is inversely proportional to plant water potential. The inverse relationship (r^2 = -0.99) between tomato water potential and pressure chamber is well-documented and was used in the studies of plant water stress (Duniway, 1971; Pardossi et al., 1991; Boyer, 1967). In the present study, plant water stress was evaluated based on the pressure measured by the pressure chamber. All plants were tested prior to conducting the biological assays. In total, 24 plants from the 50 ml water regimen and 21 plants from the 200 ml water regimen were tested.

Plant relative water content assay

A bottom-most fully-expanded leaf was collected from each plant to test plant RWC. Leaf fresh, dried and turgid weights were measured and used to calculate RWC according to Pardossi et. al., 1991. Briefly, leaf fresh biomass was measured after the excision. Leaf turgid biomass was measured after floating in distilled water for 24 hrs at 25 °C under dim light. Leaf dried biomass was measured after dried in the drying oven for 48 hrs at 65 °C.

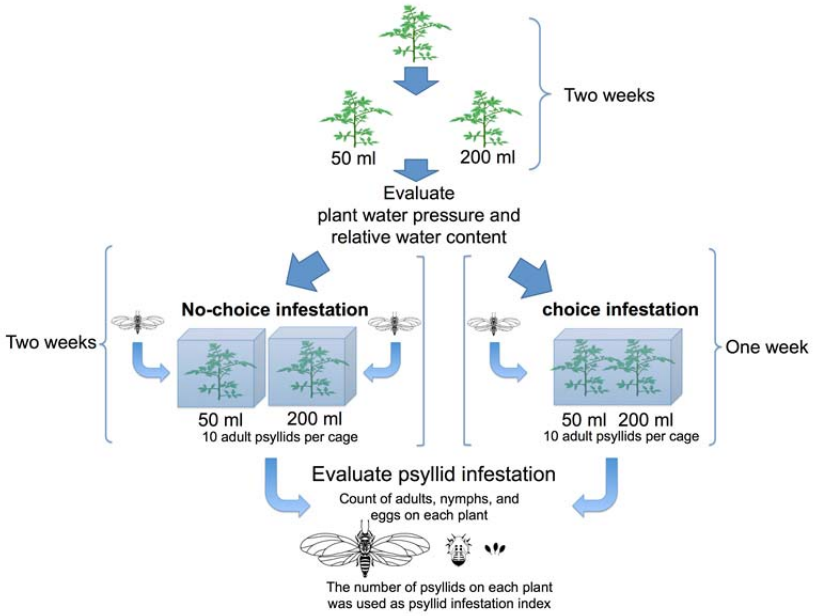

Fig 1. Experimental design

Psyllid no-choice infestation assay

After testing for water pressure and RWC, plants from each water regimen group were individually caged with ten adult psyllids from a Lso-positive colony. Psyllids were allowed to feed and oviposit eggs on each plant for two week. Each week, numbers of adults, eggs and nymphs on each plant were counted to evaluate psyllid infestation index. Plants continued to receive their weekly water

regimen during the assay. The experimental design is illustrated in **Fig. 1**. The assessment was performed using nine plants from the 50 ml and nine plants from the 200 ml weekly regimen groups.

Psyllid choice infestation assay

The assay was conducted using 32 pairs of similar size plants from the 50 ml and 200 ml water regimen groups. All plants were tested for water pressure and RWC, and one plant from each water regimen group were caged together with ten adult psyllids from the Lso-positive colony. Psyllids were allowed to feed and oviposit eggs on plants for a week. Numbers of adults, eggs and nymphs on each plant were counted to assess psyllid infestation index. The experimental design is illustrated in **Fig. 1**.

Results and Discussion

Plants were evaluated for water stress by measuring water pressure and RWC. Plants treated with 50 ml of weekly water regimen had significantly higher CP than plants treated with 200 ml of weekly water regimen ($X^2(1, N = 45)= 29.41$, $p < 0.0001$, **Fig. 2A**). On average, 50 ml treated plants required 57.7% of CP than 200 ml treated plants to push sap out of plant. This higher pressure needed to push sap out of the leaves indicated that 50 ml treated plants were on average less turgid and had lower plant water pressure when compared to 200 ml treated plants (well-watered plants). This suggested that plants in the 50 ml weekly water regime were experiencing pulse water stress. The effect of this pulse water stress allowed to test our research hypothesis because a significant difference was observed in plant water pressure but not in plant physical apparent such as wilting (see **Fig. 2B**). Wilting only occurs in extreme water stress plants (Lombardini, 2006), which was not a desirable response for this experiment since we wanted to evaluate plant susceptibility to psyllid infestation under water stress condition. Wilting would have been a confounding factor that could affect psyllid infestation because it influences psyllid infestation behavior.

An additional level of validation was performed by evaluating plant RWC to verify plant water stress. The lower the water content, the higher the water stress. Our study indicated that plants treated with 50 ml had significantly lower RWC than plants treated with 200 ml of water ($X^2(1, N = 45)= 12.12$, $p < 0.0005$, **Fig. 2C**). This result also supported that plants treated with 50 ml of water were experiencing water stressed. Thus, 50 ml and 200 ml plants were used as pulse water-stressed and control plants (well-watered), respectively.

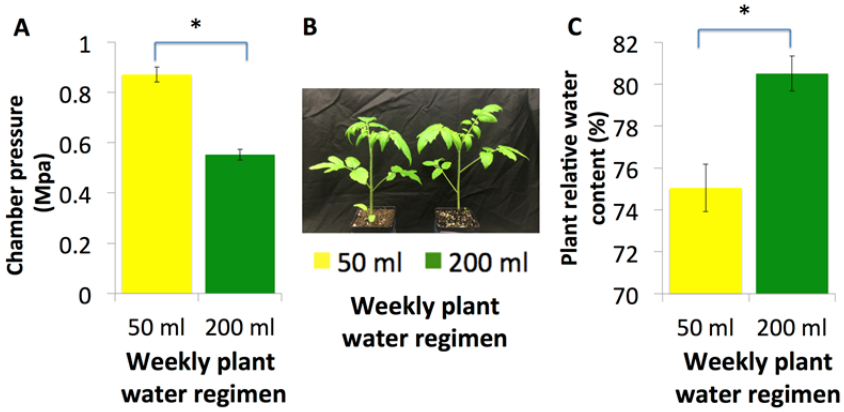

Fig 2. The effect of water regimen on chamber pressure and relative water content. **Fig. 2A** and **2C** show graphs of mean ± SE of chamber pressure and plant RWC, respectively. **Fig. 2B** shows plants treated with 50 ml and 200 ml water weekly indicating their similarities in physical appearance. Symbol * represents significant difference between the treatment groups.

The role of plant water stress on psyllid no-choice infestation was evaluated by assessing the number of psyllids colonizing plants treated with either 50 ml or 200 ml. We found that pulse water stressed plants (50 ml treated plants) were significantly more susceptible to adult psyllid infestation during one week ($F (1, 16) = 5.16$, $p = 0.0373$, **Fig 3A**) and two weeks infestation ($F (1, 16) = 16.07$, $p = 0.0010$, **Fig 3A**) than well-watered plants. In a similar pattern, the mean number of psyllid offspring (eggs + nymphs) on host plant was significantly affected by water stress after one week ($F (1, 16) =$

4.37, p = 0.0264, **Fig 3B**) and two weeks (F (1, 16) = 3.63, p = 0.0374, **Fig 3B**) of infestation in no-choice bioassays. However, the mean number of psyllid eggs on host plants was not significantly different between water-stressed plants and well-watered plants after one week (F (1, 16) = 1.78, p = 0.200, **Fig 3D**) and two weeks (F (1, 16) = 1.48, p = 0.241, **Fig 3D**) infestations. The significant difference in offspring was driven by the significant difference in the number of psyllid nymphs after one week (F (1, 16) = 14.109, p = 0.0017, **Fig 3C**) and two weeks (F (1, 16) = 3.747, p = 0.0354, **Fig 3C**). This suggested that plant pulse water stress plays a temporal dynamic role on psyllid infestation and development. Water-stressed plants were more susceptible to psyllid infestation than well-watered plants. The increase of amino acids availability in plants responding to pulse water stress is well-documented (Huberty and Denno, 2004; Hale et al., 2003). This could explain why nitrogen-seeking phloem-feeders like psyllids were more attracted to pulse water-stressed plants than well-watered plants. This attraction made water-stressed plants more susceptible to psyllid infestation. Also, water-stressed plants may have limited ability to defend against insects due to the reduction of photosynthesis and energy resource that results from the closing of stomata to conserve water.

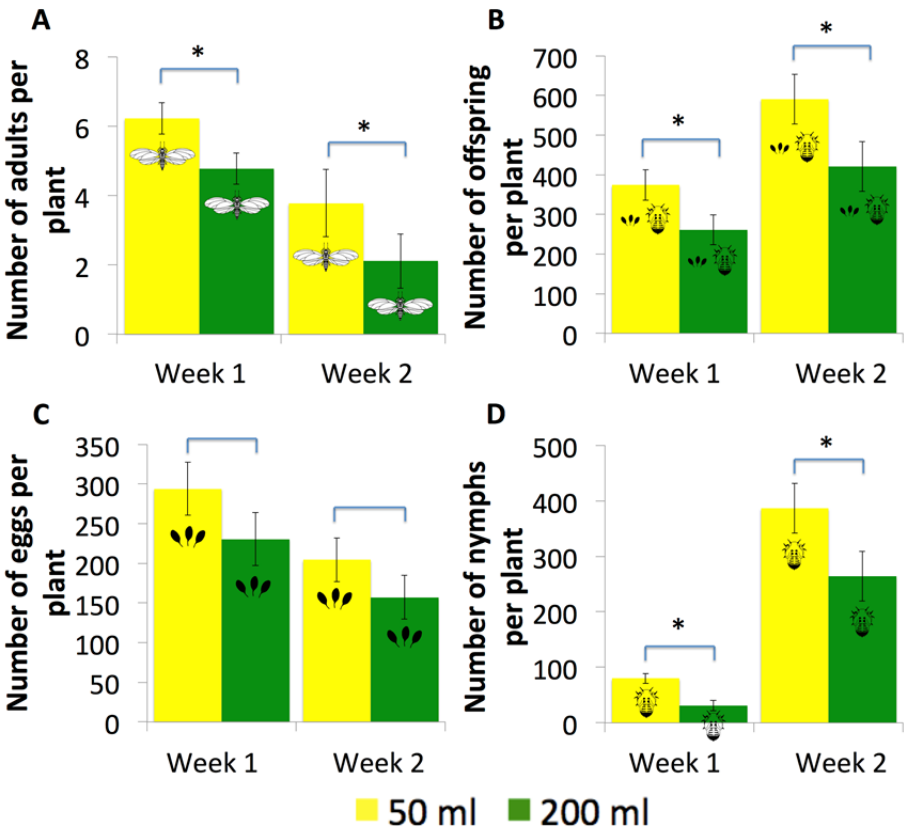

Fig 3. The role of plant water stress on psyllid no-choice infestation. **Fig 3A-B** show mean ± SE of psyllid adults, offspring, eggs and nymphs, respectively. The responses were separated by week. Pulse water stress significantly affected psyllid infestation preference by reducing the number of adults (**Fig 3A**) and offspring (**Fig 3B)** on plants. The differences were driven by the number of nymphs (**Fig 3D**), not by the number of eggs (**Fig 3C**). Symbol * represents significant difference between the treatment groups.

Contrary to no-choice psyllid infestation assays, choice psyllid infestation assay indicated that plant water-stress did not affect psyllid oviposition choice but affected psyllid settling preference. When given a choice, psyllids preferentially settled on 50 ml treated plants than on 200 ml treated plants (F (1, 62) = 5.44, p = 0.023, **Fig 4 A**). However, no significant differences in number of egg (F (1, 62) = 1.07, p =0.305, **Fig 4 B)** and the number of nymph (F (1, 62) = 0.00, p =0.995, **Fig 4 C**) were found between the treatment groups. Psyllids may preferentially feed on pulse water-stressed plants to acquire more essential amino acids for reproduction but distributed their offspring across plants to reduce competition among offspring. As explained above, plants release amino acids into the phloem to combat water stress

conditions (Huberty and Denno, 2004; Hale et al., 2003). More studies are needed to evaluate if psyllid survival on water stressed plants influence psyllid infestation preference.

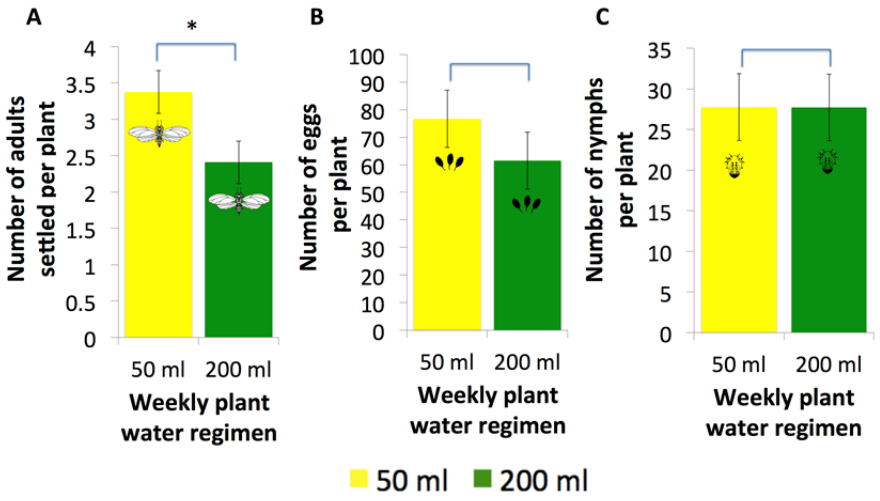

Fig 4. The impact of plant water stress on psyllid infestation choice. **Fig 4 A-C** illustrate the mean number of psyllid adults, eggs, and nymphs on plants under each weekly water regimen. Pulse water stress significantly affected the number of adult colonized plants (**Fig 4A**) but not the number of eggs (**Fig 4B)** nor the number of nymphs (**Fig 4C)**.
Symbol * represents significant difference between the treatment groups.

