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PROCEEDINGS OF THE 10TH ANNUAL ZEBRA CHIP REPORTING SESSION



F. Workneh and C. M. Rush Editors

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2010 ZEBRA CHIP REPORTING SESSION**

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**Hyatt DFW Airport
Dallas, TX
November 7-10, 2010**

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 began 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.

From November 7-10, 2010, 135 scientists, farmers, and personnel from agri-industry and potato processing companies, representing four countries, attended the 10th 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 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 and Frito Lay for covering expenses for the Welcome Reception. Finally, we appreciate the efforts of Kay Ledbetter and Donnie Parrack in recording interviews with all speakers and Advisory Board members for posting on the SCRI ZC Website.

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Resistance / Germplasm Identification Joseph Munyaneza – Session V	Molecular Biology and Physiology Jacob Price – Session VI
Survival / Alternate Hosts Don Henne – Session VII	Insect Biology Blake Bextine – Session VIII
General Discussion Session Neil Gudmestad – Session IX	

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Attendees of the
2010 Zebra Chip Annual
Reporting Session
November 7-10, 2010



2010 ZEBRA CHIP ANNUAL REPORTING SESSION



NOVEMBER 7-10, 2010



REGIONAL MONITORING OF POTATO PSYLLID POPULATIONS AND THE ASSOCIATED PATHOGEN, *CA. LIBERIBACTER PSYLLAUROS*

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Impact Statement

A regional sampling program of potato psyllid populations and its associated pathogen was conducted in the potato growing areas of Texas, Kansas and Nebraska. Psyllid pressure in Texas was similar to previous years; however the percentage of adults with *Liberibacter* was much lower, which resulted in a low incidence of zebra chip (ZC). In Kansas and Nebraska, potato psyllid populations were high, but with a very low incidence of adults with *Liberibacter*. The high population levels, especially in Imperial and Alliance, NE, were associated with direct feeding damage to potatoes called psyllid yellows. The pre and in-season sampling programs accurately predicted the final level of ZC in the tubers.

Summary

A regional sampling program of potato psyllid populations and the associated pathogen, *Ca. Liberibacter psyllarous* (Clp) was conducted in McAllen, Pearsall, Olton and Dalhart, TX; Garden City, KS; Minden, Imperial, Alliance and Scottsbluff, NE. Each week, leaf samples and yellow sticky traps were collected from test fields to assess the efficacy of the grower's IPM program and the incidence of adults that were positive for *Liberibacter*. To determine the percentage of 'hot' adults, psyllids were removed from the traps and assayed using molecular diagnostics. The information from the field and laboratory testing was reported weekly from Dec, 2009 to Oct. 2010 to more than 200 growers, consultants, researchers, and potato processors. Psyllid pressure in Texas was similar to previous years; however, the percentage of adults with *Liberibacter* was much lower, which resulted in a low incidence of zebra chip. In Kansas and Nebraska, potato psyllid populations were high, but with a very low incidence of adults with *Liberibacter*. The high population levels, especially in Imperial and Alliance, NE, were associated with direct feeding damage to potatoes called psyllid yellows. The pre and in-season sampling programs accurately predicted the final level of ZC in the tubers.

Introduction

Field studies of the potato psyllid, *Bactericera cockerelli* (Sulc), in commercial potatoes were conducted in Texas from 2006-2008 and extended in 2009-2010 to locations in Kansas and

Nebraska impacted by zebra chip (ZC) (Goolsby et al 2007, Goolsby et al. 2008). These studies have documented the basic relationship of the density of hot adults psyllids and pest management to expression of ZC in the tubers. The fields studies were conducted to determine the phenology, seasonal migration and impact of the psyllid throughout the native range, especially where it is known to cause ZC. These field studies are designed to observe and evaluate the impact of pest management practices and to use the extensive sampling effort to evaluate genotypic differences in the psyllid and putative pathogen *Ca. Liberibacter psyllarous*.

Materials and Methods

The regional potato psyllid sampling program was conducted in commercial potato fields near McAllen, Pearsall, Olton and Dalhart, TX; Garden City, KS; and Minden, Imperial and Scottsbluff, NE using the methods developed by Goolsby et al (2008). Insecticide-free control plots were established and maintained at each regional location for comparison to the commercial fields. Pre-season transects were established in each growing area using 100 yellow sticky card traps and changed weekly for 6 weeks. In season transects consisted of 5 traps were placed at intervals of 200 ft. from the field margin towards the center. Traps were scanned in the laboratory, using a dissection microscope, for identification and counts of psyllids. Psyllid adults were removed and used in molecular assays to determine if they were positive for *Liberibacter*. One hundred leaf samples were collected weekly from each field to determine density of eggs and immature potato psyllids. Sampling began at emergence from the tubers and continued until harvest. Weekly reports were transmitted to the cooperating growers detailing adult, nymph and egg densities of potato psyllids along with information about the IPM practices of the individual growers.

Results and Discussion

Adult potato psyllids were present in all locations as potato foliage emerged from tubers. Adult populations peaked twelve weeks after planting. Late season populations in Pearsall, TX and Imperial, NE were higher than the other growing areas (Fig 1). The first in-field generation was delayed due to cold and wet winter and spring conditions experienced across the growing areas. The colder than normal conditions may have reduced the Clp pathogen inoculum in the potato psyllid adults and potential wild host reservoirs.

In the Lower Rio Grande Valley, populations of adults were higher than found in the 2007-2009 growing seasons, but still far lower than the outbreak year of 2006. The percentage of adult psyllids positive for CLp, or 'hot' psyllids was slightly lower than the previous year. The low incidence of hot psyllids combined with 'current best management practices' for immature psyllids resulted in very low levels of ZC in the tubers at harvest. Best management practices used by the growers were as follows; Admire Pro® or Platinum® applied at planting followed by two consecutive foliar applications each starting at 55 days after planting of Movento®, Agrimek®, and Oberon®. Levels of ZC in the untreated control plots (UTC) planted Dec. 5, Jan 4, and Jan 25 were 55, 30 and 22 percent respectively. It appeared that the second generation of potato psyllids that emerged in the UTC plots spread the Clp which resulted in the higher levels of ZC in the tubers as compared to the treated commercial fields.

In Pearsall, populations of adults were higher than 2008-09 growing seasons. However, no hot psyllids were detected in the commercial fields or UTC. One spike of hot psyllids was detected in the pre-season transect prior to emergence of the potatoes. Pest management practices varied between fields, with greater use of pyrethroids and organophosphate insecticides in the PS1 field. Psyllid nymphs in this field rose dramatically late in the season. ZC levels in the commercial fields and UTC were zero, which correlates well with the lack of hot psyllids in the growing area.

In Olton, no hot psyllids were detected in either the commercial field or UTC in Halfway, TX. Populations of immature potato psyllids were extremely low as compared to the UTC. ZC levels in both fields were zero.

In Dalhart, adult psyllids appeared earlier than normal and low levels of hot psyllids were collected throughout the season in both the commercial fields and UTC. Normal 'best practice' pest management applications were combined with field perimeter, 'ring' treatments to control psyllid populations which are known to initially colonize field margins. ZC levels in the commercial fields were near zero as compared to 3% in the UTC. In Garden City, populations of adult psyllids were similar to 2009. Only one hot psyllid was captured late in the season. Pest management practices differed from 'best practices' with the use of several pyrethroid and organophosphate applications integrated with Movento. Immature populations in the commercial fields and the UTC remained low throughout the season, which may reflect other mortality factors such as weather or natural enemies. ZC levels were zero in all the fields including the UTC.

In Minden, no hot psyllids were detected in either the commercial field or UTC. Populations of immature potato psyllids were extremely low in all fields. Pest management practices were similar to those in Dalhart. ZC levels in all fields were zero.

In Imperial, no hot psyllids were detected in either the commercial field or UTC. However, populations of adult psyllids were extremely high. Populations of immature potato psyllids were extremely low in the commercial fields with moderate levels in the UTC. Pest management practices were similar to best practices with the addition of Abacus® and Redent® insecticides. ZC levels in all fields were zero, but some feeding damage or psyllid yellows was observed which resulted in reduced yields and some undesirable color in the potatoes.

In Scottsbluff/Alliance, only one hot psyllid was detected on the last day of sampling. However, populations of adult psyllids were extremely high late in the season. Populations of immature potato psyllids were low in the commercial fields and UTC, which may reflect other mortality factors such as weather or natural enemies. Pest management practices were different from best practices with several foliar applications of neonicotinoids, pyrethroids and organophosphates. ZC levels in all fields were zero, but some feeding damage or psyllid yellows was observed which resulted in reduced yields and some undesirable color in the potatoes.

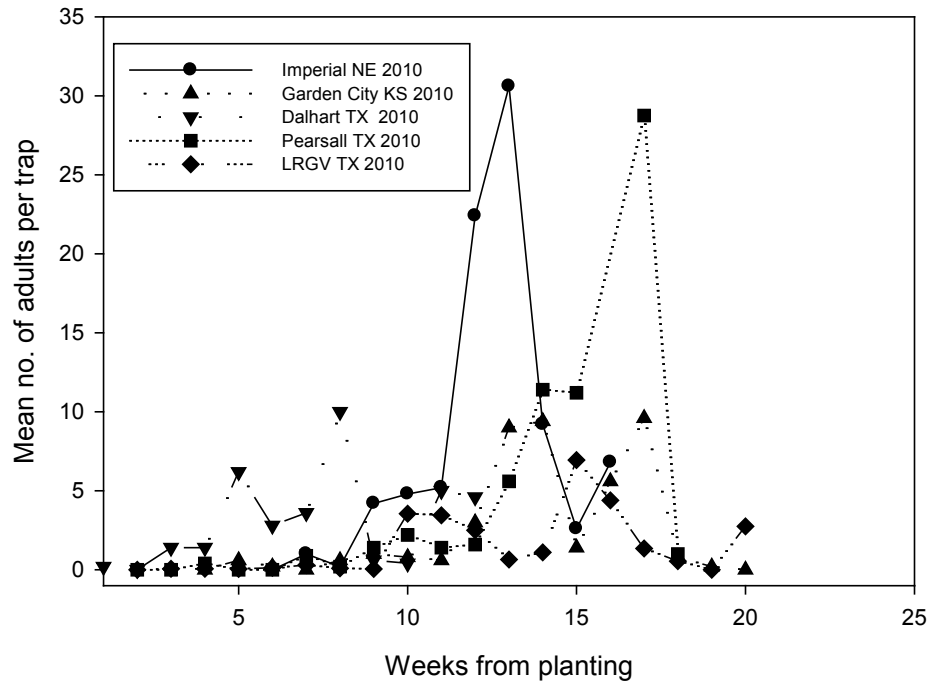


Fig. 1. Populations of adult potato psyllids in commercial fields captured in yellow sticky traps in TX, KS and NE.

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INCIDENCE OF “*CANDIDATUS LIBERIBACTER SOLANACEARUM*” IN POTATO PSYLLIDS COLLECTED IN THE SOUTH-CENTRAL UNITED STATES IN 2010.

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Summary

In 2010 more than 8,000 potato psyllids were tested for the presence of “*Candidatus Liberibacter solanacearum*”, the bacterium associated with the zebra chip disease (ZC). This was done in order to better understand the variation in the occurrence of the bacterium in populations of psyllids collected in Texas, Kansas, Nebraska, and a few other locations. All together, the incidence of the bacterium was approximately 1%. This compares with the approximately 2.5% infection rate observed in potato psyllids collected in 2009. Most of the *Liberibacter* positive samples originated in untreated control plots. Numbers of trapped insects was highest in the lower Rio Grande valley in late March and numbers increased in the northern areas as the season progressed, with high numbers in the Dalhart area in late May-early June and in Nebraska in July and August.

Impact

The relatively low incidence of the bacterium in psyllids in 2010 would appear to correlate with the low level of ZC disease symptoms observed in most potato producing areas of Texas, Nebraska, and Kansas. This work aids efforts to “predict” if populations of psyllids present in a given area pose a significant risk of inciting ZC disease and therefore should be more vigorously targeted for control.

Introduction

The potato zebra chip disease (ZC) is a serious and emerging disease of potatoes. In the last few years the disease has been associated with the presence of the potato psyllid (*Bactericera cockerelli*) within the affected fields (7,8). Even more recently the putative causal agent of ZC has been determined to be a newly described alpha-proteobacterium, “*Candidatus Liberibacter solanacearum*” (Lso) (a.k.a. *Ca. L. psyllaureus* 4,5). The bacterium is transmitted to potatoes by the potato psyllids and within a few weeks symptoms of the disease begin to develop and include chlorosis, leaf scorch, aerial tuber formation, and wilt (1,2,5).

Because of the association of the psyllid with the bacterium and subsequent development of ZC disease, a program to monitor the incidence of Lso in populations of psyllids collected in Texas, Nebraska, and Kansas begun in January of 2009. Previous work has shown that not all populations of the psyllid harbor Lso (6). Data on the incidence of psyllids in the 2009 season was presented at last years’ ZC meeting. Here we report the results of testing in 2010.

Materials and Methods

Beginning in October of 2009, yellow sticky traps were placed in and near potato production fields in five areas of the south central US: Lower Rio Grande Valley (McAllen, Weslaco), Pearsall vicinity, Texas Panhandle (Dalhart and Olton areas), southwestern Kansas (Garden City area), and southwestern Nebraska. John Goolsby was in charge of the trapping network and sticky traps from the various locations were collected weekly and sent to his laboratory in Weslaco, TX. Insects were identified, counted, and psyllids were removed from traps, placed into vials and shipped to Prosser, WA for molecular testing (PCR) for the presence of the bacterium. A few additional insect samples were received from Mexico and New Mexico and similarly tested for Lso. More specific information on the trapping sites and methods can be found in John Goolsby's report.

DNA was extracted from the psyllids, mostly as individual samples, using previously published procedures (3). During peak periods when large numbers of psyllids were received, some bulking of samples was done. Even in these cases, numerous individual psyllids from these traps/ locations were tested in order to provide reliable data on the incidence of Lso in the insects. Insect extracts were subjected to PCR analysis using previously published primers OA2/OI2c (1,5). PCR products were analyzed by agarose gel electrophoresis and presence of the predicted ~1,160 base pair amplified fragment indicated a positive sample.

Results and Discussion

In total, 8,097 psyllids were received in 2010: 7,693 potato psyllids, 91 Asian citrus psyllid, 180 prairie psyllids, 75 common brush psyllids, and 58 "other" psyllids. A listing of the majority of psyllids received and tested can be found in Table 1, below. (The full data spreadsheet is over 50 pages long). The number of psyllids collected in the lower Rio Grande valley peaked in March-April and numbers of insects increased in the more northern areas as the season progressed. The psyllids from the five main sampling areas were tested as 5,011 DNA extractions (most consisted of individual insects) and approximately 1% of these tested positive for Lso by PCR with primers OA2/OI2c. Most of the positive samples came from the untreated control plots in the lower Rio Grande valley. Also, about half of the positive samples were in insects collected in mid-March to early April. Table 2 shows the distribution of the PCR-positive psyllids. Very high numbers of potato psyllids were received from Nebraska in late summer but only one of these tested Lso positive. None of the other psyllid species tested positive for Lso.

In addition to the psyllids listed in Tables 1 and 2, 50 potato psyllids were received from Mexico and tested individually. Eleven of these were Lso positive. Similarly 2 potato psyllids from near Farmington, New Mexico were tested and both were positive for Lso.

The relatively low incidence of Lso in psyllids collected this year may directly correlate to the low incidence of ZC disease in the potato producing areas of Texas, Kansas, and Nebraska. In 2009 approximately 2.5% of the psyllids tested positive for Lso. A similar testing scheme will be conducted in late 2010 through summer of 2011. Additional sampling areas (and possibly additional state(s)) will be included.

After the next years' sampling and testing, we will have three years of such data and should have a very good idea of how the Lso incidence in potato psyllids relates to the observed incidence of ZC in growers fields.

Table 1. Number of psyllids received and tested for *Ca. Liberibacter solanacearum* in 2010.

Weeks and time period covered	Lower Rio Grande Valley	Pearsall Vicinity	Texas Panhandle (Dalhart)	Southwestern Kansas (Garden City)	Southwestern Nebraska	Lso PCR Positives	Percent of Total Positive
1-4 Oct 26 2009-Nov 24, 2009	3	0	0	0	0	0	0.00%
5-8 Dec 1, 2009-Jan 11, 2010	22	0	0	0	0	5	22.73%
9-12 Jan 20, 2010-Feb 8, 2010	30	2	0	0	0	4	12.50%
13-16 Feb 16, 2010-Mar 8, 2010	35	19	0	0	0	3	5.56%
17-20 Mar 16-Apr 5, 2010	540	360	16	0	0	29	3.17%
21-24 Apr 12, 2010-May 3, 2010	852	338	25	12	2	10	0.81%
25-28 May 10, 2010-May 31, 2010	42	297	525	30	28	1	0.11%
28-32 Jun 7, 2010-Jun 28, 2010	0	0	197	160	335	0	0.00%
33-36 Jul 5, 2010-Jul 26, 2010	0	0	298	285	1087	4	0.24%
37-40 Aug 2, 2010-Aug 23, 2010	0	0	368	116	739	3	0.41%
41-43 Aug 30, 2010-Sep 13, 2010	0	0	195	0	664	2	1.16%
Totals	1524	1016	1624	603	2855	61	0.93%

Table 2. Distribution of Psyllids testing positive for *Ca. Liberibacter solanacearum* in the 2010 season.

Weeks and time period covered	Lower Rio Grande Valley	Pearsall Vicinity	Texas Panhandle (Dalhart)	Southwestern Kansas (Garden City)	Southwestern Nebraska
1-4 Oct 26 2009-Nov 24, 2009	0	0	0	0	0
5-8 Dec 1, 2009-Jan 11, 2010	5	0	0	0	0
9-12 Jan 20, 2010-Feb 8, 2010	2	2	0	0	0
13-16 Feb 16, 2010-Mar 8, 2010	3	0	0	0	0
17-20 Mar 16-Apr 5, 2010	23	6	0	0	0
21-24 Apr 12, 2010-May 3, 2010	10	0	0	0	0
25-28 May 10, 2010-May 31, 2010	0	0	1	0	0
28-32 Jun 7, 2010-Jun 28, 2010	0	0	0	0	0
33-36 Jul 5, 2010-Jul 26, 2010	0	0	0	4	0
37-40 Aug 2, 2010-Aug 23, 2010	0	0	3	0	0
41-43 Aug 30, 2010-Sep 13, 2010	0	0	1	0	1
Totals	43	8	5	4	1

Acknowledgements

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STATUS OF REGIONAL ZEBRA CHIP INCIDENCE IN 2010 AND TEMPERATURE EFFECT UNDER CONTROLLED CONDITIONS

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Summary

In 2010 we conducted field and growth chamber studies in an effort to understand the basic epidemiology of the potato zebra chip (ZC) and associated factors. The field studies included regional assessments of its incidence and its spatial and temporal progresses coupled with the abundance of the vector of the associated pathogen (“*Candidatus* *Liberibacter solanacearum*”), the potato psyllid [*Bactericera cockerelli* (Sulc)]. The growth chamber studies consisted of determining the effect of constant temperatures (15°C, 21°C, 27°C, and 32°C) on psyllid reproduction and the pathogen titer in tubers. In the regional study there was a declining trend in ZC incidence from south to north which suggests that there may be a dilution effect as the psyllids move northward over time. The spatial and temporal study indicated that both psyllid abundance and ZC incidence progress over time faster on the edges than in the infields early in the season. The progress curves for both were near perfectly described by the logistic growth model. In the growth chamber study, approximately twice as many psyllid adults were produced in the 27°C chamber as in the 21°C chamber. In addition, there was a 10-day delay in development of new adults in the 21°C chamber compared to the 27°C chamber. No new adults were observed in the 15°C and 32°C chambers. Contrary to the adults, nymphs were produced in all temperature chambers. Tubers from all psyllid-infested plants tested positive for *Liberibacter* but tubers from the 15 °C chamber had significantly lower *Liberibacter* titer than those from the other temperature chambers.

Introduction

Potato zebra chip (ZC) is relatively a new disease and for this reason, information on factors which affect its epidemiology is virtually lacking. Thus, factor identification is the first and foremost step towards understanding of its epidemiology, which is the primary key to achieving successful management practices. Convincing evidence has now been accumulated that ‘*Candidatus* *Liberibacter solanacearum*’ (Lso), vectored by the potato psyllid, *Bactericera cockerelli* (Sulc), is associated with the disease (Liefting et al., 2009; Munyaneza et al., 2007; Secor et al., 2009). In factor characterization, as in any insect transmitted diseases, one has to consider the effect on both the psyllid and the Lso because the psyllid and the Lso may not necessarily have similar tolerance or threshold levels for all factors considered. One of our objectives in the current study was to investigate whether there are trends in ZC incidence across the region as psyllids migrate northward from overwintering areas in south Texas and transmit the putative pathogen in the process. We were also interested in finding out whether there are differences between the edges of the field and the infields in progressions of ZC incidence and psyllid abundance over time, and whether ZC incidence is spatio-temporally related to psyllid abundance. Furthermore, we were interested in investigating the effect of temperature on psyllid reproduction and Lso titer in tubers.

Materials and Methods

Regional ZC incidence assessments. Ten potato fields across the region [3 in Rio Grande Valley (TX), 2 in Pearsall (TX), 1 in Olton and Dalhart each (TX), 2 in Garden City (KS), and 1 in Bridgeport (NE)] were assessed for ZC incidence just before harvest. In each field plots of 20 m x 30 m were established at 160 m intervals around the edges and in the center of the fields. The number of plots in individual fields ranged from 14 to 32 depending on the sizes of the fields. Symptomatic plants in each plot were identified and dug up for verification by slicing the tubers on the spot. In addition, subsamples of symptomatic plants were taken into the laboratory and further verified for the presence of Lso by PCR.

Spatio-temporal assessments of ZC incidence and psyllid abundance: To further understand the dynamics of ZC incidence and psyllid abundance over time and space, additional ring of plots was established between the edge plots and the center plots in one of the regional fields (Olton) giving rise to 3 rings (edge, 100 m, and 200 m from the edge) of 16 plots each (n=48) around the center pivot. Yellow sticky traps were deployed between every other ZC plots for monitoring psyllid abundance (n=24). ZC incidence in each plot was assessed weekly as described above. The traps were removed for psyllid counts and replaced weekly.

Effect of temperature on psyllid reproduction and Lso titer. Potato plants (cv Atlantic) were grown in cages (4 plants per cage) in the greenhouse in one gallon pots (1 plant/pot) and transferred to growth chambers (2 cages per chamber: a control- and a psyllid infested cage) set at temperatures of 15°C, 21°C, 27°C, and 32°C. Thirty bacterialiferous psyllids then were transferred to each cage. Six potato leaves per plant (2 each in the lower, middle, and top part) were sampled weekly for nymph counts beginning 2 weeks after psyllid transfer. Development of new adult psyllids was monitored using yellow sticky traps (4 per cage) beginning 18 days after psyllid transfer. The tubers were harvested 49 days after psyllid transfer and tested for Lso titer levels using qPCR.

Results and Discussion

Regional ZC incidence assessments: Average ZC incidence per plot declined significantly ($R^2 = 0.33$, $P = 0.0483$) with increasing distance from south to north. ZC incidences in the Rio Grande Valley varied substantially among the three fields (~26 ° N, Fig. 1), the earlier planted field having much lower ZC incidence than the other two,

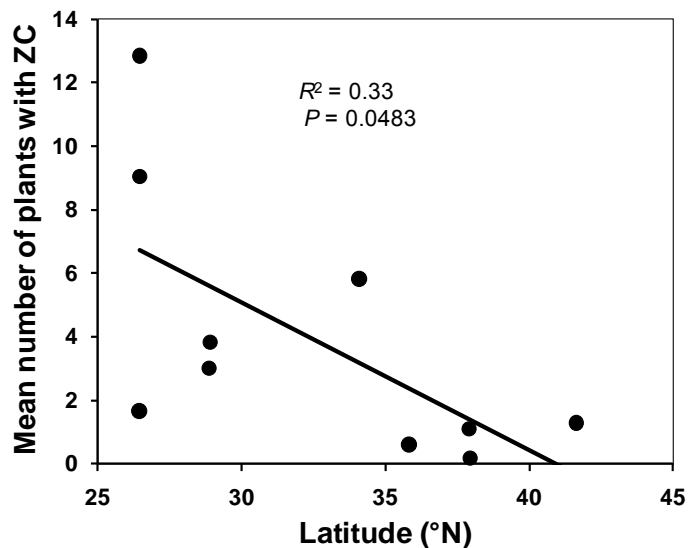


Fig. 1. Relationship between latitudinal field locations and ZC incidence

which were planted 2 to 3 weeks later. The later plantings were bordered by thick bushes which may have served as psyllid source leading to greater infection. In addition, winter temperatures in 2010 in the Valley were colder than normal, which may have affected the activities of both the psyllid and the pathogen early in the season resulting in low ZC incidence in the early planted field. Further research needs to be conducted on the effect of interactions among planting dates, locations, and winter temperatures on ZC incidence in the Valley.

Spatio-temporal assessments of ZC incidence and psyllid abundance: The Olton field was planted in the last week of March and ZC-symptomatic plants were observed for the first time on June 16 (the second week of observation; Fig. 2, left) in the two outer plot rings (the edge and the 100 m) but was not observed until the third week in the innermost plot ring (200 m). The incidence of new symptomatic plants was at its maximum on the edges on the third week in which there were greater than twice as many ZC-symptomatic plants as in the inner plots. The number of psyllids per trap was significantly greater on the edges than in the 100 m and 200 m in the first two

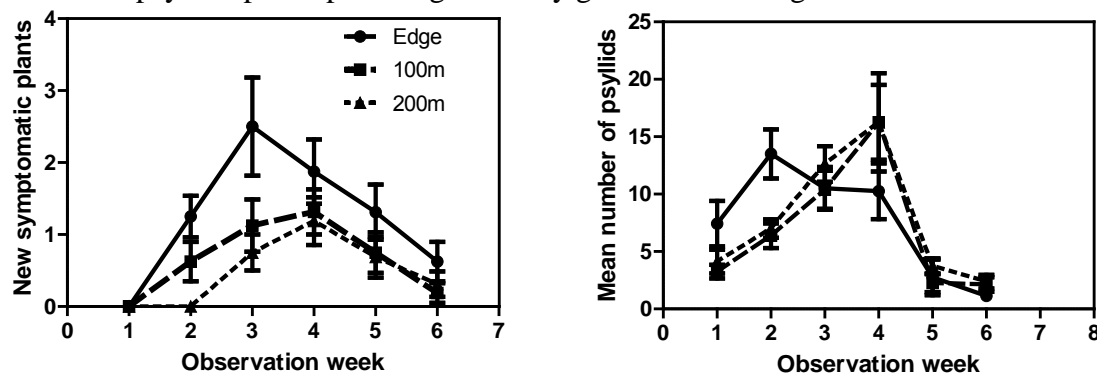


Fig.2. ZC incidence (left, observed weekly from June 9 to July 13, 2010) and psyllid abundance (right, observed weekly from May 28 to June 13, 2010) on the edge, 100 m, and 200 m infield

weeks of observations (Fig. 2, right). However, psyllid numbers on the edges declined after the second week while those in the infields continued to increase until the fourth week. The reason for this is not clear. However, a few possible scenarios may be speculated. Field edges are more frequently sprayed than the infields because of the

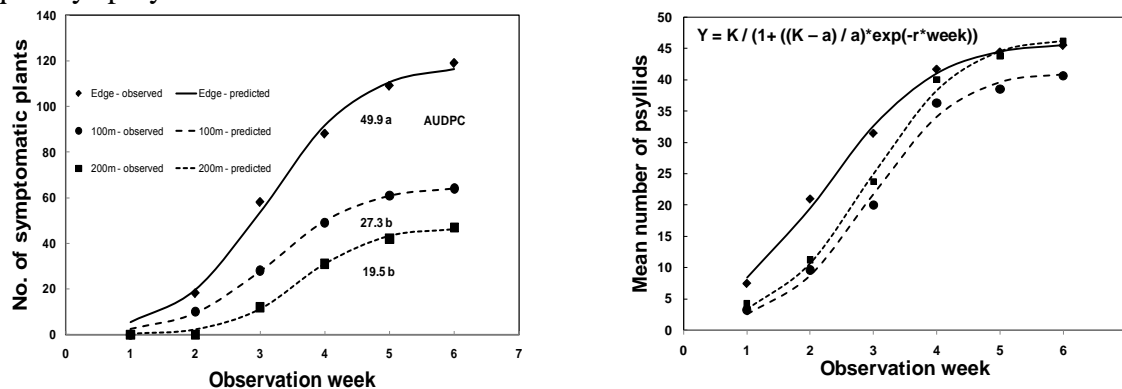


Fig. 3. ZC incidence (left) and psyllid abundance (right) progress curves for the edge, 100m, and 200 m infield

awareness that the incidence of ZC is greater on the edges than in the infields which may have had greater impact on the insect populations on the edges than those in the infields. In addition, the fact that the edges had greater psyllid densities early in the season suggest that the psyllids land on the edges, establish, and then move inward. The progress of ZC incidence over the 6-week-observation period in each ring was near perfectly described by the logistic growth model (Fig. 3, left). The model distinctly distinguished the edges from the inner plots in which the area under the disease progress curve (AUDPC) for the edge plots was significantly greater than for the infield plots indicating that the disease progresses faster on the edges leading to greater overall incidence. Progress curves for psyllid abundance in the three rings were not as distinctly separated as that of ZC but the edge again was dominant over the others early (Fig. 3, right). Both curves were significantly correlated (Fig. 4), suggesting that their distributions over time and space are similar.

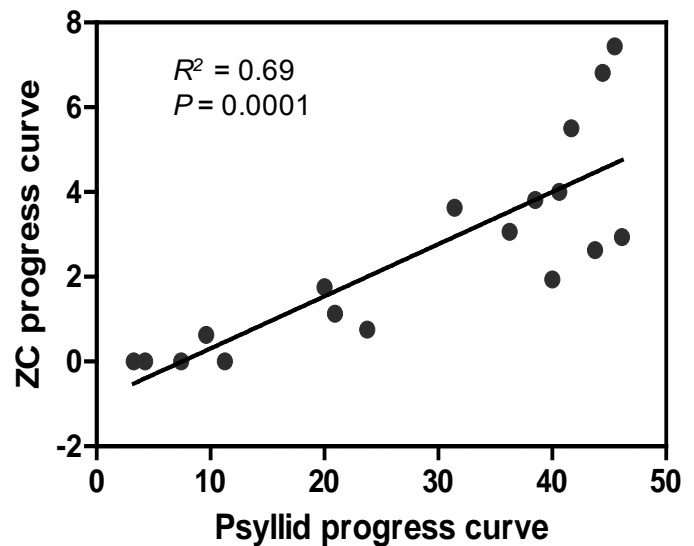


Fig.4. Relationship between psyllid abundance and ZC incidence progress curves

Effect of temperature on psyllid reproduction and Lso titer: The five temperature levels differed in their impact on psyllid reproduction. New generation of adult psyllids were observed in the 27°C

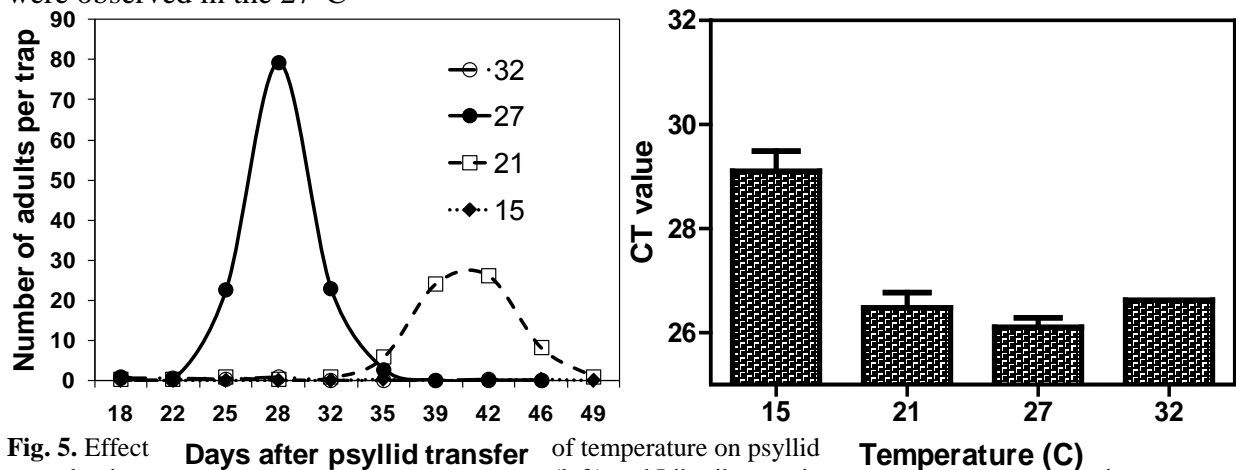


Fig. 5. Effect of temperature on psyllid reproduction (left) and *Liberibacter* titer (right)

chamber 25 days after psyllid transfer and reached maximum after 28 days (Fig. 5). However, in the 21°C growth chamber, adult psyllids were not detected until 35 days after transfer, which was a 10-day delay compared to the 27°C chamber and the total number of adults trapped were less than half of the 27°C chamber. This agrees with the previous

report that 27°C was near optimum for psyllid reproduction (List, 1938). No adults were detected in the 15°C or in the 32°C chamber 49 days after psyllid transfer at which time the experiment was terminated. These results clearly show that adult development is drastically reduced at cool temperatures. Contrary to adult development, nymphs were produced in all temperature chambers but their number was significantly greater in 27°C than in the other chambers early, but declined dramatically afterward as most of them molted into adults (data not shown). Tubers in the 15°C chamber had significantly lower Lso titer than those in the rest of the temperature chambers as depicted by the C_T (cycle threshold) values (the greater the value the lower the titer; Fig. 5). No difference in titer level was observed between the 21°C and 27°C chambers. Plants in the 32°C chamber appeared to be highly stressed, leaves becoming senescent and dropping off early, and thus, there was only one tuber that could be tested. It appears that the effect of temperature is mainly on psyllid reproduction rather than on transmission or on infectivity of the pathogen since tubers from all temperature chambers tested positive for the pathogen.

Concluding remarks

- The one-year regional ZC incidence study showed that there is low but significant trend in south-to-north ZC incidence, which means that ZC incidence in the northern regions may be predicted early from that in south Texas.
- Cool temperatures appear to slow down psyllid reproduction. This may give us a clue to further investigation of whether ZC epidemics in the spring could be predicted from winter temperatures, especially in winter psyllid breeding areas in south Texas.
- The spatio-temporal study provided information on the relationship between psyllid abundance and ZC incidence over time and space. In addition, the findings show that disease progression is faster on the edges than in the infields. The study further tends to suggest that psyllids land first on the edges and move inward as the season progresses, but further investigation is required in this regard.

Acknowledgements

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POTENTIAL IMPLICATIONS OF NEW PSYLLID SAMPLING METHOD

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Summary

We describe a simple method to extract and count nymphs of the vector of Zebra chip disease *Bactericera cockerelli* (Psyllidae) from potato leaves. Indeed, a reliable and cost-effective field sampling method is currently lacking. The proposed sampling method consists of: 1) washing infested leaves in cold water (to remove dust and sand), 2) immerse leaves in >85°C water for 5 s, 3) extract insect immatures from the water by running it through a fine mesh-filter, and 4) count insect immatures on fine mesh-filter under stereoscope. The proposed method was used to demonstrate that potato psyllid nymph density in the middle portion of the canopy showed the highest correlation with the global repartition of nymphs.

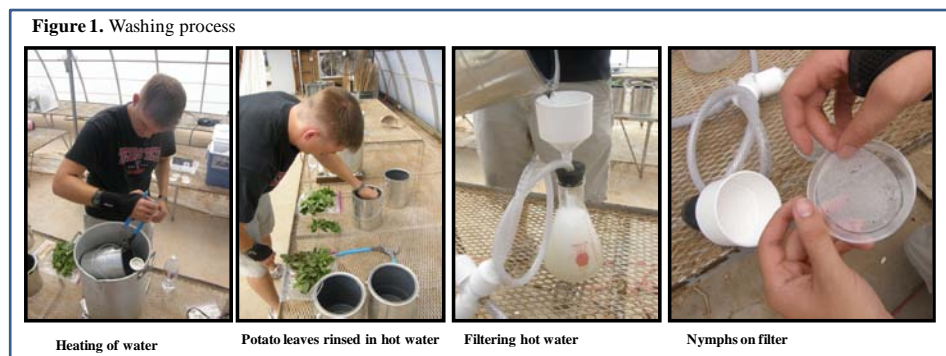
Introduction

A good sampling method is the first step to any IPM strategy. Currently there is no reliable sampling protocol for psyllid nymphs in potato crops and consequently both economic injury level and action threshold are unknown (Cameron et al. 2009). The two sampling methods presently used to assess potato psyllid *Bactericera cockerelli* Šulc incidence in crops are: 1) indirect sampling with yellow sticky cards (Tedeschi, Bosco & Alma 2002; Al-Jabr & Cranshaw 2007; Cameron *et al.* 2009; Hall 2009; Hall & Hentz 2010), and 2) visual inspection of crop leaves. Yellow sticky cards are fairly easy to deploy but are not highly attractive to psyllids, quite expensive when deployed in high numbers, and we are unaware of any studies demonstrating reliable correlations between sticky card counts and densities in the crop itself. In addition combination of wind and dust can make the sticky card unreadable (personal observation). Visual inspection of leaves under field conditions is the standard sampling method by crop consultants, who typically estimate pest population densities based on samples of 50-100 leaves from individual fields. However, it is very difficult to detect small immatures without magnification, especially if crop leaves have considerable amounts of dust and sand. Visual inspection by researchers may consist of counting immatures on crop leaves under stereomicroscope, and is highly time consuming. In this paper we propose a new direct sampling method for potato psyllid nymphs in potato plants. Based on a field application, we demonstrate how this sampling method can be used to conduct spatial population studies.

Materials and methods

Sampling System. The sampling system consisted of one turkey fryer (26.5L), one hot plate, 14 paint cans (3.8 L), one 25.4 cm channel locks, one squirt bottle, one 1/3 hp vacuum pump, three Erlenmeyer Flasks (1L) four two-hole rubber stoppers, three 70mm Polypropylene Buchner Funnels, one 1.9cm Polyvinyl chloride (PVC) cross (slip), four 2.5 cm PVC caps (slip) , three plastic and one brass 9.5 mm hose barb adapter , three 6.3 mm and one 9.5 mm high pressure braided PVC tubing, organza fabric, five 5.1 cm wide large binder clips, and five 46 cm metal wiring. 19 L of water were placed into the turkey fryer on high heat (see

further). It took roughly 1.5 hours to increase the water temperature to 99°C. The paint cans were modified near the top of the lip by drilling a rectangular opening about 2.5 cm in length by 2 cm in width. Metal wiring was cut 46 cm long, bent in half and wrapped around the large binder clip handles. The organza fabric was pre-cut into 7 cm diameter circles. A three way device used to increase the speed of the vacuum process, was made by drilling holes (1.5 cm diameter) in the centre mass of the cap(s), allowing the 9.5 mm hose barb adapter and (3) plastic fittings to fit snugly and were sealed with hot glue. The caps were placed onto the 1.9 cm PVC cross and sealed with hot glue. 5 mm holes on the rubber stoppers were drilled out to increase their diameter and allow both the Buchner Funnel as well as the 6.3mm high pressure braided PVC tubing to fit. Once the tubing was inserted into the rubber stopper, it was connected to the three way device. From the three way device, the 9.5 mm high pressure braided PVC tubing was connected to vacuum pump. Channel locks were used for gripping the paint cans to prevent the user from getting burned (Fig. 1).