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References

Adie, B A, Perez-Perez, J, Perez-Perez, M M, Godoy, M, Sanchez-Serrano, J J, Schmelz, E A & Solano, R. 2007. ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis. *Plant Cell,* 19, 1665-81.

Boyer, J S. 1967. Leaf water potentials measured with a pressure chamber. *Plant Physiology,* 42, 133-&.

Duniway, J M. 1971. Comparison of pressure chamber and thermocouple psychrometer determinations of leaf water status in tomato. *Plant Physiology,* 48, 106-&.

Hale, B K, Bale, J S, Pritchard, J, Masters, G J & Brown, V K. 2003. Effects of host plant drought stress on the performance of the bird cherry-oat aphid, Rhopalosiphum padi (L.): a mechanistic analysis. *Ecological Entomology,* 28, 666-677.

Huberty, A F & Denno, R F. 2004. Plant water stress and its consequences for herbivorous insects: A new synthesis. *Ecology,* 85, 1383-1398.

Jiang, M Y & Zhang, J H. 2002. Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *Journal of Experimental Botany,* 53, 2401-2410.

Lombardini, L 2006. Ecophysiology of plants in dried environment. *In:* D'Odorico, P. & Porporato, A. (eds.) *Dryland Ecohydrology.* Springer Netherlands.

Mattson, W J & Haack, R A. 1987. The role of drought in outbreaks of plant-eating insects. . *Bioscience,* 37, 110-118.

Pardossi, A, Vernieri, P & Tognoni, F. 1991. Evaluation of the pressure chamber method for the assessment of water status in chilled plants. *Plant Cell and Environment,* 14, 675-682.

Carrot Psyllid (*Trioza Apicalis*) Feeding Behavior on Carrot and Potato: An EPG Study

Collins, Larissa [1], Nissinen, Anne [2], Pietravalle, Stéphane [1], and Jauhiainen, Lauri [2]
[1]The Food and Environment Research Agency, Sand Hutton, York YO 411LZ, UK.
[2]Natural Resources Institute Finland (Luke),Tietotie, FI-31600 Jokioinen, Finland

Abstract
The objectives of this study were to: 1. Determine whether carrot psyllids are capable of phloem feeding on potato. 2. Determine whether there are any differences in feeding behaviour between female and male *Trioza apicalis* and on carrot and potato plants. EPG (electrical penetration graph) was used to measure the time spent by *T. apicalis* probing each plant tissue type in potatoes and carrots. More time was spent by both males and females in the start of penetration, stylet tip in parenchyma, transition to phloem ingestion and phloem ingestion/salivation on carrots than on potatoes, when compared to the time spent not probing. Both plant and sex of the insect significantly affected the duration of the non-probing phase. Only one male psyllid was recorded phloem feeding from a potato plant. Therefore, it could be possible for *T. apicalis* to transmit CLso to potato plants. However, the time spent in phloem ingestion/salivation phase in potato is very short compared the time spent in this phase the on carrot, which suggests that the probability of CLso transmission on potato is low.

Introduction
'*Candidatus* Liberibacter solanacearum' (CLso) has been previously found in several solanaceous plants and in its psyllid vector, *Bactericera coccerelli*, (e.g. Hansen et al. 2008, Liefting et al. 2008; 2009; Lin et al. 2009; Wen et al. 2009). It is associated with the potato disease: zebra chip (Abad et al. 2009; Lin et al. 2009; Wen et al. 2009). Recently, it was observed that CLso is also associated with both carrot psyllid-damaged carrots and carrot psyllids (*T. apicalis*) (Munyaneza et al. 2010a,b). Further, Nissinen et al. (2014) showed that carrot psyllid feeding is correlated with the leaf curling symptom and the bacterial infection with the leaf discoloration symptom in carrots. In addition, they showed that male and female feeding cause different severities of symptoms in carrot. As CLso is the causal agent of zebra chip disease on potato, there is a question whether carrot psyllid is able to transmit CLso to potato. Little is known about the biology and behaviour of *T. apicalis* (Meadow, 2010). Psyllids are generally host specific, although the adults can feed on plants which are not suitable for nymphal development (Hodkinson, 1974).

It is important to understand the interactions between sucking insect vectors and plants to understand the mode of transmission. The vector's stylet penetration activities can determine to a large extent whether transmission of a disease causing organism from an infected plant to a healthy plant takes place or not. The relationship between stylet penetration and the process of pathogen transmission has been elucidated for several insects and viruses (Hodges & McLean, 1969; Scheller & Shukle, 1986; Tjallingii & Prado, 2001). However, little work has been undertaken on plant bacterial pathogen transmission by psyllids and nothing was known about the stylet penetration activity of *T. apicalis*. EPG waveforms which have been correlated with stylet penetration have been published for the Asian citrus psyllid, *Diaphorina citri* (Bonani *et al.*, 2010; Cen *et al.*, 2012); the potato psyllid, *Bactericerca cockerelli* (Pearson *et al.*, 2010; Sandanayaka *et al.*, 2014); and the pear psyllid, *Cacopsylla pyri* (Civolani *et al.*, 2011). Direct current EPG (Tjallingii, 1987) was used to measure the time spent by *T. apicalis* probing each plant tissue type in potatoes and carrots. This technique records the changes in resistance which relate to the different stylet penetration activities of sucking insects. It provides information on stylet tip

position in specific plant tissues and time spent in each type of plant tissue (Tjallingii, 1985). EPG has been used to elucidate insect vectoring of pathogens (e.g. Fereres & Collar, 2001; Symmes et al., 2008; Butler *et al.*, 2012; Sandanayaka et al., 2013) by matching stylet penetration activities to acquisition and transmission of pathogens (Martin et al., 1997; Tjallingii & Prado, 2001; Stafford et al., 2009; Moreno et al., 2012). The objectives of this study were to: 1. Determine whether carrot psyllids are capable of phloem feeding on potato plants. This is an important question because CLso is a phloem limited bacterium. 2. Determine whether there are any differences in feeding behaviour between female and male *T. apicalis* and on carrot and potato plants. It has been shown that female feeding on carrot plants causes more severe symptoms than male feeding (Nissinen et al., 2014).

Materials and Methods

Carrot plants: cv. 'Autumn King' grown in a glasshouse at 18-13°C, ambient humidity, L20:D4 (with supplemental lighting). Plants used were 4-6 weeks post-sowing. Potato plants: cv. 'Fontane' grown from cuttings in a glasshouse at 18-22°C, ambient humidity, L20:D4 (with supplemental lighting). Plants used for EPG experiments were 1 plant per 9 cm pot and approximately 15cm tall. CLso infected *T. apicalis* adults were obtained from Agrifood Research Finland (MTT). The insects were reared on uninfected carrot plants, although these were infected by the psyllids. The colony was maintained in a 45 x 45 x 50 cm Perspex cage with a fan producing air circulation. Fine steel and nylon meshes covered vents and the fan to prevent escape. Culture conditions were as described by Nissinen et al. (2005; 2007) i.e. L20:D4, 20/15°C day/night, 50% RH.

The EPG recordings were made under the same environmental conditions as described above. All of the tests took place inside a Faraday cage (100 x 50 x 80 cm). The instrument used was a GIGA-4 DC EPG System (EPG Systems, Wageningen, The Netherlands). Data output was digitized using a DI-1580 analogue to digital converter (DATAQ Instruments, Akron, OH, USA) and acquired and stored using a computer. Data was recorded and analysed using Stylet^{+}d software (EPG Systems, Wageningen, The Netherlands). The insect electrode consisted of a gold wire (18 µm diameter, 5-7 cm long), attached with water based silver glue (EPG Systems, Wageningen, The Netherlands) to a copper wire (single strand from an electrical cable, 6 cm long), which was soldered to a brass nail (1 mm diameter, 19 mm long). The brass nail was inserted into the pre-amplifier (probe) and the probe connected by a lead to the GIGA-4 control box (EPG Systems, Wageningen, The Netherlands). The plant electrode, which was pushed into the soil in each plant pot, consisted of a piece of thick copper core wire from a mains electrical cable (1.5 mm diameter, 11 cm long) soldered to a lead which connected to the GIGA-4 control box. Insects were removed from the colony, placed in 1.5 ml Eppendorf tubes, sexed, and placed in a -18°C freezer until they stopped moving. They were then attached at the dorsal surface of the thorax to the fine gold wire with water based silver glue. The assemblage was then attached to the probe and the insect placed on the adaxial surface of the leaf. Recordings were started between 1000 and 1200 hours. Each psyllid was monitored for between 21 and 23 h depending on duration of the set-up procedure. A new psyllid and plant were used for each recording. A group of four psyllids, which consisted of: female psyllid on a carrot plant, male psyllid on a carrot plant, female psyllid on a potato plant, male psyllid on a potato plant were monitored during each recording period. There were six individual recordings for each of these combinations giving a total number of 24 recordings of individual psyllids. The data recorded were analysed to allocate the duration recorded for each of the following waveforms: NP – not probing**,** PA – start of penetration**,** C – stylet tip in parenchyma**,** D – stylet tip in sieve element, transition to PE1**,** PE1 - stylet tip in sieve element, phloem salivation**,** PE2 – stylet tip in sieve element, phloem ingestion**,** PG – stylet tip in xylem, xylem ingestion**,** W – walking**,** ET

– elapsed time before first attempt at probing**,** F – difficulty probing**,** U – unassigned to any waveform (used where we could not assign the observed waveform to a recognisable pattern)**.** The above descriptions are taken from a summary of published EPG waveforms by Civolani *et al.* (2011). For the purposes of data recording and analysis, each waveform was assigned a number: 1 = NP, 2 = PA, 3 = C, 4 = D, 5 = PE1, 6 = PE2, 7 = PG, 8 = W, 9 = ET, 10 = F and 11 = U

Statistical analysis. Due to the differences in total time of the recordings and that simply looking at proportions would lead to looking at non-independent variables, the *total times* spent in each activity were expressed as ratios of category 1 "NP". This category was chosen because it occurred in every recording. Categories compared were: 2/1, 3/1, 4/1, 5&6/1, 7/1, 8/1, 10/1, 9&11/1. Some categories were pooled to reduce the number of variables: PE1 and PE2 since these are both feeding from phloem; ET and U since these occurred on very few occasions and for short periods of time. The ratios were logged and a multivariate analysis of variance (MANOVA) was used to compare the means of all groups. Where a statistical difference was detected by the MANOVA, individual analyses of variance (ANOVA) were carried out to pinpoint which categories were responsible for the overall difference. The ANOVA was carried out from log-transformed data also for the *average duration* of each phase of feeding behavior.

Table 1. Average duration (SD) of each waveform in seconds for females and males on carrot and on potato.

	Female Carrot	**Female Potato**	**Male Carrot**	**Male Potato**
NP	44.2 (19.4)	951.4 (1449.1)	550.2 (658.3)	2981.0 (3401.9)
PA	6.8 (5.3)	5.7 (2.8)	4.8 (2.0)	39.7 (85.8)
C	796.7 (480.7)	796.5 (227.5)	776.3 (215.6)	468.8 (264.3)
D	155.4 (123.5)	26.7	121.6 (61.9)	47.7 (27.9)
PE1/PE2	1540.2 (491.7)	.	732.1 (497.84)	44.3 (51.9)
PG	680.0 (825.1)	2712.5 (1825.1)	1778.3 (1188.3)	329.3 (366.7)
W	34.7 (19.3)	92.5 (64.6)	59.6 (70.0)	73.9 (64.1)
F	54.2 (61.0)	42.2 (19.4)	93.0 (11.1)	105.7 (82.3)
U	494.4 (747.7)	10.2 (10.3)	21.0 (26.1)	5.8 (2.4)

Results and Discussion

Some statistical differences were found in the total time spent by males and females (taken together) between carrot or potato plants in ratios of some activities. Total time spent by both males and females in PA/NP (start of penetration) ($P = 0.009$), C/NP (stylet tip in parenchyma) ($P = 0.035$), D/NP (transition to PE1) ($P < 0.001$) and PE1-PE2/NP (phloem ingestion/salivation) ($P < 0.001$) was longer on carrots than on potatoes, which was expected. Both plant species and sex of the insect significantly affected the average duration of NP (non-probing) phase ($P_{plant} = 0.005$, $P_{sex} = 0.041$). Interaction of plant and sex was significant for average duration of W (walking) ($P = 0.040$) indicating females walked for longer times on potato plants than males. One male psyllid was recorded phloem feeding from a potato plant. Therefore, it could be possible for *T. apicalis* to transmit CLso to potato plants. However, the time spent in the phloem ingestion/salivation phase in potato was very short, less than one minute, compared with the time spent in this phase the on carrot. This may explain why even 60 individuals of

psyllids from this population per potato plant were not able to transmit the bacterium into potato in a no-choice trial (Nissinen et al. unpublished).