Potato leaf samples and potato psyllid nymph culture. Potato leaf samples (*Solanum tuberosum* L., variety: Red lasotah) were collected from a commercial center-pivot field in Olton, Texas. Each leaf consisted of five leaflets. Psyllid nymphs were cultured at the Agrilife Research Station in Lubbock, Texas.

Effects of water temperature and time on nymph counts. The effect of water temperature on nymph counts was examined over time. The materials used consisted of a small hot plate, 250mL beaker, 99 nymphs and 33 potato leaflets. The nymphs were collected and placed in groups of three onto a single leaflet, and left for 10 minutes before each bioassay. Next, we poured 200mL of water into the 250mL beaker and heated it to 50°C. To determine the temperature we used a thermometer immersed in the water. Once the water reached the desired temperature, the leaflet with nymphs was immersed into the water, and we examined the leaflet to determine the amount of nymphs that have fallen off, and continued for up to 45 s. Starting at 50°C, three replications per treatment were used and the water temperature was increased in 5°C intervals up to a maximum of 100°C.

Recovery Rate of potato psyllid nymphs. The objectives of this experiment were to estimate the recovery rate of the psyllids nymphs and to assess whether the sampled nymphs were size-biased. For the recovery rate, 140 leaves were examined in groups of 20 in which only one of the 20 leaves was experimentally infested with

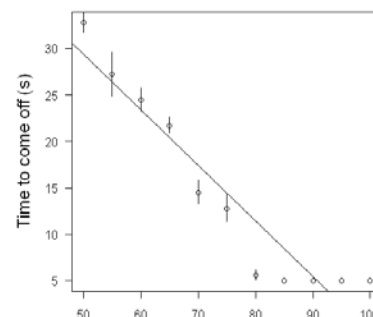


Figure 2. Time for psyllid nymphs to fall out from the leaves depending of the water temperature.

10 nymphs. Using a stereoscope, we estimated sizes of all 70 nymphs. The leaves with the 10 nymphs were marked with blue duct tape for determining if some nymphs still remained on that leaf after the sampling process was completed.

Field application: vertical distribution of potato psyllid nymphs. The experience was conducted the 27th of June 2010 in a potato field in Olton, TX. The mean temperature was 27°C, mean RH 54%, wind speed 13km h⁻¹, without rainfall. The average height of potatoes plants was 58.36 cm ($\sigma^2=5.87$, n=25). We selected a rectangle of 25m along field edge, and 20m inwards into field. Inside this rectangle we randomly chose 18 sampling points. For each of these sampling points, x and y coordinates were recorded and we took 20 leaves from three vertical positions on potato plants: top, middle and bottom (total of 54 samples). The 20 leaves were run through the sampling system, and the nymphs collected were counted under stereoscope.

Results and Discussion

Effects of water temperature over time. At 85°C and above, all nymphs were removed from potato leaves within 5 seconds (Fig.2). There was a strong correlation between the time for nymphs to fall out from the leaves and the water temperature (linear regression: $F=383.72$ $P<0.001$, $r^2=0.81$).

Recovery Rate of potato psyllid nymphs. The average length of nymphs before sampling was 1.22 mm ($\sigma^2=0.26$, Fig.3a). Of the 70 nymphs used in the experiment 64 (91%) were recovered. For each leaf took separately, percent of nymphs recovered was comprised between 80% and 100%. The average length of the nymphs recovered was 1.26 mm ($\sigma^2=0.26$, Fig. 3b).

Field application: vertical distribution of potato psyllid nymphs. We did not find any spatial trend for the psyllids in the field. However, mid portion counts showed the strongest correlation with total counts, so our data strongly suggest that sampling of potato leaves for visual inspection and/or sampling with our proposed water rinsing method should focus on leaves from the mid portion of the canopy (Fig. 4). However, additional sampling in more fields, in different phenological stages, and with different potato varieties is needed to confirm that sampling in the mid portion of the canopy provides the best depiction of the psyllid nymph density.

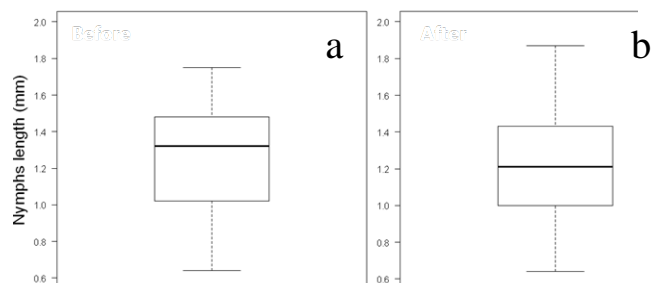


Figure 3. Length of the nymphs measured before sampling (a) and after sampling (b).

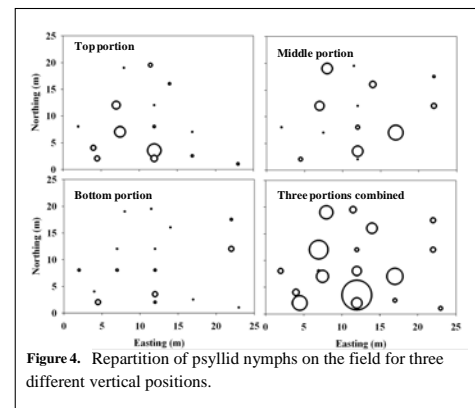


Figure 4. Repartition of psyllid nymphs on the field for three different vertical positions.

Discussion

The objective of research presented here was to develop a sampling protocol for using boiling water and filtration through organza fabric to make relative estimates of infestation levels of nymphs *B. Cockerelli* in potatoes field. The method showed high efficiency, as we were able to recover 90% of the nymphs. Moreover we did not observe any bias in the size repartition of psyllid nymphs after sampling, proving that small ones are not lost by this method. With this method, we conducted a field experiment where mid portion counts showed strongest correlation with total counts. This is an indication that these middle leaves should be taken during sampling protocol.

The price needed to build our sampling system is \$300-400. This cost is mainly due to the price of the vacuum pump and the fryer turkey. However all equipment is quite durable and could be reused many times. The only non-reusable item is the organza fabric, but the price of a square meter that corresponds to 400 circles is around \$5. This novel approach also consists of an incredible time saving. Visual inspection of 100 leaves under stereoscope take around 6 hours (Goolsby, personal communication). In comparison, for the same amount of leaves our methods take 10 min for the sampling and 50 min for the observation of the 5 Petri dishes (that count for 20 leaves) under stereomicroscope. Moreover, filters with nymphs can be kept in Petri dishes for historic record and recounted several months after sampling. In conclusion, we believe that this new, cheap, and environmentally friendly method of sampling could be a valuable tool to: 1) increase our current understanding of psyllid population dynamic in the field, 2) conduct in-depth evaluations of insecticide treatments by comparing nymph counts before and after treatment, and 3) develop a sequential sampling plan so that growers and crop consultants can make smarter decisions on when and where insecticide treatments are needed. Finally, we believe that this sampling method will enable development of an action threshold (economic injury level, EIL), which is the central pillar of a successful IPM program.

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INVESTIGATING TITER VARIATION OF CANDIDATUS LIBERIBACTER SOLANACEARUM IN INDIVIDUAL POTATO PSYLLIDS

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Summary

A study was initiated to determine the impact of temperature on titer of *Liberibacter solanacearum* (Lso) in potato psyllids. Temperature affected bacterial titer, with the highest mean titer at 28 C. However, differences in mean titer at most temperatures were not statistically significant due to extreme variability among individual psyllids. This observation led to additional studies to identify factors that impact variability of bacterial titer in individual psyllids and psyllid populations over time. Variables evaluated included colony of origin, psyllid age and sex, psyllid developmental stage, time since feeding and time of day. Mean bacterial titer and percent bacteriliferous psyllids from three sub colonies, established from the same initial colony, varied significantly from each other and also significantly changed over time. Sex of psyllids had no effect on bacterial titer but psyllid age and developmental stage did. Nymphs had significantly lower bacterial titers than adults and five day old adults had significantly higher titer than newly eclosed adults or day old adults. Bacterial titer dropped dramatically following a 24 hours starvation period and rebounded following a subsequent 2-hr feeding, but when psyllids were removed from the host plant after the two hour feeding, bacterial titer oscillated with no obvious pattern. Bacterial titer also appeared to naturally oscillate in psyllid populations, within individual colonies, during the day. Bacterial titer in individual psyllids and psyllid populations appears to be much more variable than previously recognized and bacterial titer levels appear to naturally oscillate over time.

Impact

This study detected and quantified extreme variability of Lso titer in individual psyllids from Lso-positive colonies, and the changes in bacterial titer over time. It will be important for researchers to recognize and account for this variability when conducting studies on host/pathogen/disease interactions and when evaluating the effect of independent variables on pathogen titer and disease development. Pathogen variability in psyllids populations should be taken into account when testing for Lso positive psyllids in surveys, especially when results of the surveys are used to trigger insecticide applications.

Introduction

In recent years, the incidence and severity of zebra chip (ZC) in commercial potato fields has been highly variable. The reasons for this variability are unknown but it is important to determine factors that are conducive for disease epidemics. This is complicated by the fact that ZC incidence is not only affected by vector dynamics but also interactions among vector, pathogen, host and environment. Based on observations by a number of growers, psyllids populations do not always correlate well with the incidence of ZC. This was the case in the

Texas panhandle production region in 2010, when psyllids populations were exceptionally high but the incidence of bacteriliferous psyllids, and subsequently the incidence of ZC, was very low.

The reasons for variability in Lso titer in potato psyllid populations are unknown, but studies with other insect vectors have revealed similar results. Hansen et al. (2007) showed that variability in secondary symbiont infection rates occurs in the psyllid, *Glycaspis brimblecombei*, where infection rates in 19 wild populations ranged from 0 to 75%. Whitfield et al. (2008) found substantial variation in *Tomato spotted wilt virus* titer among individual viruliferous thrips. Bressan et al. (2009) showed that the proportion of infected offspring of *Pentastiridius leporinus* testing positive for a fastidious bacterium endosymbiont that causes syndrome “basses richesses” of sugar beet varied among cohorts and ranged from 7 to 50%.

Understanding the reasons for pathogen variability in vector populations is key to understanding disease epidemiology. Among other things, the density of *Wolbachia* within their hosts can be affected by temperature, host genotype and age, and competition among the juvenile stages (Jaenike 2009). With the potato psyllid, it is unknown whether there are differences in Lso transmission ability between males and females, and whether transmission ability changes as psyllids age or Lso titer varies. It is also unknown whether Lso affects psyllid reproduction, survival, fitness or longevity. As a first step in answering these questions, a series of studies were initiated to identify factors that impacted Lso titer in psyllid populations.

Materials and Methods

The effect of temperature and other variables on titer of Lso in potato psyllids. In preliminary studies, colonies of potato psyllids were established on potato seedlings, in mesh cages, grown in incubators at constant temperatures of 4, 15, 24, 28, 32 and 36 C. After approximately four weeks, psyllids were collected from each cage and Lso titer in individual psyllids was quantified by real time PCR, as described below.

In an attempt to establish uniform, infectious populations of potato psyllids, a single psyllid colony, which had repeatedly tested strongly positive for Lso, was subdivided into three sub colonies. Twenty five psyllids from the mother colony were used to establish each sub colony. Beginning four weeks after establishment, 10 psyllids from each of the three sub colonies were collected weekly for five weeks, and Lso titer and percentage of Lso-positive psyllids was determined. Additional studies were conducted to determine the effect of psyllid sex and developmental stage on Lso titer, and the effect of starvation period on Lso titer also was investigated. In these studies, 3-6 psyllids were combined and tested as composite samples.

Traditional and Real time PCR. Conventional PCR was used for initial detection of *Liberibacter* in plants and psyllids, and for production of fragments for cloning, using the OA2/OI2c primer pair. After grinding in liquid nitrogen, total DNA from potato psyllids was isolated using DNeasy® Blood & Tissue Kit (QIAGEN), following the manufacturer’s instructions. Eluted DNA was quantified using a NanoVue Plus™ (GE Healthcare Limited, UK). PCR assays (50 µL) contained the following: 1X Ex Taq buffer, 0.2mM dNTP mix, 0.2 µM of primer OA2 and OI2c; 0.02 U of TaKaRa Taq polymerase; and 5µl of DNA template.

PCR cycles were: 2 min at 94 °C, followed by 35 cycles of 30 s at 94°C, 30 s at 66°C, 90 s at 72°C, with a final extension step for 10 min at 72°C. PCR products were then purified and ligated into pCR®2.1 vector (Invitrogen) following the manufacturer's instructions. Plasmid DNA was purified using the QIAprep Spin Miniprep Kit (QIAGEN).

Absolute quantification of *Liberibacter* was conducted using an ABI 7500 real-time machine (Applied Biosystems). TaqMan primers and probe used were designed against the 16S ribosomal sequence of Lso (NCBI GenBank no. EU980389). The reaction mix (25 µL) consisted of 1 X TaqMan Universal Master Mix (Applied Biosystems), 0.05 µM forward primer ZCf 5' CGA GCG CTT ATT TTT ATT AGG AGC – 3', and 0.05 µM reverse primer HLBf 5' – GCG TTA TCC CGT AGA AAA AGG TAG – 3', and 0.25 µM of the FAM labeled, HLBp TaqMan probe 5' – AGA CGG GTG AGT AAC GCG – 3', with a TAMRA quencher. Samples were run in triplicate. A series of standards was produced using a cloned fragment of Lso 16S rRNA gene (Accession FJ957897). Ten-fold serial dilutions from 3,000,000 to 3,000 were used to produce the standard curve for absolute quantification.

Normalization for real-time PCR was conducted by calculating the A260/280 and A260/230 ratios of each DNA extraction using a GeneQuant pro RNA/DNA Calculator Spectrophotometer (GE Healthcare, USA) and quantifying it (ng/µl). Each sample was then diluted to a concentration of 20ng/µl. Therefore the same amount of total nucleic acid extraction could be added to each reaction. Real-time reactions were replicated three times and the values for each reaction well were averaged for a qCT value for each sample. In all experiments the default run method designed by ABI for the 7500 Real-Time PCR System was used: 10 min at 95°C for denature, followed by 40 cycles of 15 sec at 95°C, and finally 1 min at 60°C. Based on results from conventional PCR, experiences by our own researchers, and recommendations from other labs, a Ct value of 30 was used as positive cut off.

Results and Discussion

Results of preliminary studies evaluating the effects of temperature on Lso titer in individual psyllids were inconclusive (Fig. 1). The lowest mean bacterial titer (highest Ct value) was recorded at 4°C and the highest mean was at 28°C, but differences were not statistically significant due to the extreme variability in bacterial titer in individual psyllids.

These results were unexpected because colonies were allowed to establish for several weeks before psyllids were sampled and plants at 15 °C and higher exhibited symptoms of ZC, so the expectation was that all psyllids would be positive. The extreme variability in Lso titer, especially in individual psyllids held at 24, 28 and 32, raised questions about the source of the original psyllids. This led to a study of whether sub colonies established from a single bacteriiferous “mother” colony would exhibit variability in bacterial titer (expressed as mean Ct value) or differ in the percentage of Lso positive psyllids over time.

Each of the three sub colonies was initially established with 25 psyllids from a single “mother” colony that had consistently tested strongly positive for Lso. Beginning at four weeks after establishment, and continuing for

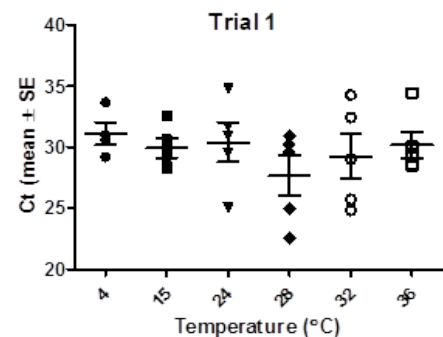


Figure 1. Effects of temperature on Lso titer (expressed as Ct value) in individual psyllids

five weeks, 10 psyllids from each sub colony were collected and individually tested by real time PCR for Lso. During the five week period of this study, both Lso titer and percent positive psyllids varied significantly within and among colonies (Fig. 2). At the first sampling, colonies A and B were similar in mean Lso titer and percent bacteriliferous psyllids but colony C had a significantly lower titer and percent positive psyllids. At the second sampling date, all three colonies had similar bacterial titer means and percent positive psyllids. However, at sampling dates three and four colonies again diverged significantly in

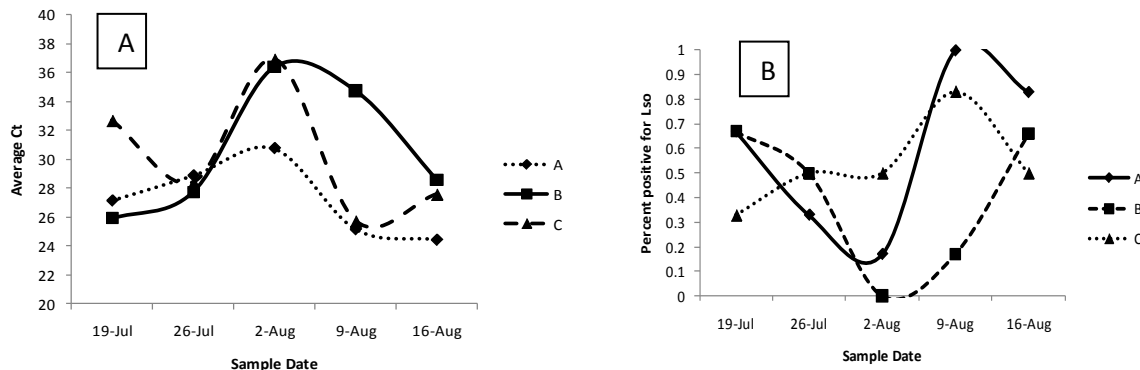


Figure 2. Variation in Lso titer (A), as indicated by mean Ct values, and percent Lso positive psyllids (B) over time, within and among three sub colonies initially established from a single “mother” colony.

both mean Lso titer and percent positive individuals. Interestingly, at sampling date three none of the psyllids from colony B tested positive for Lso, while 50% of those from colony C tested positive. However, at the same date, both B and C had the same mean Lso titer, illustrating again the extreme variability that can exist among individual psyllids from the same colony. In week four, the mean Lso titer and percent positive individuals remained very low in colony B, but from week three to week four all colonies increased in mean Lso titer and percent positive psyllids. The change was especially significant in colony A, which went from a mean Ct value of 36 to 26 and the percentage of positive psyllids increased from 20% to 100%. Likewise in colony B, from week four to week five, the mean Ct value went from 35 to 28 and percent positive psyllids rose from 20% to 70%.

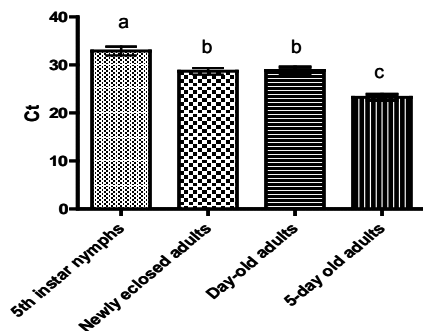


Figure 3. Effects of psyllid growth stage on mean Lso titer.

Studies were conducted to evaluate the possible effects of psyllid age and sex on Lso titer. Newly eclosed and older adults were collected from an established colony that was Lso positive, and a minimum of 10 males and 10 females from each group were tested for Lso by real time PCR. Sex of the psyllids had no effect on Lso titer but mean titer values were significantly higher in older adults than in newly eclosed adults. The study was repeated with the objective of verifying the effect of psyllid developmental stage on Lso titer. Fifth instar nymphs, newly eclosed adults, 1-day old adults and 5-day old adults were tested. Similar to the initial study, older psyllids had significantly higher mean Lso titers than younger psyllids so that mean Lso titer in 5-day old adults > 1-day old adults = newly eclosed adults > 5th instar nymphs (Fig. 3).