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References

Abad, J.A., Bandia, M., French-Monar, R.D, Liefting, L.W., and Clover, G.R.G. 2009. First report of the detection of '*Candidatus* Liberibacter' species in zebra chip disease-infected potato plants in the United States. Plant Dis. 93:108.

Bonani, J.P., Fereres, A., Garzo, E., Miranda, M.P., Appezzato-Da-Gloria, B., Lopes, J.R.S. 2010. Characterization of electrical penetration graphs of the Asian citrus psyllid, *Diaphorina citri*, in sweet orange seedlings. Entomol. Exp. Appl. 134:35–49.

Butler, C.D., Walker, G.P., Trumble, J.T. 2012. Feeding disruption of potato psyllid, *Bactericera cockerelli*, by imidacloprid as measured by electrical penetration graphs. Entomol. Exp. Appl. 142:247–257.

Cen, Y.J., Yang, C.L., Holford, P., Beattie, G.A.C., Spooner-Hart, R.N. 2012. Feeding behaviour of the Asiatic citrus psyllid, *Diaphorina citri*, on healthy and huanglongbing-infected citrus. Entomol. Exp. Appl. 143:13–22.

Civolani, S., Leis, M., Grandi, G., Garzo, E., Pasqualini, E. 2011. Stylet penetration of *Cacopsylla pyri*; an electrical penetration graph (EPG) study. J. Insect Physiol. 57:1407–1419.

Fereres, A., Collar, J.L. 2001. Analysis of noncirculative transmission by electrical penetration graphs. Virus-Insect-Plant Interaction (eds. F.K. Harris, O.P. Smith & J.E. Duffus), pp. 87–109. Academic Press, San Diego, CA, USA.

Hansen, A.K., Trumble, J.T., Stouthamer, R., and Paine, T.D. 2008. A new Huanglongbing species "Candidatus Liberibacter psyllauros" found to infect tomato and potato is vectored by the psyllid *Bactericera cockerelli* (Sulc). Appl. Environ. Microbiol. 74:5862-5865.

Hodges, L.R., McLean, D.L. 1969. Correlation of transmission of bean yellow mosaic virus with salivation activity of *Acyrthosiphon pisum* (Homoptera: Aphididae). Ann. Entomol. Soc. Am. 62:1398–1401.

Hodkinson, I.D. 1974. The biology of the Psylloidea (Homoptera): a review. B. Entomol. Res. 64:325-338.

Liefting, L.W., Rez-Egusquiza, Z.C., Clover, G.R.G, and Anderson, J.A.D. 2008. A New '*Candidatus* Liberibacter' Species in *Solanum tuberosum* in New Zealand. Plant Dis. 92:1474.

Liefting, L.W., Sutherland, P.W., Ward, L.I., Paice, K.L., Weir, B.S., Clover, G.R.G. 2009a. A new '*Candidatus* Liberibacter' species associated with diseases of Solanaceous crops. Plant Dis. 93:208-214.

Lin, H., Doddapaneni, H., Munyaneza, J.E. Civerolo, E.L., Sengoda, V.G., Buchman J.L. and Stenger D.C. 2009. Molecular characterization and phylogenetic analysis of 16SrRNA from a new "*Candidatus* Liberibacter" strain associated with Zebra chip disease of potato (*Solanum tuberosum* L.) and the potato psyllid (*Bactericera cockerelli* Sulc). J. Plant Pathol. 91:215-219.

Martin, B., Collar, J.L., Tjallingii, W.F., Fereres, A. 1997. Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. J. Gen. Virol. 78:2701–2705.

Meadow, R. 2010. The carrot psyllid, *Trioza apicalis* – biology and control. Bioforsk Report Vol. 5 Nr. 151.

Moreno, A., Tjallingii, W.F., Fernandez-Mata, G., Fereres, A. 2012. Differences in the mechanism of inoculation between a semi-persistent and a non-persistent aphid-transmitted plant virus. J. Gen. Virol. 93:662–667.

Munyaneza, J.E., Fisher, T.W., Sengoda, V.G., Garczynski, S.F., Nissinen, A., Lemmetty, A. 2010a. Association of "*Candidatus* Liberibacter solanacearum" with the psyllid, *Trioza apicalis* (Hemiptera: Triozidae) in Europe. J. Econ. Entomol. 103:1060-70.

Munyaneza, J.E., Fisher, T.W., Sengoda, V.G., Garczynski, S.F., Nissinen, A., Lemmetty, A. 2010b. First report of "*Candidatus* Liberibacter solanacearum" associated with psyllid-affected carrots in Europe. Plant Dis. 94:639.

Nissinen, A. I., Haapalainen, M., Jauhiainen, L., Lindman, M., Pirhonen, M. 2014. Different symptoms in carrots caused by male and female carrot psyllid feeding and infection by '*Candidatus Liberibacter solanacearum*'. Plant Pathol. 63:812-820.

Nissinen, A., Vanhala, P., Holopainen, J.K., Tiilikkala, K. 2007. Short feeding period of carrot psyllid (*Trioza apicalis*) females at early growth stages of carrot reduces yield and causes leaf discolouration. Entomol. Exp. Appl. 125:277-283.

Pearson, C.C., Backus, E.A., Munyaneza, J.E. 2010. Feeding biology of the potato psyllid, *Bactericerca cockerelli*. Proceedings of the 10th Annual Zebra Chip Reporting Session, (eds. F. Workneh & C.M. Rush), pp. 178–182. Dallas, TX.

Sandanayaka, W.R.M., Moreno, A., Tooman, L.K., Page-Weir, N.E.M., Fereres, A. 2014. Stylet penetration activities linked to the acquisition and inoculation of *Candidatus* Liberibacter solanacearum by its vector tomato potato psyllid. Entomol. Exp. Appl. 151:170–181.

Sandanayaka, W.R.M., Tooman, L.K., Hewett, R.E. 2013. The impact of post acquisition period on detection of *Candidatus* Liberibacter solanacearum in tomato potato psyllid. New Zeal. Plant Prot. 66:365–373.

Scheller, H.V., Shukle, R.H. 1986. Feeding behavior and transmission of barley yellow dwarf virus by *Sitobion avenae* on oats. Entomol. Exp. Appl. 40: 189–195

Stafford, C.A., Walker, G.P., Creamer, R. 2009. Stylet penetration behavior resulting in inoculation of beet severe curly top virus by beet leafhopper, *Circulifer tenellus*. Entomologia Experimentalis et Applicata 130: 130–137.

Symmes, E.J., Walker, G.P., Perring, T.M. 2008. Stylet penetration behavior of *Myzus persicae* related to transmission of Zucchini yellow mosaic virus. Entomol. Exp. Appl. 129:258-267.

Tjallingii, W.F. 1985. Electrical nature of recorded signals during stylet penetration by aphids. Entomol. Exp. Appl. 38:177–186.

Tjallingii, W.F., 1987. Stylet penetration activities by aphids: new correlations with electrical penetration graphs. Insects Plants (ed. by V. Labeyrie, G. Fabres & D. Lachaise), pp. 301–306. Dr. W. Junk Publishers, The Hague, The Netherlands

Tjallingii, W.F., Prado, E. 2001. Analysis of circulative transmission by electrical penetration graphs. Virus-Insect-Plant Interaction (ed. by FK Harris, OP Smith & JE Duffus), pp. 69–85. Academic Press, San Diego, CA, USA.

Wen, A., Mallik, I., Alvarado, V.Y., Pasche, J.S., Wang, X., Li, W., Levy, L., Lin, H., Scholthof, H.B., Mirkov, T.E., Rush, C.M., and Gudmestad, N.C. 2009. Detection, distribution, and genetic variability of '*Candidatus* Liberibacter' species associated with zebra complex disease of potato in North America. Plant Dis. 93:1102-1115.

The Mexican Conundrum of Hot Potato Psyllids: Importance of Commercial Hosts, and Surveyed Sites

Esparza-Díaz,[1] Gabriela, Villanueva, Raul T[1], Paetzold, Li [2], Henne, Don[3], Rush, Charlie[2] and Sanchez-Peña, Sergio[4]
[1]Texas A&M AgriLife Extension Service; Weslaco, TX 78596, USA, [2]Texas A&M AgriLife Research, 6500 Amarillo Blvd W, Amarillo, TX, 79106, [3]Monsanto Co., Hawaii. [3]Universidad Nacional Autonoma Antonio Narro, Saltillo, Coahuila, Mexico.

Abstract
'*Candidatus* Liberibacter solanacearum' (Lso), vectored by the potato psyllid *Bactericera cockerelli*. is the putative causal agent of potato zebra chip (ZC). *Bactericera cockerelli* is an endemic insect of the mountains of Mexico. Our objectives were to determine *B. cockerelli* in solanaceous crops, detect ZC in potato tubers, identify *B. cockerelli* haplotypes, and evaluate the proportion of *B. cockerelli* that carries Lso. *Bactericera cockerelli* was found in potato fields in the states of Veracruz and Coahuila during different periods (August and October, 2013 and May, July, September and October, 2014). Few specimens of *B. cockerelli* tested positive for Lso in Perote, Veracruz. Two *B. cockerelli* haplotypes were detected (Central and Southwestern). We found that 100% (*n*=25) of *B. cockerelli* were "hot" (positive for Lso) in a tomato field in Acultzingo, Veracruz in July 2014, whereas all samples collected in this month from potato fields in the US were negative. Also in Veracruz area, we found *B. cockerelli* in tomatillo and pepper plants. We hypothesize that potato psyllids have a continuous, and slow movement throughout the mountainous regions of the U.S and Mexico in addition to the annual migration carried by wind pattern from December to May.

Introduction
Zebra chip (ZC) is an emerging potato disease in the U.S., which was first reported from Saltillo, (Coahuila state) Mexico in 1994 ((Munyaneza et al. 2009). ZC is caused by '*Candidatus* Liberibacter solanacearum' (Lso), which is vectored by the potato psyllid *Bactericera cockerelli* (Hemiptera: Triozidae; Munyaneza et al. 2007). *B. cockerelli* is an endemic insect in the highlands of Mexico. In Mexico potato is planted from January to June and harvested from July to December across different potato growing regions. In Mexico, few studies have been conducted on Lso and its vector in regions where other solanaceous crops in addition to potatoes are cultivated (Munyaneza et al., 2009; Liefting et al., 2009). Our objectives were to determine *B. cockerelli* populations in solanaceous crops, detect ZC in potato tubers, identify *B. cockerelli* haplotypes, and evaluate the proportion of *B. cockerelli* that carries Lso in various Mexican regions.

Materials and Methods
*Sampling in Perote and Acultzingo (Veracruz)***:** Counts of *B. cockerelli* and detection of ZC in potato plants were conducted in smaples fromseveral potato fields in Perote. In 2013, we inspected 13 sites (potato fields) in Perote. Seven farms were located in Los Altos and 6 in Los Pescados. The average size of potato fields were 1.4 ha. In all the sites, potato plants were in different stages of growth. Potato varieties were Mundiales, Felsinas, Chapingo, Fianas, Mazamba, San Jose, Perote and an experimental cultivar. In each field, we collected 10 leaves and recorded *B. cockerelli* eggs, nymphs, and adults. In the same fields, we placed three yellow sticky traps /field that were collected after 2 months. Tallies of *B. cockerelli* were conducted using a hand lens (10X). Also, in each field 20 tubers were collected and ZC symptoms were observed in raw and cooked tubers. For haplotype identifications, *B. cockerelli* adults

were collected form 8 potato fields in 2013, and 4 potato fields in 2014 in Perote. Additionally, in May, July and October 2014, potato psyllids were collected in pepper, tomato, and tomatillo fields in Acultzingo, Veracruz.
*Sampling in Saltillo (Coahuila)***:** Counts of *B. cockerelli* were conducted in potato, tomato, and pepper plants in October 2013 (Villanueva et al. 2014) and July and September 2014. Detection of ZC in potato plants were conducted by cutting tubers from an experimental field and a commercial field. Two potato psyllids were collected from a potato field in Huachichil, Saltillo, and three potato psyllids from pepper plants in Saltillo and Emiliano Zapata, Coahuila. Specimens collected in July 2014 were compared with psyllids from the U.S.

Haplotypes and Lso. *B. cockerelli* specimens were analyzed for haplotypes and Lso using ViiA7 Fast 96 well real time PCR machine (Applied Biosystems) in the Texas A&M AgriLife Research and Extension in Amarillo, Texas. To detect positive psyllids, all specimens were individually tested using Absolute Quantification real-time PCR method. LsoF/HLBr primers, TaqMan® HLBp probe, and TaqMan® Fast Advanced Master Mix were applied in the PCR assay. All specimens were individually tested to identify psyllid haplotype, using HRM (High Resolution Melt) method and CO1 F1/CO1 R1 primers and MeltDoctor™ HRM Master Mix were applied in the HRM melting assay.