The high variability in mean Lso titer in psyllids from a single colony, and the observation that older psyllids had higher bacterial titers than younger psyllids raised the question of whether feeding impacted Lso titer. The results of starvation on Lso titer are shown in Fig. 4. As expected, a significant drop in Lso titer was observed in psyllids following the 24 hour starvation period, and a subsequent increase was observed after the 2 hour feeding. However, additional oscillations in titer occurred during the 10 hour period following the 2 hour feeding that is inexplicable. Furthermore, when psyllids were collected every hour of the test period, directly from plants from which the starved psyllids were originally collected, oscillations in Lso titer were also observed (Fig. 4, solid line). Each point on Fig. 4 represents a composite sample of at least six psyllids and although Lso titer in individuals can be extremely variable, this observation suggests that Lso titer naturally oscillates during the day in psyllid populations.

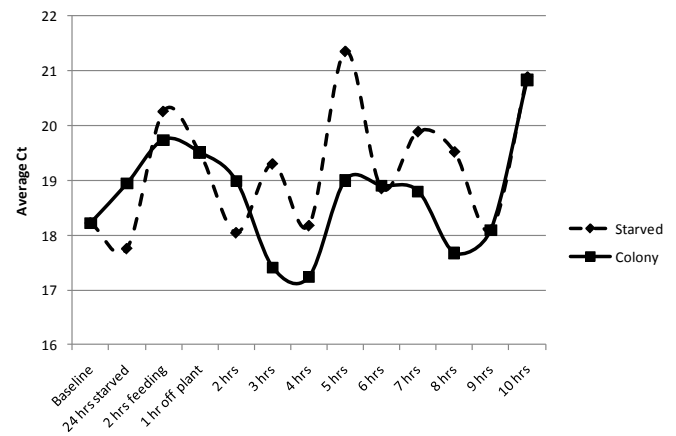


Figure 4. Oscillations in Lso titer in starved (dotted line) and unstarved psyllids over time.

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QUANTIFYING CANDIDATUS LIBERIBACTER AND OTHER MICROBES IN PSYLLIDS IN RELATION TO INSECT COLONY BEHAVIOR AND ZC DISEASE.

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Summary

Zebra chip or complex (ZC) disease in potatoes is highly correlated with the presence of *Candidatus Liberibacter solanacearum* (Ca.L) a phloem-restricted alpha-proteobacterium, that is vectored by the potato psyllid *Bactericera cockerelli*. We have discovered that psyllids with very low concentrations of Ca.L display a high rate of reproduction; however, the psyllids carrying high levels of Ca.L seem to have such slow reproduction rates that the population can even become extinct. Furthermore, entire populations of psyllids under the laboratory conditions have changed from containing high concentrations of Ca.L to undetectable levels of Ca.L or *vice versa* and those changes were accompanied by the described reproduction behaviors. We developed primers and reaction conditions for semi-quantitative real-time PCR to test if the presence of other microbes residing within the psyllid insect and their changing population dynamics in relation to the environment (abiotic or biotic stresses) could explain the observed variations of Ca.L concentrations in the psyllid insect vector. Thus far results have not yet revealed an obvious correlation between high levels of Ca.L and fluctuations of other microbes, but investigations are ongoing that include incorporation of proteomics techniques and using *Arabidopsis thaliana* as an alternative host.

Impact Statement To understand the epidemiology of ZC and for the implementation of cost-efficient control strategies, it is imperative to understand the factors that influence the dynamics of Ca.L in the psyllid vector in relation to ZC transmission, and to determine the possible contribution of other microbes. Towards this we are comparing the levels of microbial populations residing within individual psyllids either in presence or absence of Ca.L, combined with proteomic approaches in infected potato and *Arabidopsis* plants.

Introduction

Zebra chip or complex (ZC) disease is believed to be caused by the phloem-restricted bacterium, *Candidatus Liberibacter solanacearum* (Ca.L) that is transmitted by the potato psyllid *Bactericera cockerelli*. ZC was first observed around 1994 in potato crops in Mexico and by 2000 the disease was identified in Pearsall and the lower Rio Grande valley areas of Texas and then by the year 2004-2005 it had spread into the northern states (1).

Populations of psyllids containing high concentrations of bacteria can cause severe damage to the potato plants causing them to collapse and die in just a few weeks. Psyllid populations with very low concentrations of Ca.L (undetectable by PCR) only cause mild symptoms, and the plants will survive and in some cases recover. Curiously, this year (2010) high numbers of psyllids were present in potato fields, apparently with no detectable levels of Ca.L but in such high populations that their presence on the potato plants has affected the potato crop yield and quality. If those psyllids were to contain Ca.L the entire potato chip industry could have been badly impacted and economical losses would have been substantial.

For the past year and a half, we observed that only a portion of the psyllid population at any given time contains *Ca.L*, however we have identified psyllid populations where almost 90% of the individuals contain *Ca.L*, and this negatively affected the insect colony reproduction rate. As a comparison, Citrus greening disease caused by three strains of similar alpha proteobacteria, *Ca. Liberibacter asiaticus*, *africanus* and *americanus* in citrus plants have been observed in the state of Florida and the percentage of citrus psyllids containing *Ca.L asiaticus* is variable, similar to what we observed. For instance, in 1991 psyllids collected from Malaysia were only ~40% positive for *Ca. L asiaticus*, the next year a collected sample from India, showed that less than 1% of the psyllids were positive (2). It has also been observed that environmental conditions such as high temperatures affect the *Ca.L* titers in the citrus psyllids. The *Ca.L africanus* and *americanus* strains are more heat sensitive than the *asiaticus* strain (3). But, information on reproduction behavior and prolificacy affected by *Ca.L* in the citrus psyllid is lacking.

In order to begin to understand the microbial population dynamics and the changes in reproduction rates, we started by investigating the changes in *Wolbachia spp*, a secondary endosymbiont known to alter reproduction behaviors in different insects (4) and which was already reported to be present in the potato psyllid (5). To assess these changes we developed a semi-quantitative real-time qPCR method that allowed us to assess *Ca.L* levels in individual psyllids and compare those to levels of other endosymbionts such as *Carsonella ruddii*, a primary psyllid endosymbiont (6), and *Wolbachia*. We successfully designed primers for a reference gene for the psyllid DNA samples and for the endosymbionts *Carsonella* and *Wolbachia*, all primers comply with the standard MIQE guidelines for real time PCR experiments (7). The primers were used in different psyllid colonies and, thus far, no immediate changes in the levels of *Wolbachia* were evident, except for a recently acquired set of hot and cold psyllid colonies that are currently being tested more rigorously.

In parallel studies we are using proteomic analysis on healthy and infected potatoes and in the plant model *Arabidopsis thaliana*, that are exposed to psyllids of three different insect colonies. The goal is to verify *Ca.L* as the major bacterium inoculated by the psyllid insect into the plant phloem. If other microbes are released during psyllid feeding then this approach should identify them. An indirect outcome will be the identification of plant proteins that are activated by the psyllid and by psyllids containing *Ca.L*.

Materials and Methods

Detection of *Wolbachia* in psyllids by PCR. Psyllids were tested for the presence of *Wolbachia* by using the primer sets *wsp*-81F and *wsp*-691R (5). The PCR reaction contained: 1x Phusion HF buffer, 200 uM dNTPs, 0.4 uM forward and reverse primer, 2 ul of DNA and 0.02 U of Phusion Hot Start DNA Polymerase. DNA amplification was performed on an Applied Biosystems 2720 Thermocycler following the conditions of 94° C (5 min), then 35 cycles of 94° C (30 s), 55° C (1 min), 72° C (1 min), and 72° C (5 min).

Real time qPCR. Primers were designed based on sequences for *Bactericera cockerelli*, *Carsonella ruddii* and *Wolbachia*. After PCR confirmation and amplicon sequencing, qPCR primers were designed with Primer Express 3.0 (Life Technologies Corporation, Carlsbad, CA). The real time PCR reaction was performed with 1X power SYBR green mix (Life Technologies Corporation), 500 nM forward and reverse primers and 30 ng of DNA sample in a 15 ul reaction. The PCR cycle used was as recommended (95°C for 10 minutes, 95°C for

15 seconds follow by 60°C for 1 minute for a total of 40 cycles). Ct values were obtained and then normalized to the 28S reference gene. The values relative to the reference gene were represented and the standard deviation of the technical repeats was determined.

PCR efficiency test. For this, calibration curves for PCR amplification were established. Briefly, a serial dilution of known psyllid DNA sample concentrations was used and the amplification efficiency was determined from the slope of the log linear portion of the calibration curve. The PCR efficiency equals $(10^{-1/\text{slope}} - 1)$. The theoretical maximum of 1 indicates that the amount of product doubles with each cycle.

Proteomic analysis. A standard confirmatory tests for the presence of ZC disorder were carried out and tissues were subjected to starch staining, PCR and frying tests. *Arabidopsis* samples were taken from plants exposed to hot or cold psyllids. Selected tissues were macerated to a fine powdery texture in liquid nitrogen. Protein precipitation was carried out in cold TCA-mercaptoethanol solution (trichloroacetic acid 10%, acetone 90% and mercaptoethanol 0.007%). The TCA-acetone simultaneously precipitates and denatures proteins. Samples were then washed in cold 100% acetone and dried in a spin vacuum followed by resuspension in custom made rehydration buffer (8 M urea, 2 M thiourea, 2% CHAPS, 0.5% 3-10 ampholytes, 50 mM DTT, 0.005% bromophenol blue). Bradford assay was used to quantify protein concentration, 140 µg of proteins were loaded onto a 7 cm pH 4-7 IPG strip, rehydrated passively overnight and focused to 12000 vHrs at 50 mA at a maximum of 4000 V. The strips were then equilibrated and separated on a 12.5% SDS-acrylamide gel and then visualized with coomassie stain.

Results and Discussion

Detection of *Wolbachia*. The colonies reared in the labora-tory have different character-istics; C1 is a “clean colony” with undetectable levels of *Ca.L* and high reproduction rates; C2 is a “hot colony” with detectable levels of *Ca.L* but the percentage of psyllids containing *Ca.L* varies from 10 to 60% having a reproduction rate similar to C1, and C3 is a “hot colony” that usually showed a 90% of psyllids carrying *Ca.L* and low reproduction rates. In order to

corroborate the presence of *Wolbachia* in these three different populations, we screened the psyllid DNA samples for presence of the specific surface protein amplicon in *Wolbachia* (*wsp*). The amplicons obtained with the *wsp*-81F and *wsp*-691R primers were sent for sequencing and their identity was verified. *SpeI* digestion was performed to determine which *Wolbachia* strain was present (5). We found that both *Wolbachia* strains (Bac1 and Bac2) were identified in all three psyllid colonies C1, C2 and C3 (Fig. 1).

Microbial population dynamics. To assess changes in microbial populations within the psyllid we used semi-quantitative real-time qPCR to determine the concentration levels of *Ca.L*, *Carsonella ruddi* and *Wolbachia*. First, we designed and tested different sets of primers with calibration curves to identify primer pairs that were compatible and have similar

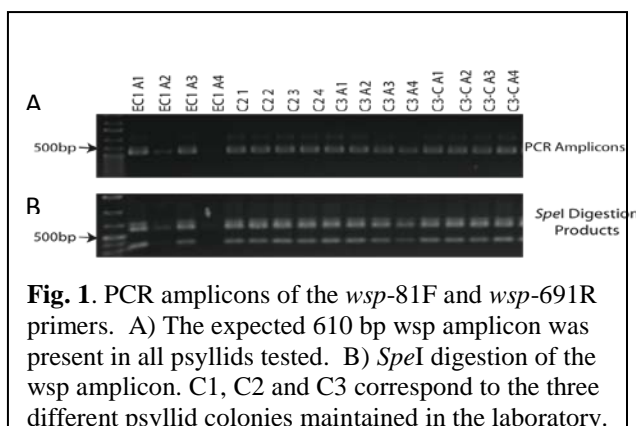


Fig. 1. PCR amplicons of the *wsp*-81F and *wsp*-691R primers. A) The expected 610 bp *wsp* amplicon was present in all psyllids tested. B) *SpeI* digestion of the *wsp* amplicon. C1, C2 and C3 correspond to the three different psyllid colonies maintained in the laboratory.

PCR efficiency to the LsoF/HLBr primer set developed by Li et al. (2009) (8). The primers that were selected for further comparison studies are listed in Table 1, and the calibration curves are depicted in Fig. 2.

Table 1. Primer sets selected for qPCR	
Primer	Sequence
28S-Bc-F	TCGGTCGTTTCCGTTGGT
28S-Bc-R	CAACATCACGCCCGAAGAC
23-16s-F	ATACTGCCCAAGAGTCCATATCG
23-16s-R	TGTGATGAGCCGACATCGA
LSOF	CGAGCGCTTATTTTAATAGGAGC
HLBr	GCGTTATCCCGTAGAAAAAGGTAG
Vent8-F	AGCTTTATGCTGGTGCTCGTTAT
Vent8-R	CATCATCTTTAGCTGCCTTACCAA
Vent1-F	AGCAAAAGCTGGTGTTAGCTATGA
Vent1-R	CGAAGTAACGAGCTCCAGCAA

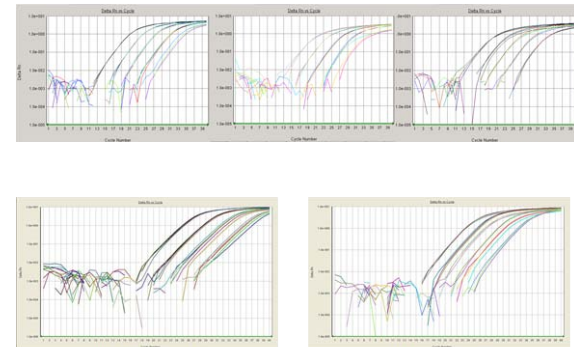


Fig. 2. Calibration curves for different primer pairs used in qPCR analysis to evaluate psyllid microbial population dynamics.

Endosymbionts evaluated in psyllid populations C1 and C2. Individual psyllids from C1 and C2 colonies were analyzed. DNA samples were standardized to a concentration of 30 ng/ul. As expected, *Ca.L* is present at different concentrations in C2 psyllids, some individuals have undetectable levels of *Ca.L* (Fig. 3). None of the C1 individuals have detectable levels of *Ca.L* (Fig. 3).

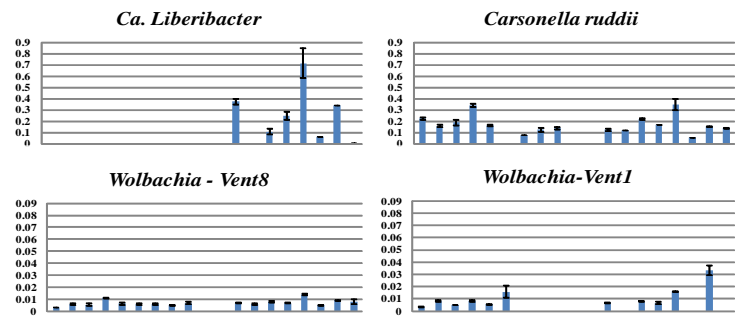


Fig. 3. Semi quantification of specific gene amplicons by real time qPCR that represent the different microbial titers in the potato psyllid vector

Carsonella ruddii, the primary endosymbiont is present at similar concentrations in both colonies. The *Wolbachia* concentrations were also similar for these two colonies, but the concentration levels were lower than for *Ca.L* or *Carsonella*. Therefore, no obvious variations of endosymbionts correlated with *Ca.L* levels.

A recently acquired set of colonies originally reared at Dalhart under greenhouse conditions was brought to the laboratory at the end of July 2010, both colonies were initially reproducing at the same rate but in October 2010 we observed a change in the reproduction rate that affected mostly the hot colony. A preliminary qPCR test showed changes in the levels of *Wolbachia* in a psyllid population that contains high titers of *Ca.L* (not shown), we are evaluating more individuals to determine the relevance of these recent findings.

Proteomic analysis in potato and *Arabidopsis*. Preliminary results indicate the presence of consistently up- and down-regulated proteins in infected potato tissues. In addition, hot psyllids were seen to recalcitrantly feed on *Arabidopsis*; however, when forced-fed, they were able to transmit *Ca.L* into these plants. This was confirmed by a PCR screen using

Lsof-OI2C primers (9). A comparative proteomics analysis of the infected and healthy plants also showed a difference in the protein profile. The differentially expressed proteins will be subjected to MALDI-TOFF mass spectrometry in order to determine their identity.

Conclusion

We consistently observe fluctuating levels of *Ca.L.* in the laboratory-reared colonies. We developed reference gene primers for qPCR analysis of psyllid microbiota composition. Based on this analysis we discovered that *Carsonella ruddii*, a primary endosymbiont in psyllids, has similar titers in the different psyllid populations indicating that changes of *Ca.L.* do not affect the levels of *Carsonella*. We also found that *Wolbachia* is a low abundant secondary endosymbiont and in some cases seems to have an effect on colony prolificacy. The use of 2D proteomic analysis in potatoes and in *Arabidopsis* should help in the discovery of pathogenic proteins that could identify other microbes involved in ZC disease, as well as host proteins that are triggered by the ZC pathogen(s).

Acknowledgments

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A SUMMARY OF NEBRASKA'S ROLE IN THE ZEBRA CHIP PROJECT DURING 2010

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Summary

Nebraska's role in the zebra chip project during 2010 was multi-faceted. We were involved with the development of the new zebra chip website, created with the purpose of highlighting the SCRI project and documenting its activities. We also collaborated with several research groups from North Dakota and Texas, primarily in the role of collecting plant and psyllid samples for testing for infection by the zebra chip pathogen. We also developed a new research technique for monitoring and sampling psyllids using traps mounted on semi-mobile towers.

Impact statement

Nebraska is uniquely positioned to continue to contribute to the project by being centrally located in the U.S. and thus subject to winds and potential insect movement from multiple directions. We additionally now have both pathology and entomology expertise to begin addressing this insect/pathogen system from both an extension and research perspective in the Central High Plains. The development of the tower traps enabled us to create our own niche and independently conduct research that is unique to Nebraska, and not being duplicated by anyone else in the SCRI project.

Introduction

The University of Nebraska (UNL) was brought into the zebra chip project as an extension presence on the technology transfer, education and training primary focus area (PFA) team. This team consisted initially of Bob Harveson with UNL and David Appel, Ron French, Kay Ledbetter, and Greta Schuster with Texas AgriLife Extension. As of January 2010, another interested member was added to the team with the hiring of an extension entomologist (Jeff Bradshaw) at the University of Nebraska's Panhandle Research and Extension Center. This enabled Nebraska to contribute both plant pathology and entomology expertise to the team. The objectives for Nebraska during 2010 were to 1) participate with the Extension team and assist with the web site development; 2) establish and implement novel research areas not being investigated by other research groups in the SCRI

Materials and Methods

ZC Website

The primary charge for extension this first year was to establish a website for dissemination of knowledge obtained through research funded by the project to stakeholders (including image galleries, and general disease and insect information). It was also desired for the website to additionally serve as a vehicle to highlight the activities of the SCRI zebra chip project and emphasize the strength of its multi-state, multi-institutional nature.

Research Activities

Nebraska also participated in independent research activities, including establishing the presence of psyllid sampling towers at several locations. Four locations were utilized for the placement of towers that represented four separate counties in western Nebraska. Two sites were in commercial potato fields (Alliance and Bridgeport), and two (Scottsbluff and Sidney) were located at research centers affiliated with the University of Nebraska, one of which was used to monitor psyllid activity in the absence of potatoes (Sidney).

Another project was monitoring each of the four psyllid sampling sites for symptoms and disease development during the season. Each field was visited at least twice during the season. Fields were walked in a “Z” or “W” pattern, with an emphasis on the outer 30-40 ft perimeters of fields. When abnormal or suspicious zebra chip-like symptoms were observed, those plants were marked with flags for later observation. Prior to harvest in September, fields were re-visited and flagged plants were dug, and tubers were sliced and observed for zebra chip vascular necrosis symptoms.

Lastly, Nebraska was also involved with several collaborative projects with some of the research groups in North Dakota and Texas. These group efforts consisted primarily of data collection for identifying both the zebra chip pathogen and vector from the Central High Plains.

Results and Discussion

ZC Website

The website has been successfully launched and contains the typical information on the disease, its symptoms, and life cycles of the pathogen and vector. However it also has links that identify and provide contacts for the various co-primary investigators and collaborators involved with the SCRI project. It will also contain a rotating section spotlighting various research participants in the SCRI and their roles in the project.

Research Activities

The establishment of the sampling towers to monitor psyllid activities will be reported separately and in more detail by Jeff Bradshaw. However, establishment of the sampling towers was successful and data were collected once a week from May – early September.

The location at Bridgeport was additionally utilized as another psyllid monitoring location in collaboration with John Goolsby (USDA-McAllen, TX). These data were collected weekly from yellow sticky traps at ground level, and were monitored for both numbers of insects and whether they were also infected with the pathogen. The tower traps were created with a different purpose in mind and were designed to trap and monitor the insects at varying heights above ground. These traps were completely passive, and were not intended to be attractive to the insects. Very few insects have been found to date harboring the pathogen from this series of tests however it will be of interest to eventually compare results from both types of traps at this site.

The Bridgeport site was one of four surveyed locations for disease identification and incidence over the course of the season, but few infected plants were identified. A weather station was further established at this site and environmental data were collected weekly in collaboration with Fekede Workneh with Texas Agri-Life Research for epidemiological studies with the pathogen.

A number of plants with suspicious symptoms were flagged during the season, but when plants were dug at harvest, very few exhibited the vascular necrosis in the tubers characteristic of zebra chip. Those few samples that did contain necrotic vascular elements were found to be negative for the pathogen after being analyzed with real-time PCR in Charlie Rush's lab. It is not known what caused the symptoms that were observed, but they may have been due to either psyllid yellows or some other virus disease such as potato leaf roll.

One last sampling project for Nebraska during 2010 was collecting solanaceous weed species and then sending to North Dakota to test for the zebra chip pathogen in collaboration with Neil Gudmestad. In addition, psyllids trapped from the towers were sent with psyllids collected from tomatoes and further tested for the presence of the zebra chip pathogen. No positive samples were obtained from the approximately 20 weed samples tested, consisting of ground cherry and black nightshade. Likewise, very few psyllids from the traps were found to be infected with the pathogen; however, about 20% of the psyllids collected from tomatoes in Scotts Bluff Co. were positive for the pathogen.

DESIGN, CONSTRUCTION, AND INSTALLATION OF A NOVEL AERIAL SAMPLING DEVICE

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Summary

An aerial sampling device was designed, constructed, and installed at four locations in the western panhandle of Nebraska. The purpose of the sampling device was to develop a way to sample aerial biota at multiple elevations. Thus we hope the trap design will facilitate the capture of migratory agents of concern for crop production. The basic trap design is modular for ease of transport and the trap clamp construction is flexible enough to accept a diversity of trapping systems. All traps were erected approximate to potato fields (UTC, Shldt, and N12 in conjunction with John Goolsby's survey) or very distant from potato production (i.e., Sidney, NE). Numerous samples have yet to be sorted. However, one sample date (last week of July) from all locations collected a total of 624 potential insect vectors of plant pathogens over one week (leafhoppers, aphids, and psyllids). Insects were collected at all sampled elevations (~10, 20, 30, 40, 50, and 60 ft), with some differences in trap distributions by location by elevation by insect group.

Introduction

Many of the most pestiferous insects of potatoes are those that transmit pathogens. Many of these insects are vectors while in their winged form. Numerous insect sampling schemes have been developed by entomologists to sample the abundance, dispersion, and dispersal of insect vectors of plant disease. One of the most common methods for regional sampling of aerial insects is through the use of suction traps. Suction traps are ideal for sampling aerial insects because of the insect's small size. Typically, these suction traps, often installed as part of a "network" of traps, are between 20 to 40 ft in height. Suction trap networks have existed or are currently in use in England, Midwestern U.S. and in the northwest U.S. The challenges with this type of sampling device is their power requirement, frequent maintenance, and the time and expense associated with sorting samples. Finally, suction traps can only sample at one elevation, thereby limiting the amount of information that could be used for understanding abundance, dispersion, and dispersal throughout a wide geographic area. The trap design proposed herein does not require power and is passive, low maintenance, and can sample at multiple elevations simultaneously (limited only by the height of the tower). Here we present the rough design plans, parts, approximate budget, and a sample data set.

Materials and Methods

A parts list with descriptions and quantities is provided on Table 1. Prior to the construction of the aerial sampling tower information was gathered regarding safety precautions and regulations that may govern the placement and height of the special communications tower and its base (e.g., needs for FAA compliance, underground service conduit locations, and safety equipment needs). Additionally, communications towers wind-speed tables were obtained and calculations were made to ensure that tie-down components (e.g., cables, fasteners, clamps, and the modified base) would support the weight of the tower under adverse conditions. The basic trap design is built onto a “Special Communication Tower” (American Tower Company, Shelby, OH). The only modification made to the basic design and installation of the tower was to have the tower base (a “roof plate” model RP-1) welded to a steel plate (Table 1, fig. 1). The steel plate was constructed (B&C Steel Co., Scottsbluff, NE) with handles and a 2-in diameter hole in each corner. The holes were made to accept steel rods (B&C Steel Co., Scottsbluff, NE) (Table 1). Details of the basic components used in the installation are described in Figs. 1—6.

Traps were set up at 4 locations (Fig. 7); 2 were placed in potato production fields, 1 adjacent to potato research plots, and 1 at a site ~35 miles away from any potato production. Traps were checked weekly and the samples were extracted from screens using HistoSol (National Diagnostics, Atlanta, GA) and stored in 90% ethanol in -20°C.

Results and Discussion

The majority of the effort this summer was devoted toward developing and installing this sampling system. We still have many samples to go through and Fig. 8 represents the one sample date that has been counted thus far. Note that for some groups (e.g., leaf hoppers, in blue) patterns in sample elevation may be evident from even a single sample week. Importantly, this single sample provides evidence that this sampling tool can be used to capture insect groups of concern as potential vectors of potato pathogens (e.g., psyllids, aphids, and leafhoppers). In total potential vectors could be collected at all elevations and it may be possible to infer the migratory status and vector potential and risk of insect populations using this tool. Additionally, it is worth noting that there appears to be a relationship between the average abundance of psyllids collected from yellow sticky cards at canopy level and the total number collected on tower traps. Work is underway to explore the sampling efficiency of this system as well as the quality of nucleic acids extracted from these samples.

Table 1. Items required to construct a tower trap and to collect samples.

Part	Description detail	Amount
Steel cable	¼-in EHS 7-strand	375 ft
Turnbuckles	~1,600 lb test strength	6
Thimbles	¼ in (protects cable from fray)	12
Cable clamps	¼ in	36
Auger anchors	39-in long	3
Steel plate	4x4 ft, ¼ in thickness (~400 bls)	1
Steel rods	39-in long, 1 in diameter, large washer welded to top, point cut at base	4
PVC pipe	2 in diameter	60 ft
PVC pipe couplings	For 2-in PVC pipe	5
PVC composite glue	For attaching PVC couplings	1
Nylon rope	For operating the trap carrying system	150 ft
Zip ties	1-ft heavy-duty for attaching PVC to tower rungs	18
Bolts	2-inch x 3/8 in diameter	21
nuts	For 3/8 in diameter bolt	21
washers	For 3/8 in diameter bolt	21
Boom lift	60 ft minimum extension	1
Custom window screen	1 x 1 ft with nylon screen	12
Sledge hammer		1
Wooden guide brackets	Custom-made guides to guide traps past cable brackets	2
Cable cutter	Strong enough to cut ¼-in cable	1
Tangle trap	“brush-on” formulation	< 1 gal
Squeegee	For spreading on tangle trap	1
Pulley	Attaches to 2-in bolt on top of tower. Needs to be big enough to accept nylon rope	1
Rubber mallet	To assist with driving bolts into tower sections	1
Clevises	>1,600 lb test strength	6
Safety harnesses	Must have for all operators within the boom bucket	2
Special Communications tower	American Tower Company. Including flat-bottom tower base	60 ft (6, 10-ft sections)
Guy wire brackets	American Tower Company. May need some custom fitting for SPC tower	2
HistoSol	High-grade citrus oil	~2 gal
Ethanol	100% ethanol (to be diluted)	5 gal
WhirlPaks	20-ml bags	~125

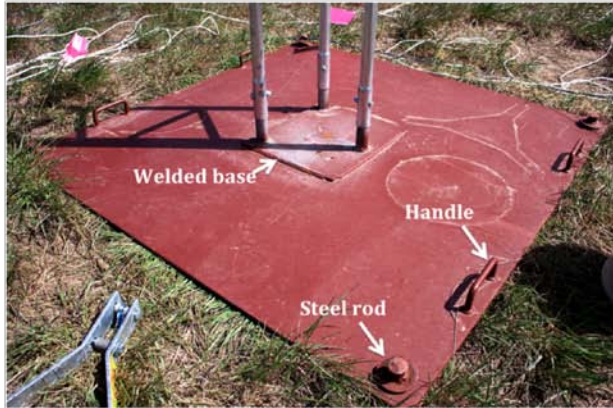


Figure 1. Modified steel-plate base of tower sampler showing the welded handles and tower base and the steel rods tacked into the ground to secure the modified base.



Figure 2. Installation of the cable bracket onto the top of the third 10-foot section of tower. Also shown is the PVC pipe lashed to this section.



Figure 3. Cable-securing components: Cable clamp (must always have 3 on each cable end), thimble (to protect the cable from fraying), turnbuckle, clevis, and the top of a 39-in auger anchor.



Figure 4. Erection of the tower showing the 60-foot boom lift (left) used for set up and the detail of the placement of the last section (right).



Figure 5. Detail of the aluminum-framed, screen trap inserted into the PVC guide.



Figure 6. Detail of the aluminum-framed screen trap outside of the PVC guide.



Figure 7. Map showing the locations of the four tower samplers in the Nebraska panhandle (~170-mile round trip between sample locations).

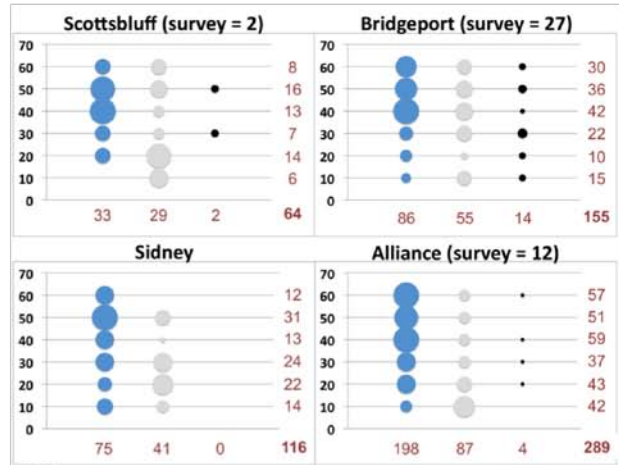


Figure 8. Samples of insects collected on tower traps for the last week of July, 2010 at Alliance (N12), Bridgeport (Shldt), Scottsbluff (UTC), and Sidney, Nebraska. The size of the dots indicate the proportional abundance of insects within a particular group for a given sample elevation (Blue dots = leafhoppers, gray dots = aphids, and black dots = psyllids). Y1 = Sample elevation (10, 20, 30, 40, and 60 ft), X = Total number of insects within an insect family of all elevations, Y2 = Total number of insects at each elevation for all insects sampled. Numbers in parenthesis indicate the average number of psyllids collected for the same time period on yellow sticky cards from John Goolsby's survey at the same locations.

DISTRIBUTION AND PHENOLOGY OF THE POTATO PSYLLID IN SOUTHERN CALIFORNIA

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Summary

The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae), is a serious agricultural pest of potatoes, tomatoes, and peppers. In the development of an integrated pest management program against this pest, it is critical that sampling plans be developed that efficiently and easily determine occurrence and population density of this insect. Such information will be important in determining action thresholds for management decisions. Our objectives for this study were to 1) document the phenology and distribution of the potato psyllid in southern California and 2) determine the distribution of potato psyllids both within commercial potato fields and within potato plants. Our methods relied on a systematic sampling design to count all of the psyllids on crop plants. Results of our study indicated that psyllids arrive within agricultural fields in different counties at different times and experience different population trajectories depending on the crop. This movement between crops clearly suggests the need for an area wide management program against this insect. To most efficiently sample potato psyllids on potatoes in southern California, we recommend starting on the edges of fields and sampling the underside of leaves in the middle of the plant.

Introduction

The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae), is a major pest of solanaceous crops in Central and North America (Cranshaw 1994, Jackson et al. 2009). While damaging outbreaks of this pest have been reported across vast geographic areas such as California, Texas, Washington State, the Central U.S.A., Ontario Canada, and Baja Mexico (Cranshaw, 1994; Zink, 1998; Al-Jabr, 1999; Ferguson et al., 2002; McGuire, 2002; Liu 2006; Munyaneza et al., 2007), there is not much information regarding the phenology of the potato psyllid in southern California within a variety of solanaceous crops and there are no studies to date regarding the distribution of the potato psyllid within a field and within a plant. Thus, the objectives of this study were to determine the phenology and distribution of the potato psyllid in southern California. Our long term goal for this research is to develop a sequential sampling plan, which will aid pest control advisors in management decisions.

Materials and Methods

Tomatoes, bell peppers, and potatoes were sampled every two weeks throughout the 2009 and 2010 growing seasons to determine the phenology of potato psyllids in southern California. These crops were sampled in Riverside, Orange, and Ventura counties. A systematic sampling design was used to determine the mean number of psyllids on the crop plants whereby every psyllid in all life stages (i.e., eggs, nymphs, and adults) were counted. The systematic sampling design consisted of examining plants every 20 m through five transects in a field for up to 80 m into the crop. These transects divided the field into the two

end rows, down the middle of the field, and the two quarter rows between the middle and ends. In addition, the location of the psyllids were recorded by further dividing a crop plant into 1): top, middle, and bottom portions; 2) by plant structure (i.e., leaf, stem, bud/flower); and 3) location on leaf (i.e., top or bottom).

Results and Discussion

Phenology. The potato psyllid was found in all three counties examined; however each location had different times of the year for when the psyllid arrived and different rates of population expansion following arrival (Figure 1). The highest density of psyllids were found in a conventional tomato field in Ventura county during the 2009 growing season. Psyllids reached a peak of 875 ± 565 per tomato plant despite repeated applications of pesticides by the grower. Population peaks were not as drastic in the other crops during the 2009 and 2010 growing seasons. These data suggest the need for area wide management of the potato psyllid to reduce population migration between crops as it is evident the potato psyllid occurs throughout the year.

Sampling. Data discussed here will only focus on potatoes. When potato psyllids arrive in a potato field, approximately 70% of the population can be found on the field edges (within 2 m; Figure 2). As the season progresses, the psyllid then becomes more evenly distributed throughout the field. In terms of the most efficient place to find psyllids within a potato plant, the majority can be found on the middle of the plant with approximately 60-80% of the psyllid population being found there in the 2009 growing season for the pre-bloom and post-bloom growth stages of the potato, and approximately 40% of the psyllid population being found there in 2010 for the bloom and post-bloom growth stages. In 2009 and 2010, close to 100% of the psyllid populations were found on the leaves of potato plants, and approximately 90% of the psyllid population were found on the bottom of potato leaves for all potato growth stages. These data suggest that to sample potatoes most efficiently samples should be taken from the edges of the field on the undersides of leaves from the middle of the plant. We plan to verify this distribution in the next field season to complete development of a binomial sampling plan.

Figure 1. Mean numbers of psyllids found in potatoes, bell peppers and tomatoes in three counties in California during 2009 and 2010.