Results and Discussion
Potato fields in Perote, Veracruz. *Bactericera cockerelli* nymphs were only found in six out of thirteen sampled sites (Fig. 1A); nonetheless there were no significant differences among populations ($F_{10, 9}$ = 0.63; p = 0.75). Adult psyllids (Fig. 1B) were found on yellow sticky cards in eight of these sites, and the numbers psyllids were significantly different among the sites ($F_{7, 16}$ = 3.04; p = 0.03). Sliced raw tubers showed ZC symptoms in all sites except two (Fig. 2A); while fried chips allowed ZC detection in all sites (Fig. 2B). The percentages of samples with ZC were above 40%, which were similar to the rates found in South Texas (Villanueva and Esparza-Diaz 2013).

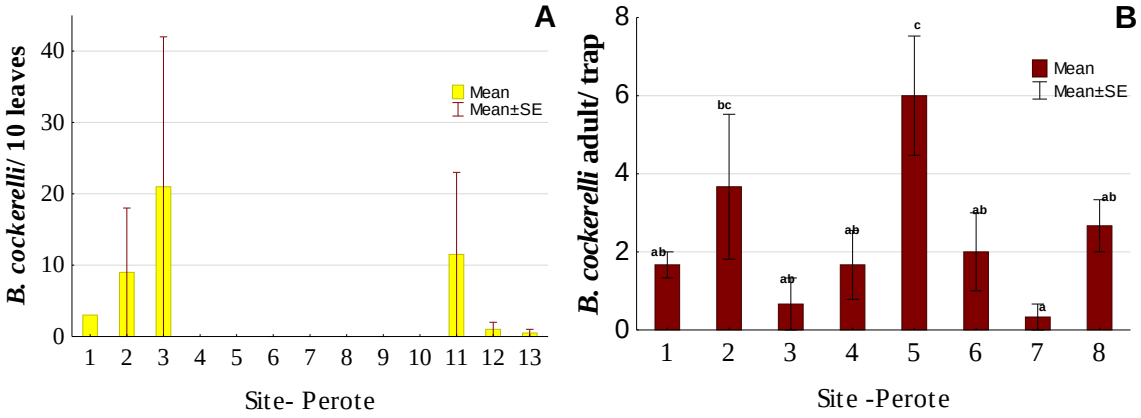

Figure 1. Mean (±SEM) of (A) *B. cockerelli* nymphs per leaf, and (B) *B. cockerelli* adults per trap in commercial potato fields in Perote, Veracruz, Mexico.

Although some specimens of *B. cockerelli* are not analyzed yet, the 2013 survey in Perote showed that there were two haplotypes of *B. cockerelli*: southwestern and , the latter being the predominant. In 2014, only the Central haplotype was found (Fig. 3A). In both years two specimens tested positive for Lso (Fig. 3B), which were 3.4 % and 6.9 % of all the samples in 2013 and 2014, respectively. In spite of

these low percentages of “hot” psyllids, all potato fields were severely infected with ZC (Villanueva et al. 2014).

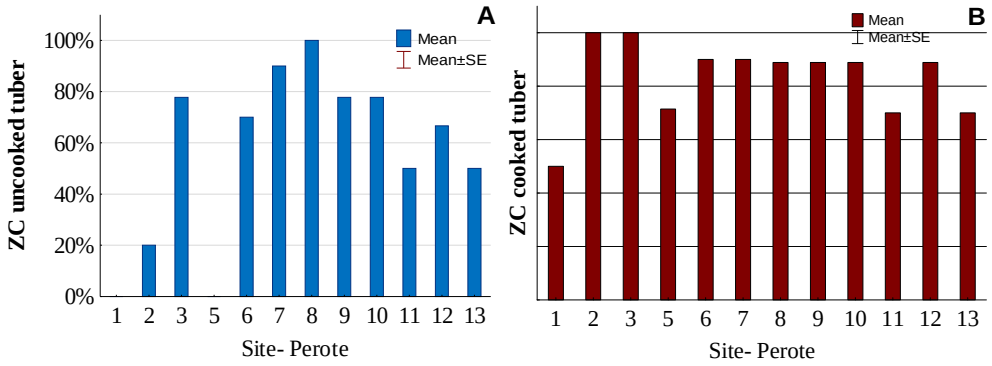

Figure 2. Mean percentages of zebra chip disease on (A) uncooked tubers, and (B) fried tubers in Perote, Veracruz, Mexico.

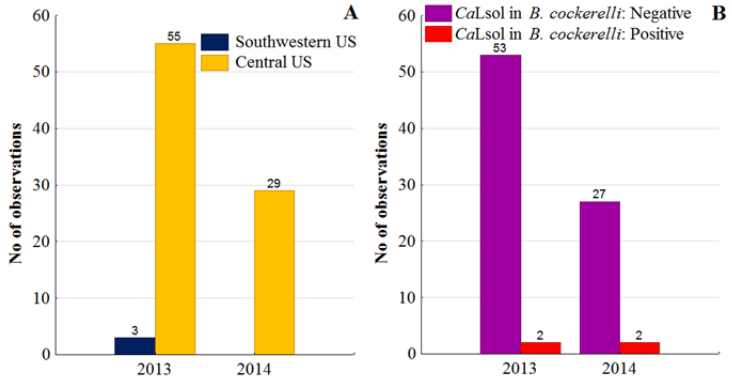

Figure 3. (A) Numbers of *B. cockerelli* per haplotype in 2013 and 2014, and (B) Numbers of positive psyllids carrying Lso (*Ca*Lsol the legend) in 2013 and 2014.

Tomato field in Acultzingo, Veracruz. In Acultzingo, 100% of *B. cockerelli* (*n*=25) were positive to Lso, and all were collected from a tomato field in July 2014 (Fig. 4). Whereas, in Perote only 3.5% of *B. cockerelli* (*n*=29) were positive to Lso, all from potato fields collected one day earlier (Fig. 4). During the same period Dr. Henne reported that from all samples collected on different regions of the USA (*n*=71) none was positive to Lso (Fig. 4). This result is important since there is a yearly influx of *B. cockerelli* into the U.S. from Mexico; and these insects not only come from potato fields but from other solanaceous hosts. In addition, Acultzingo is can be considered a humid rainforest in the subtropical region and surrounded by mountains. Acultzingo has more moderate temperatures (from 10 to 20° C) than the potato fields near Saltillo (from 4 to 22° C) or Perote (from 2 to 18° C), the two latter areas with freezing periods during winter. Acultzingo is located at 1718 m above sea level compared with the >2400 on the potato regions of Saltillo and Perote.

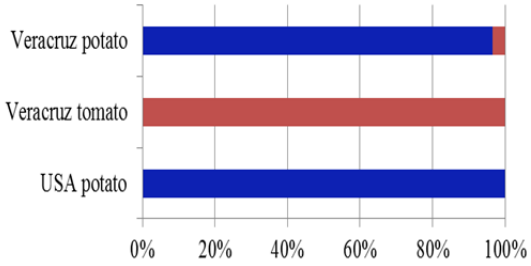

Figure 4. Positive (red) and negative (blue) percentages of *B. cockerelli* carrying Lso in Acultzingo and Perote in Veracruz and different potato regions of the U.S. in July 2014 from data reported by Dr. Henne.

Potato, pepper and tomato fields in Coahuila. In 2013, a potato field was inspected and 100% of tubers from commercial and experimental sites had ZC symptoms. From this field, two *B. cockerelli* specimens were collected (Villanueva et al. 2014); both were haplotypes, and one of them was positive to Lso. Also, two specimens collected from an experimental pepper field (Villanueva et al., 2014) were classified as Central haplotype, both testing positive for Lso. In 2014, a specimen collected from pepper plants was identified as a central haplotype, but negative to Lso. Several psyllid specimens are currently being processed from potato and tomato from this region.

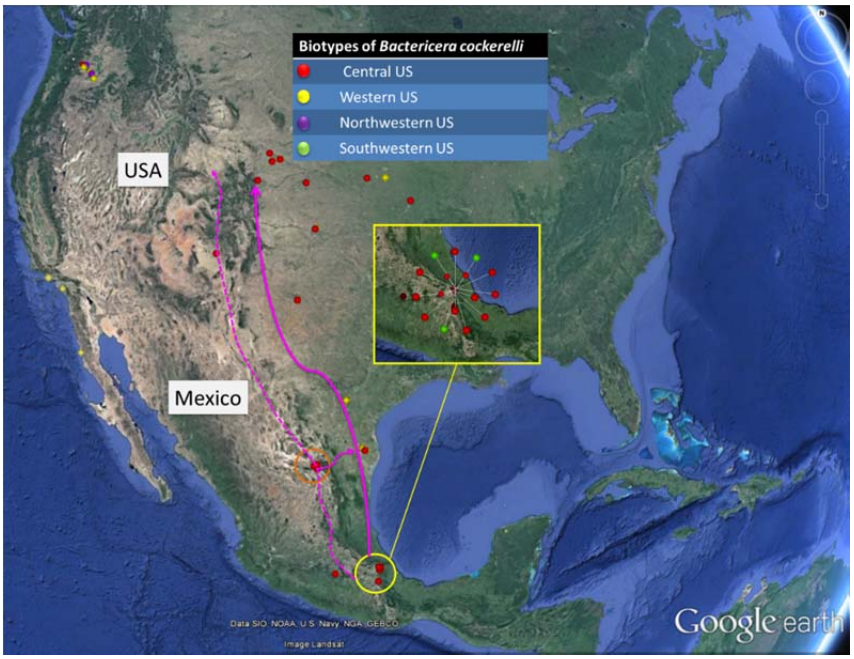

Figure 5. Geographical locations from which four *B. cockerelli* haplotypes in the US and Mexico (*Central, Western, Northwestern, and Southwestern*) were collected. Circles show the areas where this study was conducted: orange (Saltillo) and yellow (Perote and Acultzingo). The US haplotype is reported for first time in Perote. Migration o*f B. cockerelli* may also occur using a second route (dashed lines) throughout mountainous regions in the U.S. and Mexico

Conclusions

In this study, we confirmed the presence of the central haplotype in Perote. This haplotype is found in different regions of the US, even in the Pacific Northwest (Fig. 5; Liu et al., 2006; Swisher et al., 2013a; Swisher et al., 2013b). It is speculated that *B. cockerelli* has a migration route using the wind currents of the Gulf of Mexico in the spring. Here we hypothesize that these insect may use a second route passing throughout the mountainous regions of Mexico and the U.S. This latter route probably is continuous during the entire year, with a slow displacement of *B. cockerelli*. The latter might correspond to the finding of potato psyllids in the mountainous regions of the US during different months of the year even in the hot summer and frigid winter months (Romney, 1939).

The detection of 100% “hot” *B. cockerelli* specimens to Lso collected from tomatoes in Acultzingo is significant for several reasons. This area has a different ecosystem and plant diversity; thus some of the plant species in this regions (or areas with similar biodiversity in Mexico or Central America) may be the reservoir for the bacterium that causes ZC, hence the area to search for reservoir plants should be expanded. Also, the presence of other commercial solanaceous in this research indicated that this complex potato psyllid-ZC system needs further research in Mexico to understand the recent expansion

of this problem into the US The damages caused by *B. cockerelli* in tomatoes, peppers, or tomatillos are not as pronounced as in potatoes. This may be one of the reasons why the disease is poorly studied in Mexico.

Since 2001, the incidence of this pest in potatoes in western and central US and Mexico regions had increased in frequency and severity; thus the haplotype that are frequently found in the US are constantly selected for high tolerance to insecticide (Liu and Trumble 2007) in Mexico. Peppers and tomatoes are high value crops that require continuous use of pesticides, as well as in potatoes (>30 applications per growing season). Thus, *B. cockerelli* migrating into the U.S. might be selected for tolerance to a wide spectrum of pesticides, and *B. cockerelli* (i.e. from Acultzingo) adapted to more moderate temperatures might be moving north due to current climatic changes having a greater impact on the transmission of Lso.

Acknowledgments

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References

Liefting, L. W., B. S. Weir, S. R. Pennycook, and G. R. G. Clover. 2009. *Candidatus* Liberibacter solanacearum', associated with plants in the family Solanaceae. Int. J. Syst. Evol. Microbiol. 59: 2274–2276.

Liu, D., and J. T. Trumble. 2007. Comparative fitness of invasive and native populations of the potato psyllid (*Bactericera cockerellii*). Entomol. Exp. Appl. 123: 35–42.

Liu, D., J. T. Trumble, and R. Stouthamer. 2006. Genetic differentiation between eastern populations and recent introductions of potato psyllid (*Bactericera cockerelli*) into western North America. Entomol. Exp. Appl. 118: 177–183.

Munyaneza, J. E., J. M. Crosslin, and J. E. Upton. 2007. Association of Bactericera cockerelli (Homoptera: Psyllidae) with "Zebra Chip," a New Potato Disease in United States and Mexico. J. Econ. Entomol. 100: 656–663.

Munyaneza, J. E., V. G. Sengoda, J. M. Crosslin, G. De la Rosa-Lozano, and A. Sanchez. 2009. First Report of *Candidatus* Liberibacter psyllaurous in Potato Tubers with Zebra Chip Disease in Mexico. Plant Dis. 93: 552.

Romney, V. E. 1939. Breeding Areas of the Tomato Psyllid, *Paratrioza cockerelli* (Šule). J. Econ. Entomol. 32: 150–151.