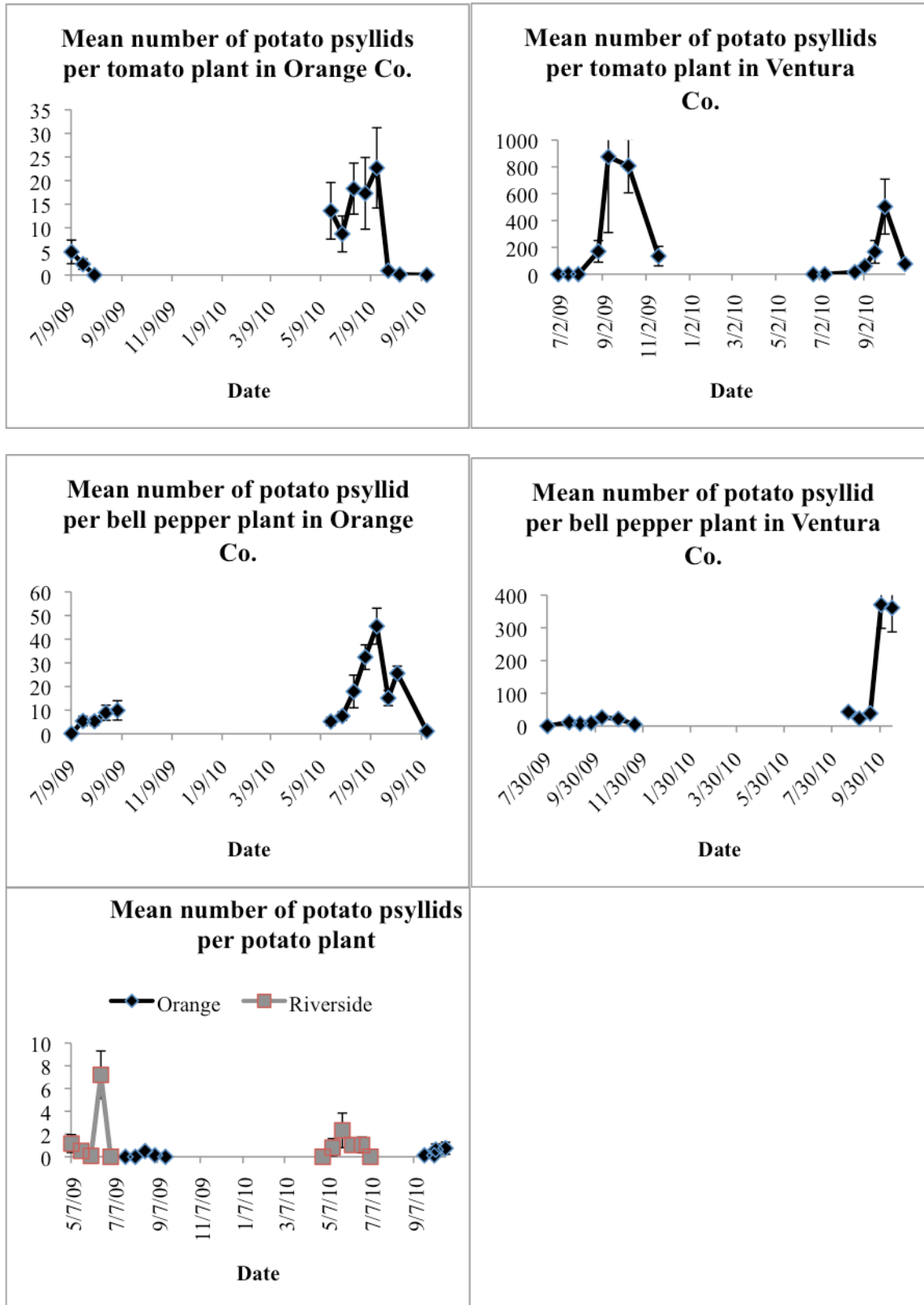
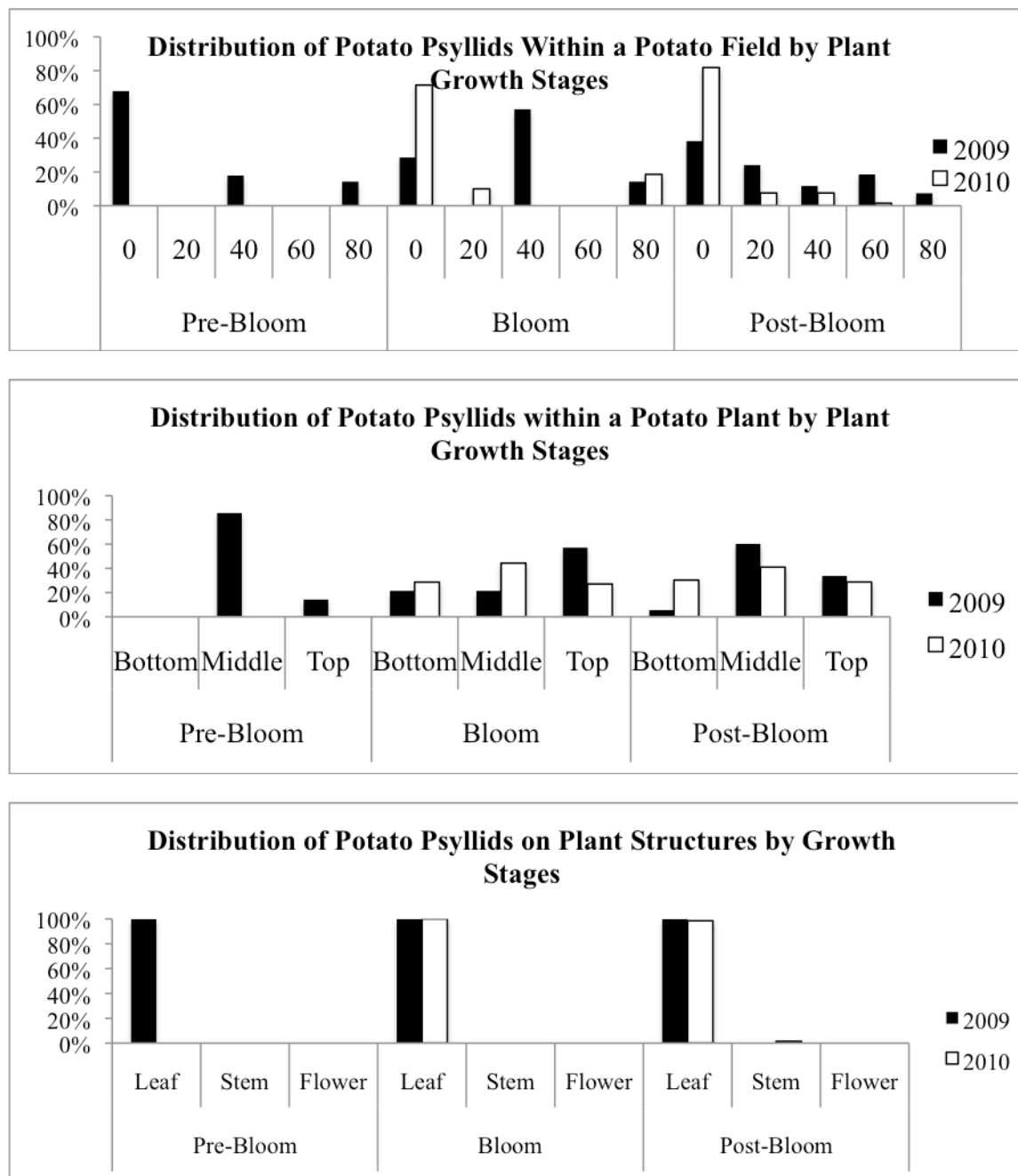


Figure 2. Distribution of potato psyllids within potato fields, and within tomato plants, over two cropping seasons.



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HIGHLIGHTS ON ZEBRA CHIP MEXICAN EXPERIENCES

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Summary

Mexican potato growers for seed, chipping or table markets have experienced devastating effects of zebra chip (ZC) disease during the last 7 years. Nevertheless, some of them have learned to deal with this issue, based on research as well as on “trial and error” experiences. High, medium and low risk areas have been identified in Mexico: Nuevo León and Coahuila being high, Chihuahua medium and Sonora and Sinaloa with almost no ZC prevalence. Monitoring methods have been developed by potato growers to quantify and manage potato psyllids populations in order to minimize negative impacts on yield and frying quality. Defining best suitable planting dates has also proved to be relevant: in Nuevo León and Coahuila planting in May and early June reduces crop exposure to potato psyllids. Chihuahua potato psyllids populations are significantly lower than those of Nuevo León and they increase from south to north as summer progresses. Infected seed-tubers have been identified as an important source of inoculum. It has also been determined that maximum dispersion distance from inoculum source is 80 to 100 meters.

Introduction

Zebra chip (ZC) has caused devastating epidemics in potato growing areas of Northern Mexico, especially in 1994 and 2004. In Nuevo León and Coahuila states, disease presence varies from 20 to 60% of potato planted surface every year, while in other growing areas such as those from Chihuahua, Sonora and Sinaloa states, disease presence is lower than 10% (Hernandez *et al.*, 2006). Potato psyllids role in the transmission of purple top disease, which included ZC symptoms in tubers, was established by Mexican researchers since 2002 (Leyva *et al.*, 2002, Flores and Lira, 2008). In 2009, Almeyda *et al.* as well as Munyaneza *et al.* identified *Ca. Liberibacter psyllaurcus* in potato fields from Nuevo León state. Since then, chemical spraying has been heavily used as a primary control strategy. But it appears to be costly and has shown low efficiency rate when used as a corrective measure. Covering the plant with insect-proof fabrics has resulted in good disease control (Flores and Lira, 2008), but it has not been a cost-effective strategy either. Although perennial weeds have been identified as over-wintering sites for potato psyllids, and seed tubers have been confirmed as ZC inoculum source, transmission to potatoes from over-wintering hosts and their importance for ZC epidemics have not been determined. This paper describes potato psyllids monitoring results in Chihuahua and Nuevo León as well as research on epidemiology of ZC in Mexico.

Materials and Methods

Two different data collection methods were implemented in Nuevo León, Coahuila and Chihuahua states since 2004. On one hand, potato psyllids populations were measured in Nuevo León since 2004 and in Chihuahua since 2010 through i) yellow sticky traps and ii)

swipe net use every other day at commercial fields. On the other hand, experimental trials were established in 2007 and 2009 at Universidad Autónoma Agraria Antonio Narro (UAAAN) in Saltillo, Coahuila for inoculum sources identification and dispersion pattern modelling.

1. Potato psyllids population measurement at commercial fields

a. Sticky traps

Yellow sticky traps were used from 2007 to 2010 in 3 different potato growing plots located in Nuevo León and Coahuila states. Three yellow sticky traps were placed at potato canopy level in each plot. Traps were replaced every week. Captured insects in sticky traps from all 3 plots were quantified and averaged to calculate number of insects per trap per week. Weekly data were added to calculate the cumulative number of insects trapped per year. In Chihuahua 2010, yellow sticky traps were not effective to trap the very low population of potato psyllids present.

b. Sweep nets

Potato psyllids were sampled over an average planted surface of 500 Has from 2005 to 2010 in AgroJaba farm in southern Nuevo León state. A person walked through the field every other day and did 100 net sweeps in four different sampling areas per 65 Has pivot. Potato psyllids were quantified per sampling date during crop development in order to obtain an average weekly population per 100 sweeps. Tuber samples were fried and total defects were measured and reported under PepsiCo's procedure. This methodology was also applied to Chihuahua commercial fields in 2010 over 400 planted hectares. Weekly populations were plotted versus timeline.

2. Experimental trials

Potato seed and *Lycium berlandieri* as inoculum sources (UAAAN – Saltillo Campus)
In 2007, four rows, 3 meters long, were planted with tuber seeds from diseased plants with ZC symptoms. In 2009, 2 *Lycium berlandieri* potted specimens of this native plant were transferred to a potato experimental field. In both years, a 50 m long plot was established with mini-tubers of Giant Variety. Every 10 m from the inoculum source to the end of the plot, 10 leaf samples were taken at 30 and 57 days after plant emergence. Samples from 2007 were frozen at -80C degrees, stored and tested by PCR in 2009 for *Ca. Liberibacter psyllae*. An epidemiological model (Campbell and Madden 1990) was fitted to the data and maximum dispersion distance was estimated using the model equation.

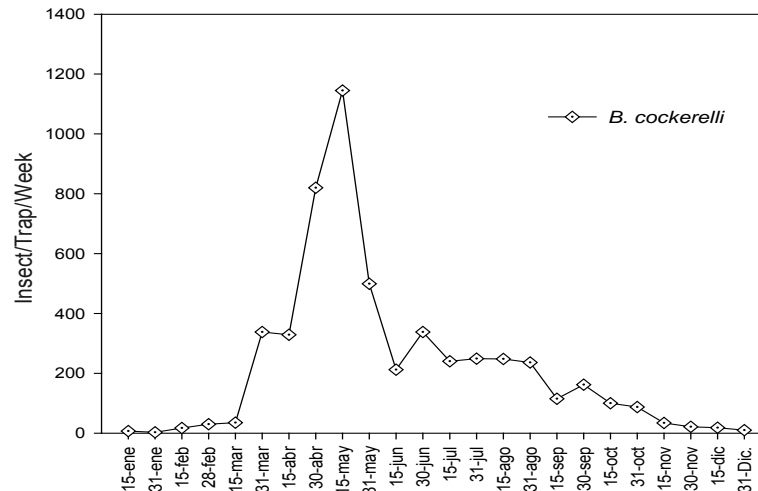
Results and Discussion

1. Potato psyllids population measurement at commercial fields

a. Sticky traps

Potato psyllids populations range in Nuevo León and Coahuila fluctuated between none in late January to 1200 insects per trap per week in mid-May from 2004 to 2007, and peak occurred between March 15 and June 15 (Fig. 1). Coincidence between potato psyllid population peaks and most susceptible young plants can then be avoided through planting in late May and June. Early planting dates are already known as high ZC infection risk. Potato growers are following recommendations from Fundación Produce, a government funded agency, and moving to later planting dates. Chihuahua potato psyllids populations were not detected with sticky traps, suggesting that lower ZC incidence compared to Nuevo León state is related to low insect populations.

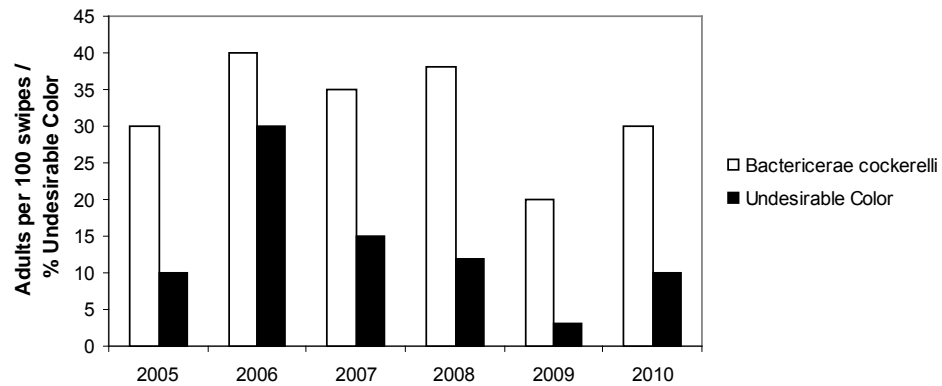
Figure 1. Potato psyllids population in Coahuila and Nuevo León. Average of 3 potato fields and 4 growing seasons (2004 – 2007).



b. Sweep nets

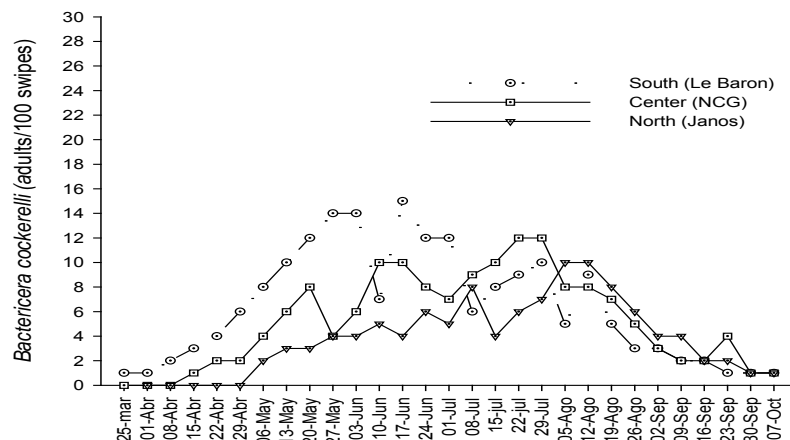
In Nuevo León, potato psyllids presence per 100 sweeps varied from 20 to 40 insects from 2005 to 2010 (Fig. 2). When compared to ZC incidence in tubers, measured as undesirable frying color, we observed that highest and lowest potato psyllids population matched highest and lowest frying defects levels. However, intermediate figures were not correlated with ZC incidence, which suggest that other factors such as hot/cold potato psyllids mix, temperatures and planting dates might be playing a role in the transmission of ZC pathogen.

Figure 2. Annual potato psyllids population and undesirable frying color in Nuevo León.



For Chihuahua 2010, average potato psyllids per 100 swipes varied from 0 to 16 insects (Fig. 3). Highest populations were observed from mid-May to early August depending on region latitudes. Planting dates to avoid potato psyllids presence peaks should consider planting location. Early planting dates may reduce ZC infection risk at Janos, in northern areas, while late planting dates may be better for Le Baron, a southern location in the state.

Figure 3. Potato psyllids population observed at 6 commercial fields in Chihuahua.

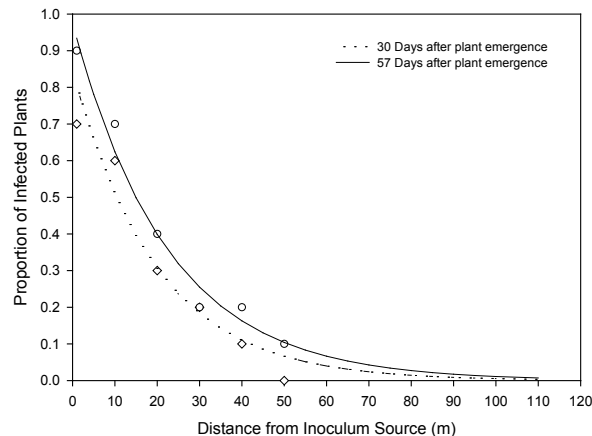


2. Experimental trials

Seed tubers. A steep disease and infection gradient was observed 30 and 57 days after plant emergence. No symptomatic and no infected plants were observed at 40 and 50 meters after exposing disease-free plants to inoculum source. Results suggest that insects transmitting the disease do not move far away from the inoculum source. Fitted model to the data indicates that maximum dispersal distance of ZC inoculum source was 80-100m (Fig. 4). Therefore, controlling volunteers and alternate hosts in and around potato fields is a strategy that may reduce disease incidence. Steep gradients also indicate that infected seed-tubers are an important source of inoculum and that using *Ca. Liberibacter psyllae* free-tested tubers may reduce ZC infection risk.

Lycium berlandieri. No disease gradient was observed when infected *Lycium berlandieri* plants were used as inoculum sources. This suggests that potato psyllids do not transmit ZC pathogen from this host to potatoes; specific transmission experiments are needed to remove *Lycium berlandieri* from native inoculum potential sources.

Figure 4. ZC infection gradient from an inoculum source; plants from infected tubers.



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FACING THE BACTERICERA COCKERELLI/CANDIDATUS LIBERIBACTER COMPLEX IN HONDURAS.

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Summary

The potato psyllid, *Bactericera cockerelli* was first detected in Honduras in 2002. Since then, it has spread all over the potato growing areas, causing significant losses related to direct damage and its associated disease. A study to determine *B. cockerelli* phenology and the incidence of potato zebra chip disease was started in March 2010 in the Departments of Intibucá and Ocotepeque. Adult psyllids were monitored with yellow sticky traps, whereas immature and disease incidence were monitored by direct counts. Psyllids were observed in all sites under study, but only in one of three sites in Intibucá there were plants with clear foliar symptoms and discolored tubers associated to *Liberibacter solanacearum*. In Ocotepeque, no fields have been monitored because growers are reluctant to plant potatoes due to heavy losses associated to the *B. cockerelli*/*L. solanacearum* complex in the past two years. The information gathered indicates that both the disease and its vector are present in the two main potato growing areas of Honduras, but the disease is not as widespread in Intibucá as it is in Ocotepeque.

Introduction

In Honduras, potatoes are grown at altitudes over 1300 meters above sea level, and, thus, it is concentrated in the mountainous areas of the Departments of Intibucá (ca. 2000 ha), Ocotepeque (ca. 800 ha) and Francisco Morazán (ca. 250 ha). Due to the country's climatic conditions, potatoes are grown all year round, however, most of the production is done from November through May (dry season) when the pressure of late blight and other diseases is lower. About 60% of the growers are small holders, with 0.2 ha or less per grower, under a low input production system. Ten percent of the growers operate areas of 1 ha or larger, with a high input production system. All production in Honduras is for fresh consumption.

The potato psyllid and its related disease were first reported in Honduras in February 2002, when potato growers in Ocotepeque reported significant losses to a condition they called “papa negra” (black potato) (H. Espinoza, unpublished data), which had the symptoms of what, at the time, was regarded as psyllid yellows of potato (APS 1981). However, no psyllids were observed then. In 2006, presence of potato psyllids was reported from Intibucá, when Ocotepeque was already reporting heavy losses related to the insect/disease complex. By 2008, the psyllid and the disease were found in Francisco Morazán, attacking potatoes and other solanaceous crops.

Materials and Methods

In March 2010, we started a study to determine *B. cockerelli* phenology and the incidence of potato zebra chip disease. Five cylindrical (9 cm diameter, 19 cm height), sticky traps with a reticulated yellow card (Al-Jabar 1999) with 280 cm² of effective area were deployed per

field to monitor adult psyllid population. Traps are serviced weekly, replacing the exposed yellow card, which is taken to the laboratory for counting the trapped psyllids. Immature psyllids (eggs and nymphs) were monitored in foliage. Five samples of five leaves (one mature leaf per plant) were taken weekly in each field, starting three weeks after emergence. Disease incidence is also monitored weekly, recording plants with symptoms in five samples of ten plants per field. At harvest, five tuber samples of one row-meter were collected in each field and sliced to determine presence of discoloration due to *Liberibacter* disease. Non cultivated solanaceous plants in the surrounding of the selected fields are also observed for psyllids and symptoms of the disease.

During the first semester of 2010, observations were conducted in three sites in Departamento de Intibucá: **Site 1**, at El Pelón, Municipio de Yamaranguila (1902 meters above sea level), 600 m² planted on week 6, 2010 with the cultivar Caesar. **Site 2**, at Santa Catarina, Municipio de La Esperanza (1783 masl), 0.8 ha planted on week 6 with the cultivar Arnova and **Site 3**, at Santa Catarina (1635 masl), 1.0 ha planted on week 8 with the cultivar Caesar. Pest management included an application of imidacloprid at planting and a rotation with pyrethroid, neonicotinoid and organophosphate insecticides, directed, mainly, at potato psyllid management. All plantations received regular applications of fungicides for late blight management.

Results and Discussion

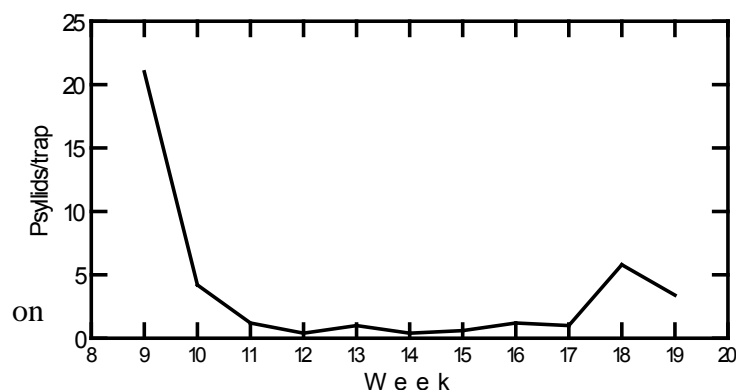


Figure 2. Average capture of Potato Psyllids in yellow, sticky traps in potato field at El Pelón, Yamaranguila, Intibucá, Honduras.

Site 1. When traps were deployed (week 9), an adjacent potato field had just been mowed, prior to harvest. As a result, there were high counts of psyllids, especially in the two traps next to the mowed field. Also, we could observe high number of adult psyllids and eggs foliage that decreased as we moved away from the older potato field. After that, trap counts decreased and stayed below 2 psyllids/trap until week 18 (Fig. 1). Traditionally, potato growers stop

treatments for pest and disease management three to four weeks before harvest, which may be the reason for the increase in psyllid captures during weeks 18 and 19. The weekly observations did not reveal the presence of plants with symptoms that could be clearly attributed to *Liberibacter solanacearum*. A sample of tubers collected at harvest did not have any symptoms of the disease, either, in spite of the high number of adult psyllids observed initially. At this site, a plant of *Datura stramonium* with interveinal yellowing symptoms was observed. Tissue samples from this plant tested positive for *Liberibacter solanacearum* (J. K. Brown, unpublished data).

Site 2. Average trap captures remained below one psyllid/trap, with two consecutive weeks (weeks 11 and 12) without any capture. No plants with unequivocal *Liberibacter* symptoms were observed throughout the potato cycle. A sample of tubers collected at harvest did not have the discoloration characteristic of *Liberibacter* infection.

Site 3. At this site, psyllids were captured in the traps every week (weeks 10 – 21), even before plant emergence. However, only on weeks 14 and 20 the average capture of psyllids per trap was 1 or higher. By the end of the cycle, 11% of the plants had symptoms as described by Crosslin et al. (2010). Fourteen percent of the tubers sampled at harvest had discoloration that could be attributed to *Liberibacter* disease.

This study was designed to gather information from Intibucá and Ocotepeque, the two main potato growing areas of Honduras. This report includes only information from Intibucá, since no potatoes have been planted in Ocotepeque since the beginning of this study. Potato growers in Ocotepeque have not grown potatoes this year because of heavy losses associated to the *B. cockerelli*/*Liberibacter* complex in the past two years. However, in March 2010, at the beginning of the study, three potato fields at the end of the cycle were observed. All of them had psyllids in all stages of development and there were plants that presented symptoms characteristic of *Liberibacter* disease. The information gathered indicates that both the disease and its vector are present in the two main potato growing areas of Honduras, but the disease is not as widespread in Intibucá as it is in Ocotepeque.

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PHYTOPLASMA INFECTION OF CHIPS POTATOES IN ROMANIA AND SOUTH RUSSIA (2008 – 2010)

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Summary

In 2008, Fitolab Ltd, Hungary undertook a contracted multi-year research project for Pepsico International, UK, to investigate the occurrence of phytoplasma in potato crops in Romania and South Russia.

The aim of the project: to clarify the causal agent(s) and look for the potential vector(s) of the disorder, partially resembling zebra chip disease reported in USA. The overall objective of the study was to provide new crop management and control strategies to avoid the losses of processing potatoes to Pepsico businesses in these sensitive regions.

The present report summarizes the results of the activities completed in selected topics of the research, conducted between 2008 and 2010. In the course of the three years more than 100 000 insect specimens were collected and counted. Additionally, 955 plants and 2003 insects were analysed by PCR in the frame of the topics below.

Monitoring of insects, symptomatology and identification of the causal agent. On designated potato fields of the southern part of Romania and Russia (in Krasnodar and Rostov regions) insects were monitored by using yellow sticky traps, changed weekly. Insects were also collected by sweep netting at regular intervals. Plants and insects were tested by conventional PCR and RFLP and/or by real-time PCR for the identification of the causal agents and confirmed with sequencing. Appearance and types of symptoms on plants were also recorded. Symptoms typically found on the potato fields both in Romania and Russia were: leaf rolling, purple top and aerial tubers. Spongy tubers were observed under the heavily infected plants. It is important to emphasize that, unlike the symptoms of zebra chip in USA, the surface of the fresh cut tubers did not show zebra chip patterns, the discoloration only appearing on the fried chips. Stolbur phytoplasma was identified in the symptomatic plants and in the insects from both Romania and Russia. Results of molecular tests on sweep netted insects showed that Stolbur was present mostly in cixiid plant hoppers. It could be concluded that the potential vector species of Stolbur phytoplasma in South Romania and South Russia belong to Cixiidae, but the abundance of plant hopper species varied by the different locations and by years. The results obtained indicate that annual monitoring is essential to provide basic data for the development of effective crop management protocols and strategies for the potato chip industry.

Vector transmission experiments. In 2009 membrane feeding experiments was applied to screen 21 insect species of Cixiidae, Delphacidae, Cicadidae, Miridae, Psyllidae, all of which

had been found in high numbers at some time during the course of the 3-year investigation. These were screened for the eventual ability to transmit Stolbur phytoplasma. In 2010 the experiment was set up with specimens of *Reptalus quinquecostatus*. The results of the 3-year vector transmission experiments revealed that *R. quinquecostatus* is an effective vector of Stolbur phytoplasma in the case of potato. The study of the biology of this species is one of the tasks of the next years in the development of new crop management strategies.

Anti Vector Activity Trial. In 2010, ten treatments + the untreated control, in 2 replicates, total 22 plots, 360 potato plants/plot were involved in the trial. There was no strong correlation between the insecticide treatments, their efficacy and frequency of Stolbur disease. It can be concluded that an intensive insecticide program will not give acceptable protection against Stolbur infection. Therefore other new methods shall be involved in the next years' trials in order to reduce the losses of chip potato industry.

Impact statement. Infection of commercial potato crops with phytoplasmas (Stolbur) is becoming increasing by frequent within the PI region and particularly at latitudes encompassing South Central and Eastern Europe and into South Russia. Significant and sometimes catastrophic crop losses can occur, reflecting similar experiences in North and South America and New Zealand where similar organisms are involved. Key business developments in The Balkans and S Russia will be impacted by future phytoplasma infection and has already resulted in certain regions being unsuitable for sustainable potato production.

Since 2003, early-crop potato supplies from infected areas have been severely impacted. In Romania over 70% of the crop has been affected during this period, whilst in Russia, for the same period about 40% of the crop was lost. Overall, the financial impact has been significant.

Introduction

In recent years, severe symptoms have been observed in the potato fields and on fried potato chips produced from infected tubers in South Romania and in southern parts of Russia, resembling zebra chip disease which occurs in Central America, the US and New Zealand. The only difference is that in Europe the ZC discoloration does not appear on the fresh cut (raw) potato slices. Since high economic losses are being recorded by the chips industry of Romania and Russia, in 2008, a research project was contracted by Pepsico International, (UK) to clarify the causal agent(s) and identify the potential vector(s) of the disease, in order to develop crop management strategies that would mitigate the losses of the potato industry. The multi-year research plan has been designed and conducted annually by the Fitolab Plant Pest Diagnostic and Advisory Ltd, Budapest, Hungary. The primary research objectives were: monitoring of potato fields for determination of the causal agents and potential vectors; vector transmission experiments; anti vector activity trial; tuber transmission studies; evaluation of chip and potato varieties for susceptibility to Stolbur phytoplasma infection. In this proceeding we report on the results of insect monitoring and symptomatology, identification of the causal agent, the vector transmission activities, and the anti vector activity trial.

Material and Methods

Monitoring of insects and symptomatology

South Romania. Annually, two potato fields were designated for monitoring in Fundulea (2008) and Radovanu (2009, 2010).

South Russia. Two-four fields/year were monitored in South Russia in the following regions: Krasnodar region: Gulkevichi (2008-2010); in Rostov region: Azov (2008, 2009), Niva (2009), Mayak (2008, 2009), Manitek (2010), Zolotovskoye (2010). Flying period of the insects were monitored by placing out eight yellow traps per potato field: 4 marginal traps at 4 points of the compass/field, 3-5 m from the potato field edge and 4 inner traps: 50 m from the edge of the field. The yellow traps were replaced every 7 days. Insects were also collected weekly on the potato fields by sweep netting and stored in 96% ethanol at 4°C. Additional sweep netting was carried out 2-3 times during vegetation period on surrounding crops in the vicinity. The yellow traps and the sweep netted insects were delivered to Fitolab regularly for morphological identification and molecular diagnostic purposes. The symptoms observed on the potato fields were recorded and symptomatic and asymptomatic plants were sampled for laboratory analyses.

Identification of the causal agents

Morphological determination of insects and molecular analyses for phytoplasma infections of plant and insects were performed by Fitolab. Plants were individually tested. Depending on the size of the insects, 2-5 specimens of the same species were grouped in batch or used individually for testing. DNA extraction both from plants and insects were carried out with CTAB (3%) method, developed by Daire *et al.* (1997). In 2010, Roche High Pure Tissue Kit was used for DNA extraction from insects.

Phytoplasma detection, PCR-RFLP analysis. Universal phytoplasma primers were applied in nested PCR: P1/P7, (Smart *et al.*, 1996; Deng & Hiruki, 1991), R16F2n/ R16R2 (Lee *et al.*, 1995) or/and U3/U5 (Lorenz *et al.*, 1995). In RFLP R16F2n/R2 nested-PCR products were digested with *Tru1I* (*MseI*), *RsaI*, *AluI*, *HhaI*, *HhaII* (Fermentas) restriction enzymes. Real-time PCR: map gene specific mapBN-F and mapBN-R primers and mapBN-VIC reporter labelled TaqMan LNA probes were used (Pelletier *et al.*). For DNA sequence analyses, direct sequencing of R16F2n/R16R2 amplicons of selected positive samples was performed.

Vector transmission experiments

Membrane feeding experiments, 2009. Twenty one species belonging to Cixiidae, Delphacidae, Cicadidae, Miridae, Psyllidae were collected by sweep netting on potato fields under high infection pressure by Stolbur phytoplasma in Radovanu, Romania and in Monorierdő, Hungary. The insects, caught alive, were individually put into tubes, where insects could feed through the parafilm membrane from a sterile sucrose media. After a feeding period of 1-5 days the insects and the feeding media were tested for phytoplasmas.

Classical vector transmission experiment, 2009. Specimens of selected potential vector species belonging to Cixiidae (*Reptalus quinquecostatus*, *R. panzeri*), Cicadidae (*Empoasca pteridis*, *E. decipiens*, *Anaceratagallia ribauti*) or Miridae (*Lygus rugulipennis*, *Adelphocoris lineolatus*) were collected by sweep net on Stolbur infected potato fields in South Romania and in Hungary. The live insects were selected by species or genera and put into insect cages in a growing chamber at Fitolab. Three to fifty insects were released per cage, where 4 or 6

potted healthy potato plants were grown in order to feed the insects. The cages were stored under laboratory conditions (16 L/8 D, 25° C). Visual observations of plants were carried out regularly. At the end of the experiments all plants were separately tested for phytoplasma. *Classical vector transmission experiment, 2010.* The experiment was set up under laboratory conditions (16 L/8 D, 25° C) in cages. Specimens of *R. quinquecostatus*, collected on Stolbur infected potato field in Radovanu, were released in six cages (60 insects/cage, ten insects per healthy potato plant). Control cages, without insects, were also set up. Analysis of plants was performed by conventional and real-time PCR for determination of phytoplasma infection on the 3rd, 5th, 7th, 14th and 52nd day after release. Visual observations of plants for phytoplasma symptoms were carried out weekly.

Anti vector activity trial

In 2010, ten treatments + the untreated control, in 2 replicates, total 22 plots, 360 potato plants/plot were involved in the trial. The following treatments were applied alone and in combination: 3 neonicotinoids (Acetamiprid, Tiametoxam, Tiaklopid); 2 biopesticides (Abamectin, Spinozad); 1 ketoenol (Spirotetramat); 3 additives (Biofilm, Plantafos Universal, Spur). Four biostimulants were applied alone: Amalgerol, Plantafos Universal, Kendal and Bion. From plant emergence to harvest the insects were collected weekly on yellow traps, and by sweep netting. Disease symptoms caused by viruses, bacteria, fungi and phytoplasmas on potato plants were assessed and recorded on six occasions during the growing season. The species of insects was determined based on morphology and their infection rate by PCR. Randomly selected individuals of the potential phytoplasma vector species and of the potato plants were tested by PCR. Fry tests were carried out on 20 tubers collected from under symptomatic plants and 20 from under asymptomatic plants for each plot, and each replicate.

Results and Discussion

During the 3-year study, more than 100 000 insect specimens were identified and counted. In total 955 plants, 2003 insects were analysed by PCR in the frame of the topics reported below.

Monitoring of insects and symptomatology

South Romania. Leaf rolling, purple top and aerial tubers were the main characteristic symptoms on the potato fields in South Romania. Spongy tubers were also observed in under the heavily infected plants in 2008-2010. Stolbur phytoplasma was identified in the symptomatic plants and in the insects. Density of cixiids on potato fields was high in all the 3 years. *Reptalus* specimens were found dominant among cixiids in South Romania: in 2008: *Reptalus* spp. (82%), in 2009: *R. quinquecostatus* (76%), 2010: *R. quinquecostatus* (99%). The peak of their flying period varied, between beginning of July (2008) and end of June (2009, 2010). High Stolbur infection rate was determined in the specimens of *R. quinquecostatus*: 72% (2008), 40% (2009) and 53% (2010). *South Russia.* At all the locations the same symptoms were typical as in Romania in the three years of the project and zebra chip symptoms of the tuber slices have been observed only after frying. In Krasnodar region, at Gulkevichi *Hyalesthes obsoletus* was found the most frequent cixiid species and the peak of the flying period was at the beginning of July (2008, 2010) or in the middle of July (2009). In Rostov region, monitoring of insects was less intensive in 2010 than in 2009. Different cixiid species were present on potato fields (*Hyalesthes obsoletus*, *Reptalus quinquecostatus*,

Reptalus cuspidatus, *Pentastiridius* sp.) but their population ratio varied by locations. In Mayak *Pentastiridius* sp. was the most abundant (56% of cixiids), while in Niva *Reptalus quinquecostatus* (42%) and *H. obsoletus* (33%). At the different locations of Rostov region the flying period of cixiids was similar: from the beginning of June to the beginning of August. The peak of the flying period was at the beginning of July in Mayak and in middle of July in Niva.

Stolbur phytoplasma was identified in the symptomatic plants and in the insects both in the Krasnodar region and at all the testing sites of the Rostov region. Results of molecular tests of sweep netted insects showed that Stolbur was present mostly in cixiid plant hoppers.

Based on the results, we may conclude that Stolbur phytoplasma was the main causal agent, responsible for the severe losses of chips potato in South Romania and South Russia in 2008-2010. The abundance of plant hopper species varied with different locations, but it can be concluded that the potential vector species of Stolbur phytoplasma in South Romania and South Russia belong to the Cixiidae. The results indicate that annual monitoring is essential to provide basic data for the development of effective crop management strategies for the chip industry.

Vector transmission experiments

Membrane Feeding experiments, 2009. In the experiment we could determine that *Reptalus panzeri* and *R. quinquecostatus* were able to transmit the Stolbur phytoplasma.

Classical vector transmission experiment, 2009. After 3 weeks of release symptoms appeared on the plants, fed by *Reptalus* spp. and PCR confirmed that *Reptalus* spp. were able to transmit Stolbur from infected potato plant onto healthy ones.

Classical vector transmission experiment, 2010. The typical purple top symptoms appeared on 83% of the plants on the 5th week after release of *R. quinquecostatus* specimens onto the healthy potato plants. All the plants showed severe symptoms on the 8th week. Real-time PCR was able to detect Stolbur in 85% of the plant on the 3rd day, and 100% of the plants proved infected on the 14th day after release. Based on these results we may conclude that *R. quinquecostatus* is an effective vector of Stolbur phytoplasma in case of potato. The study of the biology of this species is essential to the development of new crop management strategies.

Anti vector activity trial

On the basis of results of this year, we can conclude that there is no strong correlation between the insecticide treatments, their efficacy and frequency of Stolbur disease. An intensive insecticide program will not give acceptable protection against Stolbur infection. Conventional insecticides, even when frequently used, will not control the incidence of Stolbur disease because; phytoplasma transmission occurs faster than insecticides can act, there is a constant migration of vector insects from surrounding habitats, and, a continuous presence of infection and vectors from within the planted potato fields themselves. This is why other new methods will be tried in the next years in order to avoid the losses of chip potatoes.

NON-INSECTICIDAL BASED APPROACHES TO PSYLLID MANAGEMENT

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Summary

Although insecticides constitute the most important responsive actions against potato psyllid [*Bactericera cockerelli* (Sulc)] infestations in potatoes, the sustainability of medium – and long – term management plans will rely heavily on including other components, such as: use of biological control agents, potato plant resistance, and development of “smart” farming practices, which enable manipulation of potato psyllid behavior. Here, we present preliminary results from on-going research activities, which are intended to complement insecticide-based control tactics.

Introduction

Similar to many other important pests on row crops, potato psyllids [*Bactericera cockerelli* (Sulc) (Hemiptera: Trioizidae)] are considered poly-phagous with confirmed ability to oviposit and complete development on a wide range of both agricultural and native host plants. On one hand, polyphagy by psyllids complicates development of successful control/management tactics, because psyllid populations may migrate into potato fields from many sources – on the other hand, it may also be viewed as an opportunity, because it means that potato psyllids have many choices and therefore should only chose potato plants when these are comparatively more suitable hosts. So, two important questions should be addressed as part of a comprehensive and ecologically-based approach to development of successful IPM strategies: What do we know about host selection by potato psyllids? Can we – pro-actively - lower the risk of potato psyllid infestations? Here, we partially address these questions with some preliminary data from a combination of greenhouse and field studies. When choice tests are conducted under field conditions, many environmental factors may interfere with the outcome. On the other hand, it may be argued that choice tests conducted under controlled laboratory conditions provide data that are too artificial – not reflecting what occurs under natural conditions. We wish to emphasize that the presented experimental setup was chosen because of a strong interest in generating data with a limited budget and within a short time frame.

Materials and Methods

Plant material. The following potato varieties in the early flowering stage were used in a 6-choice study with potato plant material cultivated in a greenhouse at the AgriLife Research and Extension Center in Lubbock, TX: two ZC-susceptible varieties: Atlantic (ATL) and Russet Norkotah (RUSS) and four ZC-resistant varieties: ATX85404-8w (ATX), BTX1749-1W/Y (BTX), NDTX059632-1W (NDTX1), and NDTX059828-2W (NDTX2). These varieties were selected based on chipping and frying results over a multi-field-season research conducted by Dr. Miller. A 5-choice study was conducted with potato plant material

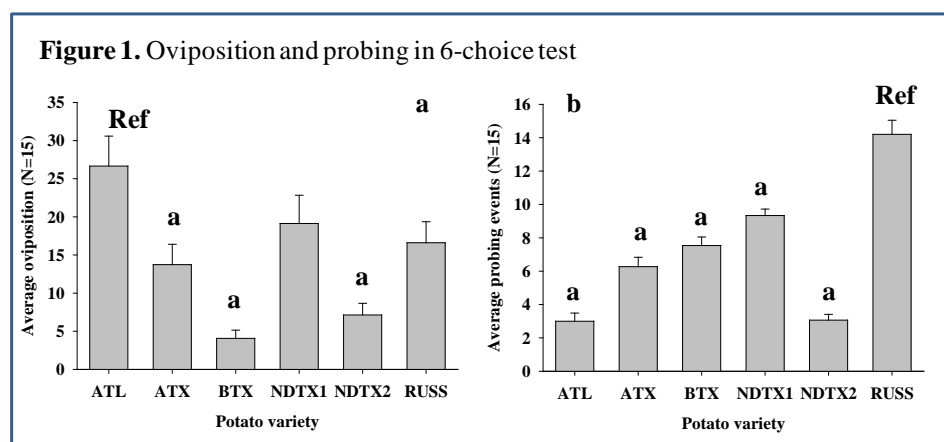
from a center-pivot field trial at the AgriLife Research and Extension Center in Amarillo, TX: ATL, ATX, BTX, NDTX1, and NDTX2 with plant material being collected June 9, 28, and July 14 and 27, 2010.