Swisher, K. D., J. E. Munyaneza, and J. M. Crosslin. 2013a. Temporal and Spatial Analysis of Potato Psyllid Haplotypes in the United States. Environ. Entomol. 42: 381–393.

Swisher, K. D., V. G. Sengoda, J. Dixon, E. Echegaray, A. F. Murphy, S. I. Rondon, J. E. Munyaneza, and J. M. Crosslin. 2013b. Haplotypes of the Potato Psyllid, *Bactericera cockerelli*, on the Wild Host Plant, *Solanum dulcamara* , in the Pacific Northwestern United States. Am. J. Potato Res. 90: 570–577.

Villanueva, R. T., and G. Esparza-Diaz. 2013. Psyllids, Whiteflies and Environmental Factors Affecting Potato Trials in S. Texas: 2010-2012. Proc. 12th Annu. 2012 ZEBRA CHIP Report. Sess. 163–167.

Villanueva, R. T., G. Esparza-Díaz, I. Garay-Peralta, and S. Sanchez-Peña. 2014. Intricacies on the Control of the Potato Psyllid in Mexico and South Texas. Proc. 13th Annu. 2013 ZEBRA CHIP Report. Sess. 81–86.

Sequence Analysis of '*Candidatus* Liberibacter Solanacearum' (Lso-C) Isolated from Carrot Psyllids Collected in Scandinavia

McCue, K.F.[1], Lazo, G.R.[1] and Munyaneza, J.E.[2]
[1]Crop Improvement and Genetics Research, USDA-ARS Western Regional Research Center 800 Buchanan Street, Albany, CA 94710
[2]Fruit and Vegetable Insect Research, USDA-ARS 5230 Konnowac Pass Road, Wapato, WA 98951

Abstract
The fastidious prokaryote '*Candidatus* Liberibacter solanacearum' (Lso), transmitted by the tomato potato psyllid (*Bactericera cockerelli*), is associated with the zebra chip disease of potato. Plants infected with Liberibacter may experience significant yield losses and these plants also serve as potential reservoirs for Liberibacter to spread to other psyllid hosts with alternate crop feeding preferences, potentially introducing new epidemiological focal points among crops. New associations between Liberibacter species and crop plants have been detected in different parts of the world, presenting concerns about the potential roles of these strains in causing disease. Carrots (*Daucus carota*) showing damage from feeding of carrot psyllids (*Trioza apicalis*) were reported to be infected with Lso. Using sequence information generated from Lso positive psyllids collected in Finland, Norway and Sweden we have constructed a metagenomics map for this new Lso haplotype LsoC. We have compared the genomes of the LsoC to the closely related LsoB haplotype, the only Lso whose genome sequence is currently available. In addition differences between the Lso sequences derived from samples from each country were identified. These differences will aid in the monitoring of the regional populations.

Introduction
Psyllids are common pests of agricultural crops. Psyllids and their nymphs can cause damage during feeding and can also transmit bacterial and viral pathogens. Transmission of the fastidious bacteria '*Candidatus* Liberibacter' species is associated with several emerging plant diseases of citrus and solanaceous crops. Three species are implicated in citrus greening disease (Huanglongbing), '*Ca*. L. asiaticus' (Las), '*Ca*. L. africanus' (Laf), and '*Ca*. L. americanus' (Lam), transmitted by the Asian citrus psyllid, *Diaphorina citri* Kumayama and the African psyllid *Trioza erytreae* Del Guercio. The potato zebra chip disease is associated with two haplotypes A and B (Central and North America and New Zealand) of '*Ca.* L. solanacearum' (Lso) (syn. '*Ca*. L. psyllaurous'), vectored by the tomato potato psyllid *Bactericera cockerelli* Sulc. Recently, carrots showing symptoms of psyllid feeding damage were confirmed to be infected by a third haplotype C of Lso in Scandinavia (Nelson et al., 2011). In this study we used high throughput sequencing to examine the relationship and differences of Lso haplotype C from Scandinavia to Haplotype B from America.

Materials and Methods
Sample preparation. Carrot psyllids were collected from symptomatic and asymptomatic carrot plants from fields in Finland, Norway and Sweden. DNA was extracted using a Qiagen Kit. DNA was prepared at a final concentration of 100 ng/µl for a total of 10 µg. Following DNA isolation DNA samples were screened by conventional PCR to confirm the presence or absence of Lso (Munyaneza *et al*., 2010). Illumnia standard and mate pair libraries were prepared according to manufacturer's instructions.

Sequencing genomes of psyllids collected from symptomatic / asymptomatic carrot plants. High-throughput sequencing was conducted on psyllid samples which included a diverse set of location origins and the presence/absence of the Lso phenotype. The sequences were assembled and resulting contigs were screened against microbial reference genomes to detect potential Lso presence. Candidate matches were then sought in the other geographic samplings to potentially identify strain differentiation among samples.

Table I. The total number of sequences generated from each psyllid DNA sample.

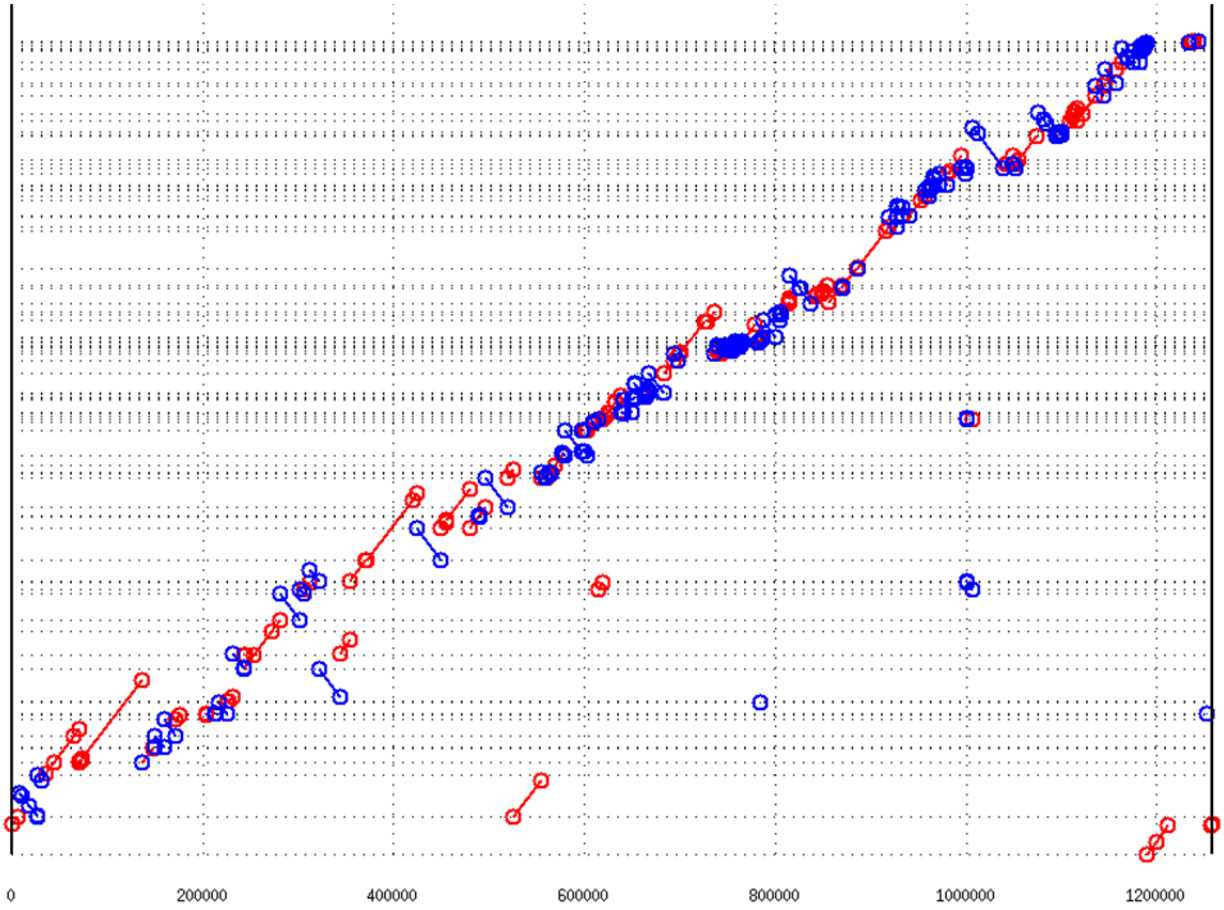
Sample	**Asymptomatic**	**Symptomatic**
Finland	51,812,097	99,993,754
Norway	66,610,113	48,403,014
Sweden	78,719,118	49,913,432

Extracting candidate microbial species from psyllid sequence data. Sequences were assembled from the raw Illumina reads using VELVET (version 1.2.08) as the assembler software (Zerbino and Birney, 2008). Since the psyllid samples may also have a high-abundance of microflora-associated sequences and since the sequences were assembled in an un-filtered state, there may be other k-mer parameters which may be beneficial in selecting out different genome constituents. Potential k-mer distributions were reviewed using the KmerGenie software tool (Chikhi & Medvedev 2013). k-mer peaks were generally surveyed between the 15- and 41-mer range for test assemblies (data not shown). Other optimal settings for assembly may be desirable to distinguish between insect and microbiome; however, the initial assembly indicated that a k-mer setting of 29 matched many microbial genomes including Liberibacters, *Wolbachia*, and related *Agrobacterium* and *Rhizobium* matches. All data was reassembled using a k-mer = 29 and the results presented below.

Table II. Analysis of contig formation for symptomatic and asymptomatic DNA samples using uniform k-mer assembly settings (29) for each DNA sample.

Country	**Assay**	**N50**	**MAX**	**# of Contigs**	**Depth**
Finland	Symptomatic	147	11,888	7,552,276	3.2
Finland	Asymptomatic	69	5,649	1,455,778	3.5
Norway	Symptomatic	123	6,601	1,792,425	6.3
Norway	Asymptomatic	96	5,661	1,976,517	3.7
Sweden	Asymptomatic	87	47,013	2,775,458	3.6
Sweden	Symptomatic	135	8,277	2,061,652	4.0

A.



B.

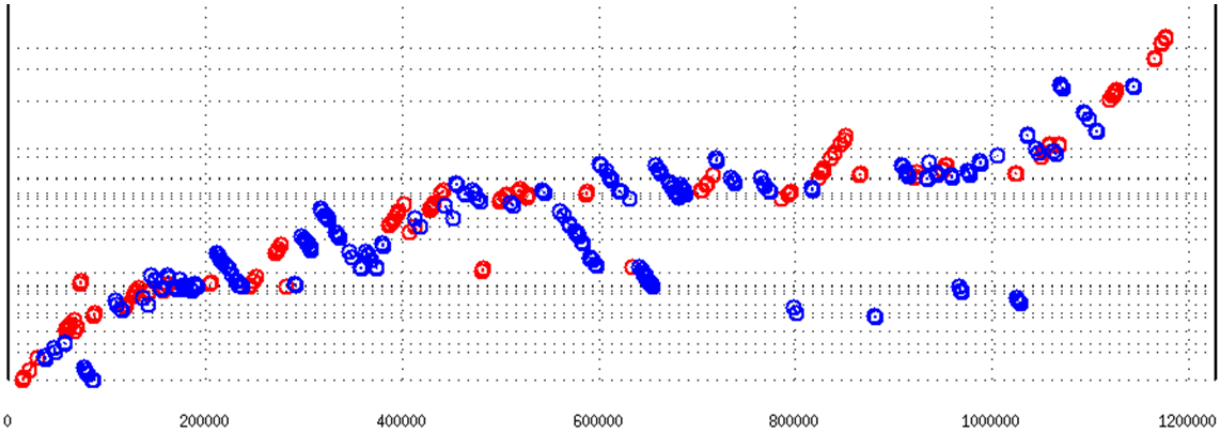


Figure 1. Alignment of k-mer 29 assembled contigs of the Norway symptomatic sample to the Lso (A.) and Las (B.) reference genomes (X-axis).

Assembled contigs were BLASTN compared against representative microbial genomes commonly found in earlier studies of psyllid microflora (Saha *et al.*, 2012). The numbers in Table II reflect the matches detected from contigs derived via the optimum k-mer setting with the most microbial matches.

Number are given for contigs of psyllid DNA sequences collected from both asymptomatic and symptomatic plants. There is generally 10 fold more contigs aligned to microbial genomes in the symptomatic plant samples.

Table III. Total number of k-mer=29 contigs from asymptomatic and symptomatic DNA samples that matched the 5 different bacterial reference genomes for each DNA sample.

Sample	**Asymptomatic**	**Symptomatic**
Finland	12	1,278
Norway	25	394
Sweden	123	271

Reference genomes identified as being closely related to microbial genomes (GenBank accession numbers) detected in other psyllid genomes were: *Candidatus* Liberibacter solanacearum CLso-ZC1 (CP002371), *Candidatus* Liberibacter asiaticus str. Psy62 (CP001677), *Wolbachia* endosymbiont (AM999887), *Agrobacterium tumefaciens* C58 (AE007869), and *Rhizobium etli* CFN 42 (CP000133).

Table IV. Total number of k-mer=29 contigs with significant identity to the five different bacterial reference genomes for each DNA sample.

Sample	**Assay**	**Lso**	**Las**	**Wolbachia**	**Agrobacterium**	**Rhizobium**
Finland	Symptomatic	1,055	27	8	30	17
Norway	Symptomatic	226	190	4	5	7
Sweden	Symptomatic	2,585	144	8	18	9

Comparing candidate species to reference genomes. Genome coverage was significantly matched to the CLso-ZC-1 strain when detected (Lin *et al*., 2011). Assembled contigs from optimized k-mer settings were used to match genome differences between the carrot candidates and the CLso-ZC-1 strain used as a reference genome. Longer contigs were formed against the Lso reference strain (CP002371) than with the Lso reference strain (CP001677), and was also exemplified with abundantly more matches (Figure1).

Developing genome specific probes to differentiate candidate microbial species. Differences detected were generally distinct from the Lso reference genome; however, those of Scandinavian origin seemed closely matched. If these are indeed pathogenic strains they may represent newer variants of the pathogen. A true Koch's postulate has not been validated in this case; however, the molecular data is convincing that a new candidate variant may be involved as a causal agent of psyllid-transmitted disease. Alignments to the reference genomes and SNP determinations were done using the MUMmer 3.0 software package (Kurtz *et al*., 2004). SNPs were filtered down to represent 20-nt stretches conserved with the reference which were not due to insertion/deletion or too short of an assembled sequence available which did not match the reference.

Table V. Total number of SNPs per country-derived assemblies compared to the Lso reference genome.

Symptomatic (k-mer)	**Finland (27)**	**Norway (29)**	**Sweden (23)**
SNP candidates called	3,414	19,353	1,996
Excluded due to length (<20)	1,799	61	595
Excluded due to INDEL	61	250	4,283
Remaining candidates	1,554	15,009	1,151

Results and Discussion

In total > 1Mbp of sequence aligned along the length of the LsoB reference genome. There were 24 contigs > 10 kbp genome totalimg of 551 kbp. The largest contig was 53.5 kbp. The most notable difference is the deletion of the prophage region at the end of the reference, along with numerous small insertions and deletions and small translocations and rearrangements. There were a total of 15,637 SNP alignments to the Lso reference. 15,009 are to the Norway symptomatic contigs (this is the most because the genome basically fully assembled); 1,151 are to Sweden symptomatic contigs; and 1,554 are to Finland symptomatic contigs. 47 SNPs differentiated all three Scandinavian strains from the Reference strain. 1,984 differentiated two of the three Scandinavian strains from the Reference. 13,611 differentiated only one of the Scandinavian strains from the Reference. Most studies in developing Lso-specific probes have focused on ribosomal-based probes. In the screening of potential SNP-variation between the Scandinavian strains and the Lso reference genome, other candidate loci were identified. These regions represent potential new targets to differentiate Liberibacter between different regions.

Acknowledgements

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References

Chikhi, R., and P Medvedev 2013. Informed and Automated k-Mer Size selection for Genome Assembly. (http://arxiv.org/pdf/1304.5665v1.pdf).

Kurtz, S, Phillippy, A. Delcher, A.L., Smoot, M., Shumway, M., Antonescu, C. and Salzberg, S.L. 2004. Versatile and open software for comparing large genomes. Genome Biol 5:R12

Lin, H, Lou, B., Glynn, J.M., Doddapaneni, H., Civerolo, E.L., Chen, C., Duan, Y., Zhou, L. and Vahling, C.M. 2011. The complete genome sequence of '*Candidatus* Liberibacter solanacearum', the bacterium associated with potato Zebra Chip disease. PLoS ONE 6:e19135.

Munyaneza, J.E., Fisher, T.W., Sengoda, V.G., Garczynsk, S.F., Nissinen, A. and Lemmetty, A. 2010. Association of "*Candidatus* Liberibacter solanacearum" With the Psyllid, *Trioza apicalis* (Hemiptera: Triozidae). Eur J Econ Entomol 103(4):1060-1070.

Nelson, W.R., Fisher, T.W., and Munyaneza, J.E. 2011 Haplotypes of "*Candidatus* Liberibacter solanacearum" suggest long-standing separation. Eur J Plant Pathol 130:5-12

Saha, S., Hunter, W.B., Reese, J., Morgan, J.K., Marutani-Hert, M., Huang, H. and Lindeberg, M. 2012. Survey of Endosymbionts in the *Diaphorina citri* metagenome and assembly of a *Wolbachia* wDi Draft Genome. PLoS ONE 7:e50067.

Zerbino, D.R., and Birney, E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res 18:821-829.

Analyzing Expenditures for Control of *Bactericera cockerelli* in U.S. Potatoes

Greenway, G., McIntosh, C., and Guenthner, J.
Department of AERS, University of Idaho, Moscow, ID 83844-2334, USA

Abstract
In 2000, the potato psyllid as a labeled target made up just one half of a percent of all insecticide expenditures in U.S. potatoes (Fig. 1). By 2013 the psyllid was the second largest pest category comprising over 21 percent of U.S. potato insecticide expenditures (Fig. 2). Data on actual insecticide applications were collected from growers in Texas who provided data from 2009-2014 as well as growers in Kansas and Nebraska who provided data from 2009-2013. The most widely used insecticides in all three states were Movento and Admire. Texas growers spent an average of $280 per acre on psyllid control over the six year time frame. Kansas growers spent a five year average of $283 per acre, and Nebraska growers spent an average of $228 per acre.

Introduction
Potato psyllids vector '*Candidatus* Liberibacter solanacearum' the putative causal agent of potato zebra chip (ZC). The economic analysis part of a USDA-funded project focuses on the project's primary goal of the development of a comprehensive, environmentally responsible ZC management program. The three specific objectives of the economic analysis part are:
1. Estimate grower losses due to costs of current ZC control practices
2. Estimate ZC losses due to poor tuber quality
3. Evaluate the economics of alternative disease management strategies

Materials and Methods
To estimate ZC control costs (Table 1.) we relied on data provided by cooperating potato growers and the Texas A&M Potato Psyllid Survey Report from 2009-2014. The data tracks insecticide use in 89 commercial potato fields in Texas, Kansas and Nebraska. When application rates were not reported by growers we assumed the highest label rate for individual applications complying with total application limit restrictions. During each year we obtained insecticide and application cost estimates using university surveys, and by conducting our own surveys of agricultural chemical dealers (North Dakota State University 2010; Patterson & Painter 2012, 2013; Texas A&M University 2013).

To analyze the impacts of ZC on profitability in major potato producing states we modify and develop enterprise budgets for seven potato producing regions in six states. In the states of Idaho, Wisconsin, and Colorado, potato enterprise budgets are already developed each year by extension professionals (Patterson 2012; Barnett, 2012, CSU Extension, 2012). We make small modifications to the already developed budgets in these states. In the Rio Grande Valley of Texas, Columbia Basin of Washington and Oregon, and Klamath Basin of Oregon and California we estimate the budgets using the most recently available budget as a guide, updating each line using grower input, potato production guides, land value guides, and input cost surveys, (Texas ASFMRA, 2012; Patterson & Painter, 2012). Price and yield data for each region are obtained from USDA/NASS; we apply the three year average of both.
To analyze the dollar per acre impact of ZC on grower profits we consider two scenarios. Under the first scenario costs and returns without ZC are compared to costs and returns with a ZC infestation. The ZC infestation in Scenario 1 is represented by increasing costs by the six year average $280 per acre cost encountered by Texas growers and reducing revenue by 10 percent to capture both quality and yield

effects. In the second scenario we compare a no zebra chip situation to a less severe infestation by increasing costs by $185 per acre and decreasing revenue by 5 percent.

To estimate average annual ZC losses in Texas due to poor tuber quality, a Delphi survey is used to obtain expert opinion from potato experts in the state knowledgeable about zebra chip. We apply results of the survey to 2010-2012 USDA-NASS marketing year average price data, and 2010-2012 USDA NASS Production estimates for the state.

Results and Discussion

Texas growers used a total of 25 different insecticides for the 2009-2014 crops. Currently, the most widely used product is Movento. In Texas, during the last four years, 100 percent of fields have been treated with Movento. Consideration of resistance issues will likely play a key role in ZC management moving forward.

Regardless of location, the number of insecticide applications and costs of protecting the crop remains high (Table 1). The six year average number of applications for control of ZC in all Texas locations was 9.2 In Kansas and Nebraska the five year average was 9.4. The six year average cost was about $280 per acre in all Texas locations. The five year average cost in all Kansas locations was $283 per acre. The five year average cost in all Nebraska locations was $228 per acre.

Growers in the largest potato producing states operate on thin margins. In order to be profitable they must achieve high yields and capture quality incentives, both of which are jeopardized by ZC. According to the Southern Idaho Potato Cooperative, the Eastern Idaho contract for processing potato prices in 2010, 2011, and 2012 were $6.02, $6.80, and $7.55, per cwt. Costs of production in the same region were estimated at $6.29, $7.16, and 7.40 per cwt (Patterson 2012). The Idaho fresh potato market is characterized by volatility. The statewide 2010 marketing year average price was $9.55 per cwt, dropping to $8.05 per cwt, in 2011, and $5.30 per cwt in 2012 (USDA-NASS 2012).

If growers in Idaho and the Columbia Basin faced a 10% reduction in revenue due to quality and yield issues, while dedicating an average of 9 insecticide applications to the crop, the economic impacts would be far reaching (Fig. 3). Using 2010-2012 average (fresh and processed category) prices of $7.47 per cwt, losses could range from $357 per acre to as high as $597 per acre (Fig. 3).The Klamath Basin region of Oregon and California faces high production costs similar to Idaho and the Columbia Basin. Under ZC conditions with (2010-2012) average prices of $8.11 per cwt, grower profits could be reduced to just $111 per acre. Texas growers benefit from warmer temperatures and are often able to market early potatoes at prices higher than the national average. A price of $13.80 per cwt, combined with a 10% revenue loss due to ZC infestations would reduce profits by more than 60% of what they would have been without psyllid and ZC pressure (Fig. 3). If Texas prices were to fall to the 3-year US average price of $9.00 per cwt, profits would erode further and losses would occur. Wisconsin and Colorado producers benefit from higher (2010-2012) average prices at $9.93 and $9.96 per cwt. The higher prices in combination with lower costs of production than growers in other regions help cushion the magnitude of impact.

Using the same 2010-2012 average price data with a less severe 5% revenue reduction and $180 per acre increase in cost still produces losses in the major potato producing regions of Idaho and the Columbia Basin of Washington, though the magnitude of loss is much less severe (Fig. 4). Timing of psyllid arrival

Fig.1. Insecticide Expenditure in Potatoes 2000

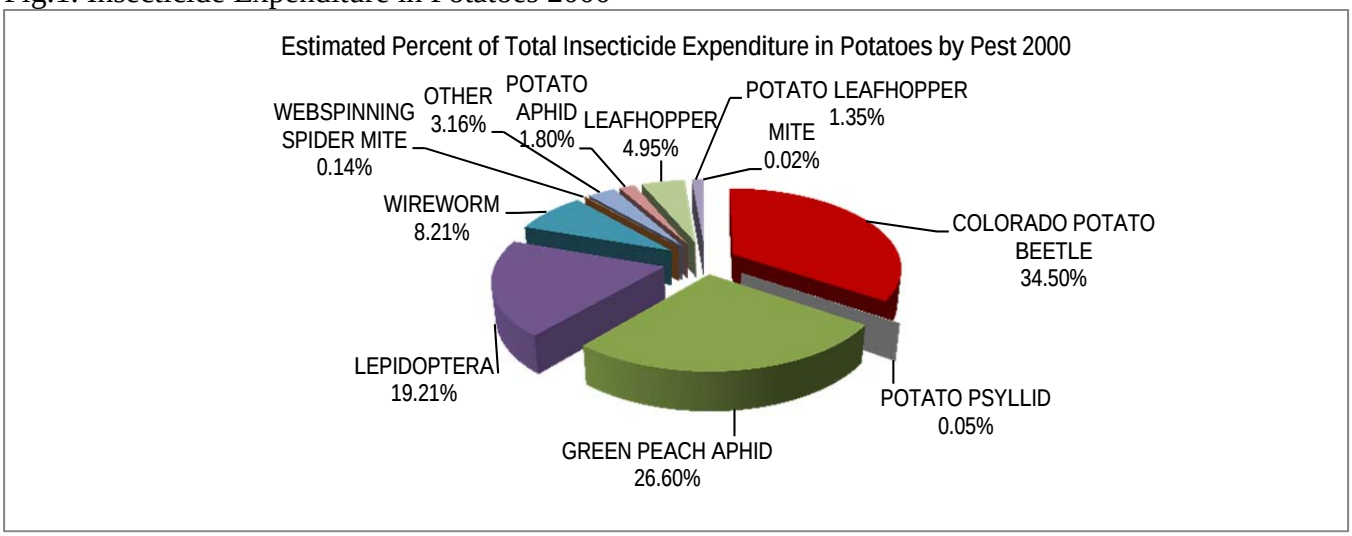


Fig. 2. Insecticide Expenditure in Potatoes 2013

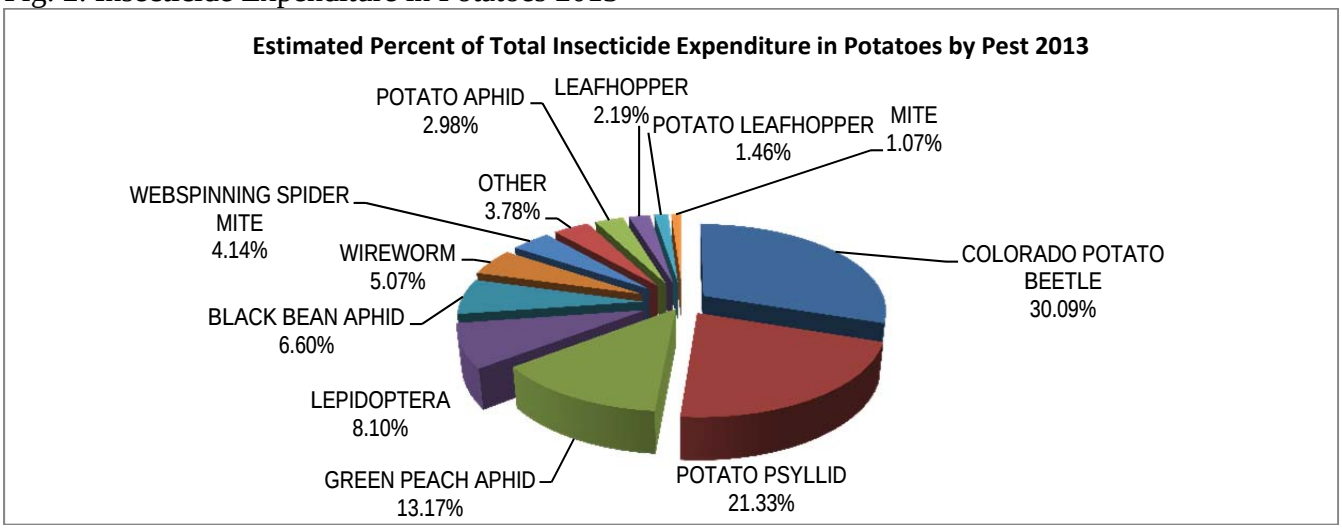
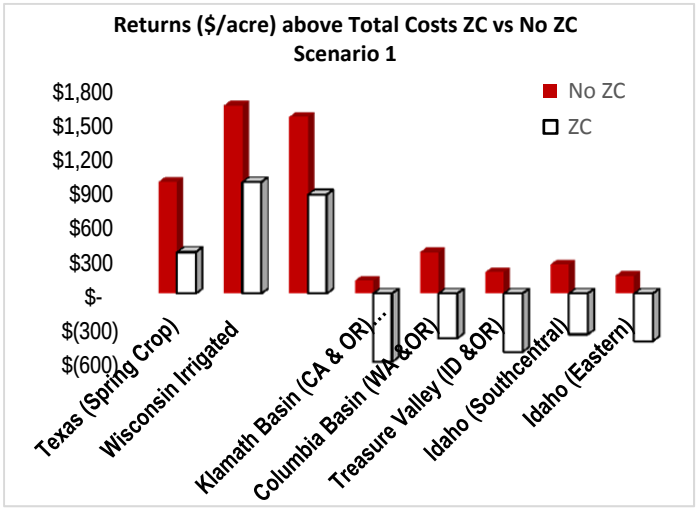


Fig. 3 & 4. Estimated returns above total costs ($/acre) by region with and without ZC



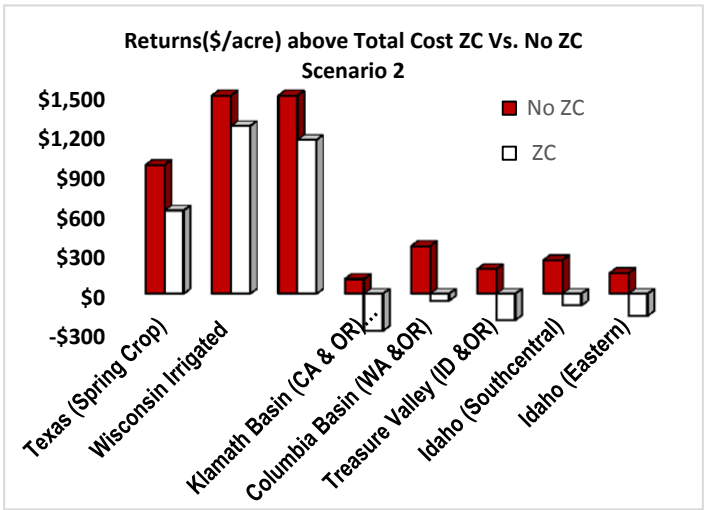


Table 1. Insecticide costs for ZC and psyllid control, 2009-14

Year/Locations	Fields	Lowest Cost ($/ac)	Highest Cost ($/ac)	Simple Average ($/acre)
2009				
Kansas, Garden City	3	$ 214	$ 241	$ 223
Texas, Dalhart	2	$ 286	$ 292	$ 289
Texas, McAllen	4	$ 296	$ 344	$ 319
Texas, Olton	1	$ 223	$ 223	$ 223
Texas, Pearsall	3	$ 214	$ 452	$ 358
2010				
Kansas, Garden City	3	$ 303	$ 399	$ 367
Nebraska, Alliance	3	$ 296	$ 354	$ 315
Nebraska, Imperial	3	$ 321	$ 515	$ 397
Nebraska, Minden	3	$ 131	$ 191	$ 153
Texas, Dalhart	2	$ 323	$ 388	$ 355
Texas, McAllen	6	$ 176	$ 499	$ 362
Texas, Olton	1	$ 270	$ 270	$ 270
Texas, Pearsall	3	$ 151	$ 226	$ 180
2011				
Kansas, Garden City	1	$ 443	$ 443	$ 443
Nebraska, Alliance (Angora, Bridgeport)	2	$ 146	$ 231	$ 188
Nebraska, Minden	1	$ 31	$ 31	$ 31
Texas, Dalhart	3	$ 252	$ 358	$ 304
Texas, McAllen	4	$ 229	$ 338	$ 274
Texas, Olton	2	$ 240	$ 516	$ 378
Texas, Pearsall	3	$ 154	$ 401	$ 279
2012				
Kansas	2	$ 339	$ 355	$ 347
Nebraska	2	$ 183	$ 341	$ 262
Texas, Dalhart	2	$ 313	$ 425	$ 369
Texas, McAllen	5	$ 147	$ 594	$ 317
Texas, Olton	2	$ 328	$ 531	$ 441
Texas, Pearsall	3	$ 250	$ 340	$ 299
2013				
Kansas, Garden City	1	$ 318	$ 318	$ 318
Nebraska, Bridgeport	1	$ 253	$ 253	$ 253
Texas, Dalhart	2	$ 179	$ 211	$ 195
Texas, McAllen	4	$ 161	$ 391	$ 183
Texas, Olton	1	$ 124	$ 124	$ 124
Texas, Pearsall	2	$ 227	$ 298	$ 262
2014				
Texas, Dalhart	2	$ 223	$ 247	$ 235
Texas, McAllen	8	$ 238	$ 238	$ 238

will have a significant impact on the costs of insecticides needed to protect the crop in various regions and the level of risk associated with length of exposure to psyllid pressure.

Quality losses due to ZC present a wide range of estimates. In less severe cases growers will lose quality incentive premiums or face penalties, but in the early years of Zebra Chip many Texas growers lost more than half of their paid yield due to rejected loads. On average, Texas potato experts estimated a 23 percent decrease in paid yield caused by ZC. Applying this estimate to 2010-2012 USDA-NASS marketing year average price data, and 2010-2012 USDA NASS production estimates generates 17,000,000 annual average loss in Texas due to poor tuber quality caused by ZC.

References

Barnet, K. 2012. Potato processed irrigated budget for Wisconsin. Retrieved from: http://cdp.wisc.edu/vegetable.htm

Colorado State University Cooperative Extension 2012. Estimated costs and returns-irrigated potatoes in northeastern Colorado. Retrieved from: http://www.coopext.colostate.edu/ABM/neirpts12.pdf

North Dakota State University. 2010. Insecticide price list. Retrieved from: http://www.ag.ndsu.nodak.edu/aginfo/entomology/entupdates/ICG_09/25_price_list09.pdf

Patterson, P. 2012. Cost of potato production comparisons for Idaho commercial potato production. Retrieved from: http://web.cals.uidaho.edu/idahoagbiz/files/2013/01/2012-Cost-of-Potato-Production-Report.pdf

Patterson, P. and K. Painter. 2012. Idaho crop input price summary. Agricultural Economics Extension Series No 729. University of Idaho.

Patterson, P. and K. Painter. 2013. Crop input cost summary. Agricultural Economics Extension Series No 13-03. University of Idaho.

Texas A&M University AgriLife Extension. 2013. 2013 Texas agricultural custom rates. Retrieved from http://agecoext.tamu.edu/files/2012/05/CustomRateSurveyMay2013.pdf

Texas Chapter of the American Society of Farm Managers and Rural Appraisers. 2013. Texas rural land value trends 2012. Retrieved from http://recenter.tamu.edu/data/rland/trends/ASFMRA_2012.pdf

United States Department of Agriculture. 2103. Potatoes 2012 summary. Retrieved from: http://usda01.library.cornell.edu/usda/current/Pota/Pota-09-19-2013.pdf

Determination of Potato Zebra Chip Tolerance of Six Advanced Potato Lines under Field Conditions in the Toluca Valley, Mexico

Rubio-Covarrubias, O.A.[1], Cadena-Hinojosa, M.A.[1], Flores-López, R.[1], Munyaneza, J.[2], Prager, S.[3] and Trumble, J.[3]
[1)]Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP)
Programa de Papa, Conjunto SEDAGRO s/n, Metepec, Estado de México. C.P. 52140.
[2] USDA-ARS, Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road Wapato, WA, 98951.
[3] Department of Entomology, University of California Riverside, 900 University Ave., Riverside, CA 92521.

Abstract
Zebra chip, a new disease of potato, is threatening potato production in Mexico, Central America, the United States, and New Zealand. The disease is caused by the phloem-limited '*Candidatus* Liberibacter solanacearum' (Lso), vectored by the potato psyllid, *Bactericera cockerelli*. Currently, the only means to manage zebra chip is by conducting numerous insecticide applications targeted against the potato psyllid. A field study was carried out during three years in the Toluca Valley, Mexico, to assess the zebra chip resistance of six advanced potato breeding lines. In addition, the commercial variety "Fianna" was included as a control. There were no significant differences in yield and number of potato psyllid nymphs per plant among the seven potato genotypes. However, significant differences were observed in the percentage of healthy tubers, area under disease progress curve in the foliage and in the severity of the internal tuber discoloration. The 6 potato clones showed higher tolerance to ZC symptoms than Fianna.

Introduction
Zebra chip (ZC) disease is one of the main potato production constraints in Mexico, New Zealand, the United States, and Central America (Munyaneza, 2012). The symptoms of ZC, also known as 'potato purple-top' and 'internal tuber browning' in Mexico, resemble those caused by infection of phytoplasma in potato.

In Mexico, ZC is ubiquitous throughout the country (Rubio-Covarrubias et al., 2006). The Toluca Valley, located in the central plateau of Mexico, used to be an important seed-potato producing region. Because of ZC, seed potato production no longer exists in this region (Rubio-Covarrubias et al., 2011). Prior to the discovery of the association of ZC with the newly described bacterium '*Candidatus* Liberibacter solanacearum' (Lso) (Hansen et al., 2008, Liefting et al., 2008), the disease was believed to be caused by potato purple top phytoplasmas in Mexico, and vectored by the potato psyllid, *Bactericera* (= *Paratrioza*) *cockerelli* Sulc (Leyva-Lopez et al., 2002; Rubio-Covarrubias et al., 2006). Later studies showed that Lso was indeed widespread in Mexico and associated with the observed symptoms in potato crops (Munyaneza et al., 2009, Rubio-Covarrubias et al., 2011).

Currently, ZC management is mainly based on insecticide applications targeted against the potato psyllid. This control strategy is expensive and pesticide intensive (Butler and Trumble, 2012; Guenthner et al., 2012), underscoring the need for development of potato varieties that are resistant to Lso and/or potato psyllid. A number of breeding programs for ZC resistance are underway in ZC-affected countries and are focused on the generation of varieties (Cadena-Hinojosa et al., 2003; Butler et al., 2011; Anderson et al., 2012; Butler and Trumble, 2012; Diaz-Montano et al., 2014; Rubio et al., 2013). While a few potato lines have been found to be tolerant to ZC, no tolerant or resistant varieties had been

released so far. The present study reports results from ZC screening trials of six advanced potato breeding lines with good agronomic and commercial characteristics that have shown tolerance to ZC internal tuber discoloration.

Materials and methods

The six advanced potato breeding lines (8-65, 5-10, NAU, 99-38, 8-29 and 2-75) used in the present study were selected from 800 lines previously screened under field conditions in the central part of Mexico. These advanced potato lines were selected based on their agronomic and marketable characteristics, in addition to ZC tolerance. Among the selected lines, the clones 8-65, 99-38, 8-29 and 02-75 are tolerant to late blight (*Phytophthora infestans*), the clone NAU has good commercial characteristics for the fresh market, whereas the tubers of the clone 5-10 have shown good chipping quality. During three years (2010-2012), the six potato genotypes and Fianna, a commercial variety used as control, were field tested at the experimental station of INIFAP in Metepec, Mexico State, Mexico. This site is located in the Toluca Valley, which is a place with a high density of *B. cockerelli* and high ZC infection pressure (Rubio-Covarrubias et al., 2011, 2013).

During the 3 years of the study, whole seed-tubers of each of the seven genotypes were planted in a randomized complete block design with 6, 10 and 4 replications in 2010, 2011 and 2012, respectively. Applications of insecticides were made weekly during the first 5 weeks post-emergence to help plant establishment and promote tuber setting and production. No further insecticides were applied to allow natural infestations of *B. cockerelli* and Lso infection under normal field conditions.

The *B. cockerelli* population was weekly monitored by using yellow sticky traps. At the end of each potato growing season approximately 60 *B. cockerelli* adults from each plot were collected and shipped to USDA-ARS Wapato, WA and tested for Lso by PCR. Three months after planting, when most of the tubers had a commercial size, each plant was clipped at the base and the number of *B.cockerelli* nymphs of all instars was scored.

The variables used to measure ZC resistance in the plants and tubers were: number of *B. cockerelli* nymphs per plant, Area Under Disease Progress Curve (AUDPC), potato yield, percentage of healthy tubers per plant, and severity of internal tuber discoloration. The percentages of the foliage with ZC symptoms were recorded weekly for each plant and used to calculate the AUDPC according to Shaner and Finney (1977).

After harvest, the tubers were weighed and stored at room temperature for 5 months, after which the tubers developed sprouts. The number of tubers per plant with normal sprouts (healthy tubers), wire sprouts, and without sprouts was recorded. Then, each tuber was cut in cross section and the severity of internal tuber browning was visually scored in raw slices using a scale from 0 to 5, with 0 indicating no discoloration and 5 corresponding to severe discoloration. The severity of internal tuber discoloration was calculated by averaging the scores of the diseased tubers per plant.

Data were analyzed with generalized mixed models (GMM) fit with appropriate probability distributions (negative-binomial or Poisson) and performed with the package glmmADMB in R 3.1.1. Data are presented as Analysis of deviance (type II tests). Significant models were further examined using contrasts comparing the mean of the control with the means of the 6 clones.

Results and discussion

The general average of the percentage of healthy tubers was 31.7%, which means that the 68.3% of tubers were ZC infected. This high disease incidence is related with the high population of *B. cockerelli* in the experimental site, which was determined by the population dynamics (Figure 1) and the presence of nymphs on the plants (general average of 41 nymphs per plant). Besides the high insect population,

the Lso infection rate of the adult insects may contribute to explain the high ZC incidence. The analysis of insects collected in the experimental plot showed that 22, 2 and 7% were positive to Lso in 2010, 2011 and 2012 respectively. Low Lso infection rates of the adult insects are enough to spread the disease in an entire potato field because it has been demonstrated that the adult potato psyllids are highly efficient vectors of Lso (Buchman et al., 2011).

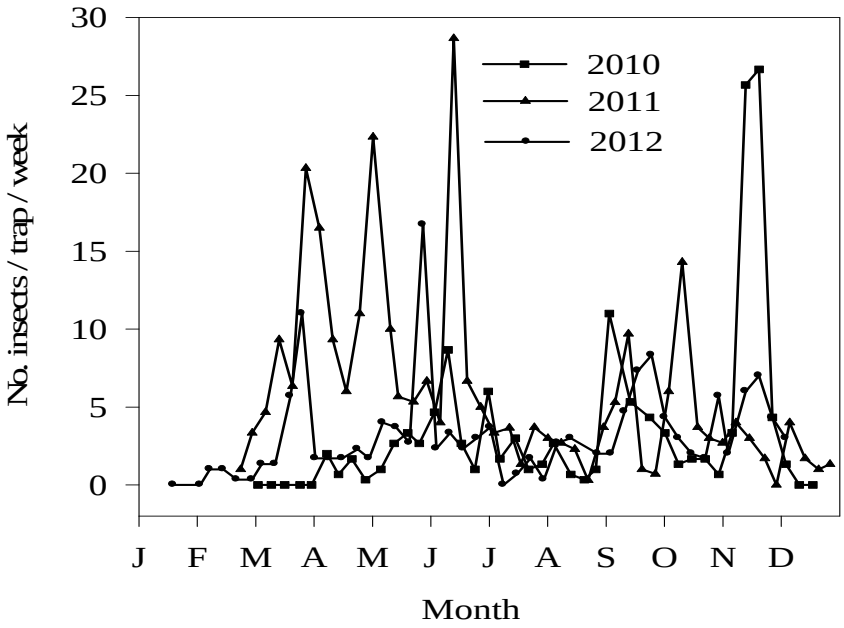


Fig. 1. Potato psyllid captures on yellow sticky traps over 3 years in The Toluca Valley, Mexico.

The analysis of deviance indicated that there were statistical differences among the 7 genotypes in the percentage of healthy tubers, AUDPC and tuber discoloration, nevertheless no differences were detected in yield (general average was 1.26 kg/plant) and number of nymphs (general average was 41 nymphs per plant). Based on these results, the means of the 3 significant variables were further analyzed.

The means of the 3 variables that presented statistical differences (% of healthy tubers, AUDPC, and severity of tuber discoloration), were analyzed by comparing Fianna with each clone (Fig. 2). This figure shows significant differences between Fianna with 8-65 and 99-38 in percentage of healthy tubers, with 8-65, 5-10, 8-29 and 02-75 in AUDPC and with all clones in tuber discoloration. Collectively, these results indicate that the 6 clones performed better than the commercial variety Fianna. The clone 8-65 presented the highest % of healthy tubers, the lowest AUDPC and the lowest severity of tuber discoloration. The response of all these variables suggests that, among the 6 clones, 8-65 possesses the highest tolerance to ZC.

In conclusion, the high numbers of nymphs in the 7 genotypes, the capture of adult insects during the entire year and the presence of Lso in the insects confirms the highly ZC infective conditions in the experimental site. Compared with the commercial variety Fianna, the 6 clones presented higher tolerance to ZC symptoms in the tubers and they possess commercial characteristics that make them candidates to be released as varieties. In the present study it was not possible to clarify the exact mechanism of the tolerance exhibited by the tested clones and further studies are needed to elucidate this issue.

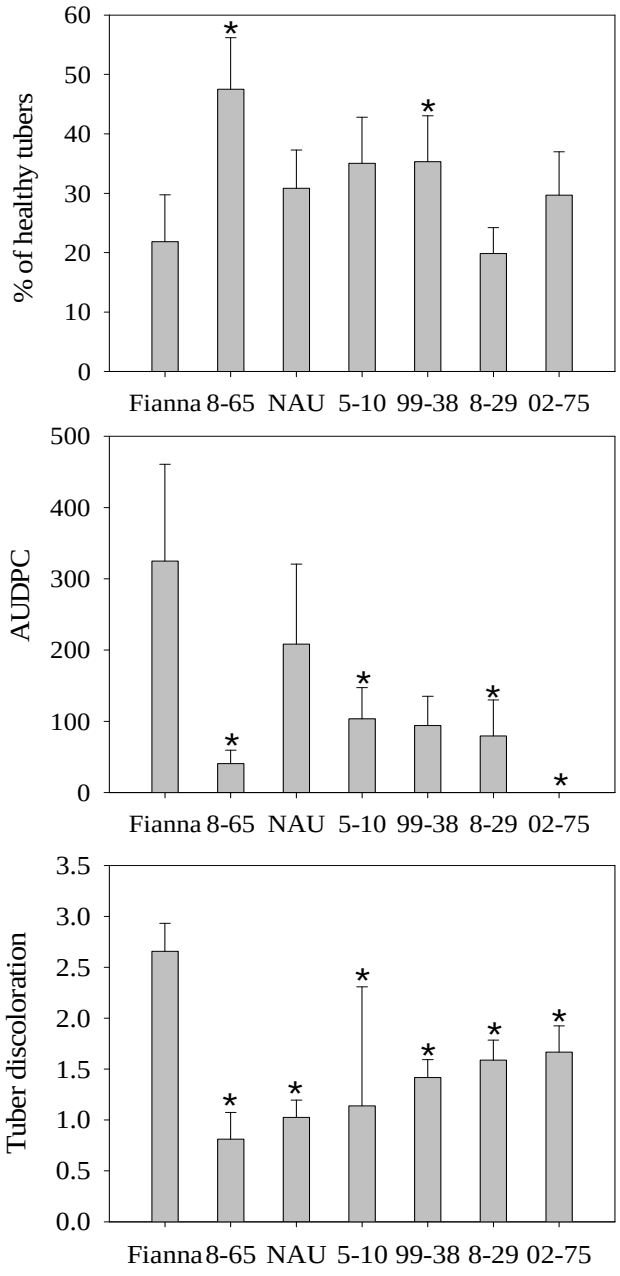


Fig. 2. Means of percentage of healthy tubers, AUDPC and the severity of tuber discoloration in the 7 potato genotypes. * Significant difference compared with Fianna, contrast test $P<0.05$.

References

Anderson, J.A.D., G.P. Walker, P.A. Alspach, M. Jeram, and P.J Wright. 2012. Assessment of susceptibility to Zebra Chip and *Bactericera cockerelli* of selected potato cultivars under different insecticide regimes in New Zealand. Am. J. Potato Res. 90:58-65.

Butler, C.D. and J.T. Trumble. 2012. The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae): life history, relationship to plant diseases, and management strategies. Terrestrial Arthropod Reviews 5:87-111.

Butler, C.D. B. Gonzalez, K.L. Manjunath, R.F. Lee, R.G. Novy, J.C. Miller, and J.T. Trumble. 2011. Behavioral responses of adult potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), to potato germplasm and transmission of *Candidatus* Liberibacter psyllaurous. Crop Protection 30:1233-1238.

Buchman, J.L., V.G. Sengoda, and J.E. Munyaneza. 2011. Vector transmission efficiency of Liberibacter by *Bactericera cockerelli* (Hemiptera: Triozidae) in zebra chip potato disease: effects of psyllid life stage and inoculation access period. Journal of Economic Entomology 104:1486:1495.

Cadena-Hinojosa, M.A., I.R. Guzmán-P., M. Díaz-V., T.E. Zavala-Q., O.S. Magaña-T., I.H. Almeyda-L., H. López-D., A. Rivera-P., y O.A. Rubio-C. 2003. Distribución, incidencia y severidad del pardeamiento y la brotación anormal en los tubérculos de papa en Valles Altos y Sierras de los estados de México, Tlaxcala y el Distrito Federal, México. Revista Mexicana de Fitopatología 21:248-259.

Diaz-Montano, J., B. G. Vindiola, N. Drew, R. G. Novy, J. C. Miller, and J. T. Trumble. 2014. Resistance of Selected Potato Genotypes to the Potato Psyllid (Hemiptera: Triozidae). Am. J. Potato Res. 91:363-367.

Guenthner, J., J. Goolsby, and G. Greenway. 2012. Use and cost of insecticides to control potato psyllids and zebra chip on potatoes. Southwest. Entomol. 37: 263–270.

Hansen, A.K., J.T. Trumble, R. Stouthamer, and T.D. Paine. 2008. A new huanglongbing species, '*Candidatus* Liberibacter psyllaurous' found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). Applied and Environmental Microbiology 74:5862–5865.

Leyva-López, N.E., J.C. Ochoa-Sánchez, D.S. Leal-Klevezas, and J.P. Martínez- Soriano. 2002. Multiple phytoplasmas associated with potato diseases in Mexico. Can. J. Microbiol. 48:1062-1068.

Liefting, L.W., Z.C. Perez E., G.R.G. Clover, and J.A.D. Anderson. 2008. A new '*Candidatus* Liberibacter' species in *Solanum tuberosum* in New Zealand. Plant Disease 92: 1474.

Munyaneza, J.E. 2012. Zebra Chip Disease of Potato: Biology, Epidemiology, and Management. Am. J. Pot Res. 89:329-350.

Munyaneza, J.E., V.G. Sengoda, J.M. Crosslin, G. De la Rosa-Lozano, and A. Sanchez. 2009. First report of ´*Candidatus* Liberibacter psyllaurous´ in potato tubers with zebra chip disease in Mexico. Plant Disease 93(5):552.

Rubio-Covarrubias, O. A., I. H. Almeyda-León, A. M. Cadena-Hinojosa y R. Lobato-Sánchez. 2011. Relación entre *Bactericera cockerelli* y la presencia de *Candidatus* Liberibacter psyllaurous en lotes comerciales de papa. Revista Mexicana de Ciencias Agrícolas. 2(1):17-28.

Rubio-Covarrubias, O.A., I.H. Almeyda-León, J. Ireta-Moreno, J.A. Sánchez-S., R. Sosa-Fernández, J.T. Borbón-Soto, C. Hernández-D., J.A. Garzón-Tiznado, R. Rocha-Rodríguez, y M.A. Cadena-Hinojosa. 2006. Distribución de la punta morada y B*actericera cockerelli* Sulc. en las principales zonas productoras de papa en México. Agricultura Técnica en México. 32(2):201-211.

Rubio-Covarrubias, O.A., M.A. Cadena-Hinojosa., y G. Vázquez-Carrillo. 2013. Manejo integrado de la punta morada de la papa en el Estado de México. Folleto Técnico No. 2. Sitio Experimental Metepec INIFAP. México.

Shaner, G. and R. E. Finney. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67: 1051-1056.