Hyperspectral imaging of potato leaflets. Immediately before being used in 9-choice tests (see below), individual potato leaflets were placed on a platform and subjected to hyperspectral imaging to obtain relative reflectance profiles from each potato variety. The principal research justifications for deploying this analytical approach are that: 1) reflectance profiles provide insight into the biochemical composition of the leaf tissue and 2) visual cues are known to be important in host selection by psyllids. We followed methodology described in Nansen et al (2010), in which imaging data were acquired with a line-scanning hyperspectral camera with 640 sensors in a linear array (PIKA II, www.resonon.com) and a wavelength range from 405 to 907 nm. The camera was mounted on a 35 cm aluminum tower, and hyperspectral imaging data were acquired at a magnification representing about 100 hyperspectral profiles (pixels) per mm² inside the greenhouse in which the maize plants were grown with sunlight as the sole light source. Dark calibration was conducted at the beginning of the data acquisition, and we conducted white calibration (using white Teflon) immediately before each image acquisition to account for subtle changes in light conditions. Based on dark and white calibration, reflectance values from hyperspectral image cubes were converted into proportions ranging from 0-1.

Choice-tests. We used plastic containers with screened lid to conduct either 5- or 6-choice tests with terminal potato leaflets from the varieties described above. Each terminal leaflet was placed with the petiole inserted into a 20 ml plastic vial with water, and parafilm with small hole was used as lid to prevent psyllids from falling into the water. A total of 20-30 adult unsexed psyllids were used in each trial with leaflets being exposed to adult psyllids for 72 hours. Subsequently, the number of eggs laid on each terminal leaflet was recorded, and in the initial choice test we also counted the number of probing events in each leaflet according to a modification of the McBryde staining technique (McBryde 1936). In brief, each leaflet is placed in a plastic petri dish with McBryde's stain solution consisting of 95% ethanol and glacial acetic acid in a 1:1 ratio and 2% (by weight) acid fuchsin for 48 hours. Subsequently, each leaflet is transferred to a glass petri dish with lactic acid, glycerol, and water (1:1:1 ratio) and heated in an oven (70 °C) for 2-3 hours. After McBryde staining, stylet sheaths turn distinctly purple and can be counted under stereoscope (×100).

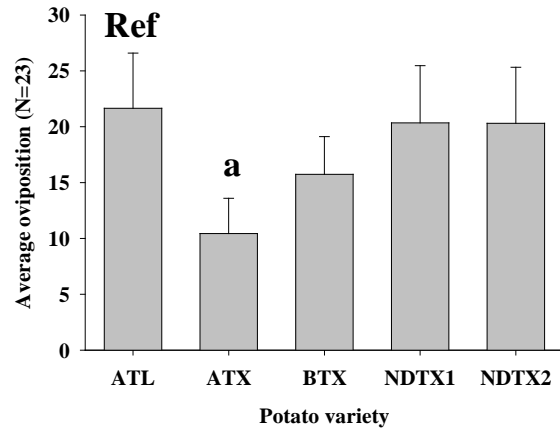
Results and Discussion

Choice tests. Based on 6-choice tests with potato leaf material from plants in early flowering stage grown under greenhouse conditions, we found that ATL was the most preferred oviposition host and that, on average about



5 times less eggs were laid on BTX, which was the least preferred oviposition host (Fig. 1a). The distribution of eggs was significantly different from random ($df = 5$, $\chi^2 = 26.90$, P -value < 0.001), and in pairwise comparisons with ATL as reference numbers of eggs laid on ATX, BTX and NDTX2 were all significantly lower ($P < 0.05$). Russ was the host with the highest number of probing events, and it received about 7 times more probing events than ATL and NDTX2 (Fig. 1b). The distribution of eggs was significantly different from random ($df = 5$, $\chi^2 = 70.34$, P -value < 0.001), and in pairwise comparisons with RUSS as reference the number of probing events was significantly lower on the other 5 varieties (ATL, ATX, BTX, NDTX1, and NDTX2) ($P < 0.05$). These findings suggest that potato varieties are not equally attractive, and that critical piece of information may be used by breeders to develop less attractive varieties. Based on 5-choice tests with potato leaf material from plants grown under center-pivot at the AgriLife Research and Extension Center in Amarillo, TX, we found that, again, most eggs were laid on ATL, but only oviposition on ATX was significantly lower than on leaflets from ATL (Fig. 2).

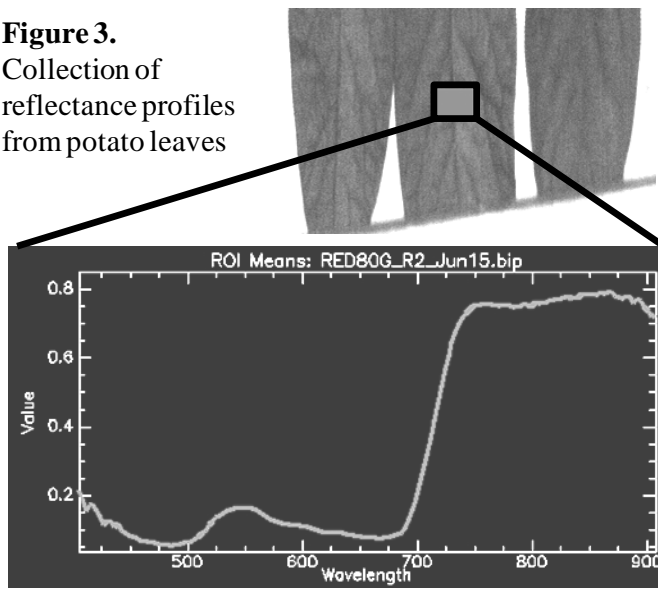
Figure 2. Oviposition 5-choice test



Hyperspectral imaging of greenhouse plants. Fig. 3 shows potato leaflets on a horizontal

Figure 3.

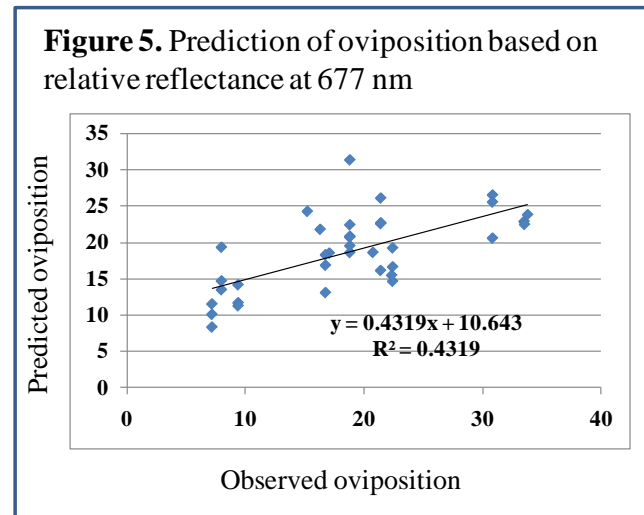
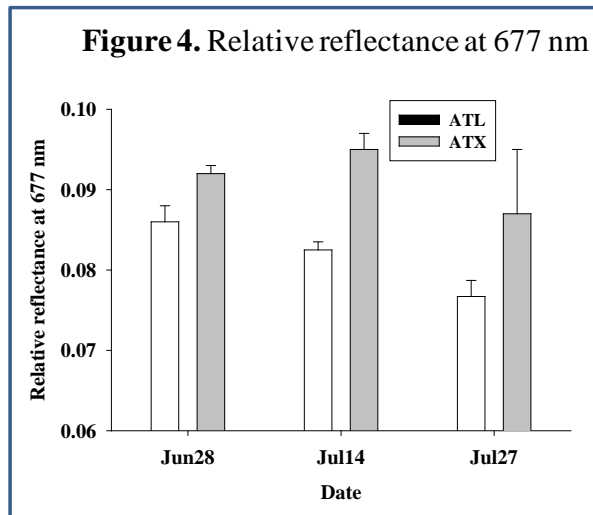
Collection of reflectance profiles from potato leaves



platform and the average reflectance profile acquired from potato leaflets. Due to the apparent difference in oviposition preference between ATL (attractive) and ATX (less attractive), we acquired reflectance profiles from these 2 potato varieties from field-collected leaflets on 3 of the sampling dates (June 28 and July 14 and 27). We used stepwise-regression analysis to identify the spectral band (out of 160 from 405-905 nm) which contributed the most significantly to the distinction between ATL and ATX. Most significant difference was found at 677 nm, and there was a significant

effect of both variety ($df = 1,14$, F -value = 43.77, P -value < 0.001) and date ($df = 2,14$, F -value = 10.44, P -value = 0.002) (Fig. 4). It is seen that, compared to ATX, the attractive oviposition host (ATL) had comparatively lower reflectance at both wavelengths. We used reflectance values from the 3 sampling dates (June 28 and July 14 and 27) at 677 nm to predict number of eggs laid on potato leaflets from all 5 potato varieties grown under center-

pivot at the AgriLife Research and Extension Center in Amarillo TX and obtained a highly significant linear regression fit ($df = 1,35$, adjusted R^2 -value = -0.415, F-value = 25.84, P-value < 0.001) (Fig. 5). Thus, we have demonstrated that oviposition appears to be negatively associated with reflectance at 677 nm and thereby provided a tentative indicator of oviposition by psyllids on potatoes.



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MANAGEMENT OF ZEBRA CHIP OF POTATO: A PATHOGEN-BASED APPROACH

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Summary

Zebra chip, a potato disease associated with the bacterium '*Candidatus Liberibacter solanacearum*' and vectored by the potato psyllid, *Bactericera cockerelli*, is mainly managed through the use of insecticides. The focus of this study was to look at alternatives that would target the pathogen by using antibiotics or chemistries that could end up being bactericidal or by using chemistries that could trigger a plant defense response. Based on results from this year's study and previous trials, the potential exists for antibiotics, such as streptomycin sulfate, and plant activators, such as acibenzolar-s-methyl, to potentially play a role in the management of zebra chip.

Introduction

Zebra chip (ZC), a disease causing economic losses to the potato industry in the United States, Mexico, Central America, and New Zealand, was associated with the phloem-limited bacterium '*Candidatus Liberibacter solanacearum*' (Abad et al., 2009; Liefting et al, 2008; Liefting et al., 2009). Recent research studies have documented that the potato psyllid, *Bactericera cockerelli*, is associated with zebra chip (Munyaneza et al., 2007; Hansen et al., 2008).

Efforts to manage ZC have centered in controlling or managing this insect vector through the use of insecticides. Efforts are on the way to understand if planting date, tolerant varieties, repellents, and other alternative may be suitable additions or alternative to managing this disease. Rather than focusing on cultural practices, vector management, or plant resistance, two other options are to potentially prevent symptom development by targeting the pathogen and the ability for the potato plant to trigger its own defense responses or mechanisms against this pathogen.

Targeting this phloem-limited bacterial pathogen could involve the use of antibiotics. In citrus greening (Huanglongbing), a disease caused by three other species of '*Ca. Liberibacter*', leaf symptoms were reduced with tetracycline hydrochloride in several countries and relatively successful control was obtained with penicillin carbendazin (Abdullah et al, 2009). However, this was achieved by injection of antibiotics into the citrus trees and may require the use of high capacity compressors. The use of plant activators, such as acibenzolar-s-methyl, have been documented to suppress viruses such as the *Tomato spotted wilt virus*, the oomycete *Pythium ultimum* on cucumber, and the bacterium *Xanthomonas campestris* pv. *pruni* on peach (Csinos et al, 2001).

For this study, the use of antibiotics, plant activators, and potentially bactericidal compounds were evaluated on potatoes against ZC and/or the causal agent, *Ca. Liberibacter solanacearum* in field plot experiments.

Materials and Methods

Field Site. Field experiments for 2010 were conducted from 30 March until 24 August near Springlake, Texas, on a commercial potato production farm. This site had a previous history with both potato psyllids and *Ca. Liberibacter solanacearum*. The soil was Tivoli fine sand. A total of 15 treatments were arranged in a randomized complete block design and were replicated 4 times. Each replicate consisted of 2-row plots of nine potato plants per row, for a total of 18 plants per replicate. Potato seed tubers ‘Russet Norkotah 223’ were used for this study. Potatoes were planted on 30 Mar, vines were killed on 16 Aug, and potato crop was harvested on 24 Aug.

Field Treatments. For this study, the treatment numbers and treatments were: 1) Untreated, 2) Firewall (Streptomycin Sulfate), 3) Prophyt (Potassium phosphate), 4) K-Phite (Potassium salts of phosphorous acid), 5) Keyplex KPX-B2 (Yeast extract hydrolysate), 6) Phostrol (Phosphite), 7) Actigard (Acibenzolar-s-methyl), 8) Firewall plus SAver (Salicylic Acid), 9) Firewall plus SAver, 10) K-Phite plus Actigard, 11) K-Phite plus Firewall, 12) K-Phite plus SAver, 13) KPX-B2, 14) KPX-B2 plus Actigard, and 15) Renew (Phosphate plus Phosphite). Chemistries for this trial were applied as foliar sprays on 28 May, 11 June, 22 June, 2 July, and 15 July. All chemistries were applied with a backpack CO₂ sprayer at 35 psi using a 3 and/or 4 nozzle aluminum spray boom. Fungicide, herbicide, and insecticide applications were applied by the producer as needed and consistent with commercial production practices. Fungicides used include: Bravo, Dithane, Quadris, and Super Tin. Herbicides included Sencor, Roundup, and Treflan. Insecticides used include: Movento, Rimon, Admire Pro, Fulfill, Agrimek, Tracer, Venom, and Abacus.

Assays for detection of zebra chip or *Ca. Liberibacter solanacearum* were conducted by visual ratings of cut, raw tubers at harvest and by tuber frying. The presence of the pathogen was confirmed by PCR (Li et al, 2009).

Results and Discussion. Field plots had lower temperatures than usual at planting and throughout April, which delayed plant establishment and higher than usual rainfall in the third week of April, third week of May, and the first week of July. Plots received a moderately severe hail in May while higher than normal temperatures were recorded for June. Psyllid populations were high. All these factors may have contributed to low yields in comparison to previous years. One replication had lower yields than the other replicates and may have been partially due to factors such as location in the field and soil parameters such as compaction and drainage. By the end of the season, all plants exhibited psyllid damage (ie. yellowing of tissue, leaf curling, inflated nodes, and/or purpling).

Yield. There was no significant difference between treatments and the control (untreated) in terms of total yield, individual yield for tubers weighing 4-6 oz., 6-10 oz., 10-18 oz., under 4

oz., and over 18 oz. (data not shown). However, the removal of one replicate allowed for some significant differences between some chemistries and the untreated control (Table 1). For total yield of all tubers harvested, only the application of Firewall gave higher yields than the untreated control while treatments with K-Phite plus Actigard, K-Phite plus Firewall, K-Phite plus SAver, and KPX-B2 plus SAver, ended up with significantly lower yields (Table 1). For total yield of 4-18 oz. tubers (U.S. No. 1), only the application of Firewall and Firewall plus Actigard gave higher significant yields, while KPX-B2 plus SAver, Prophyt, and Phostrol plus Firewall, ended up with significantly lower yields (Table 1). For tubers weighing less than 4 oz., only the treatment with Prophyt ended up with significantly higher yields, while K-Phite plus Firewall, K-Phite + SAver, and KPX-B2 + SAver, ended up with significant lower yields (Table 1). For 4-6 oz. tubers, only the application of Firewall plus Actigard ended up with significantly higher yields while K-Phite plus Firewall and KPX-B2 ended up with significant lower yields (Table 1). For 6-10 oz. tubers, the application of Firewall, Firewall plus SAver, and Firewall plus Actigard, ended up with significantly higher yields while K-Phite plus Actigard, K-Phite plus SAver, KPX-B@ plus SAver, KPX-B2 plus Actigard, Prophyt, K-Phite, and Phostrol plus Firewall, had significantly lower yields (Table 1). For 10-18 oz. tubers, there was no significant difference amongst treatments. Unlike similar trials conducted in 2009 (data not shown), this year there were no tubers larger than 18 oz. for any treatments.

Although trials conducted this year had good psyllid pressure and Zebra chip was present in the plots (data not shown), other factors such as initial low temperatures, higher rainfalls in three months, high temperatures in June, and hail, affected potato production and may not have allowed for effects of the chemical applications for this trial to be properly accounted for. In trials conducted in 2009 with some similar chemistries, applications of streptomycin sulfate gave significantly higher yields for total U.S. No. 1 tubers (4-18 oz.) while the treatment with Actigard was the only one that had tubers weighing over 18 oz. Based on data for the three replications, Firewall allowed for significantly higher yields for total tuber yields and for total yields of 4-18 oz tubers. By itself, or in combination with Actigard, SAver, or K-Phite, Firewall also provided for significant higher yields of other tuber weight categories.

Chemistries for this study were applied as foliar sprays and would be an ideal method for application of chemistries in the field especially if they can be translocated to the phloem. Unlike antibiotics and fungicides that can be applied to trees as injections, this is not an option for potatoes. Experimentation with substrates that can facilitate chemical availability to the phloem needs to be conducted for foliar sprays and also for potential applications at the root or crown level. Future trials will attempt to achieve this and replicating treatments at least six times will allow for better comparison and statistical analyses. Although it may be difficult to label an antibiotic for foliar applications in potato field production systems, streptomycin sulfate and other antibiotics are used as seed treatments, but could further be labeled for a single foliar or soil application at plant establishment.

Table 1 *Total yield, total yield of U.S. No.1, under 4 ounce, over 18 oz, and culls/No.2 potatoes for three replicates of 15 Treatments on 'Russet Norkotah 223' potatoes grown near Springlake, Texas-2010.*

Treatment No. and Treatment	Total Yield Cwt/A	U.S. No. 1 Cwt. Per Acre				Over 18 oz	Under 4 oz.	Culls/ No.2
		Total Yield	4-6 oz	6-10 oz	10-18 oz			
1-Untreated	186.7	140.7	104.6	31.5	4.6	0.0	46.0	0.0
2-Firewall (Fwl)	224.6	182.7	115.0	52.1	15.6	0.0	41.9	0.0
3-Prophyt	166.8	103.5	89.4	10.5	3.6	0.0	63.3	0.0
4-K-Phite (KPh)	182.7	125.5	110.4	15.1	0.0	0.0	57.2	0.0
5-KPX-B2 (KB)	204.6	146.8	102.6	31.5	12.8	0.0	57.8	0.0
6-Phostrol + Fwl	149.8	104.4	90.3	10.8	3.3	0.0	45.4	0.0
7-Actigard (Act)	159.3	118.9	89.0	21.4	8.5	0.0	40.4	0.0
8-Fwl + SAver	194.1	152.0	103.5	48.4	0.0	0.0	42.2	0.0
9-Fwl + Act	210.7	179.2	119.7	55.0	4.4	0.0	31.5	0.0
10-KPh + Act	148.8	116.9	103.9	6.2	6.9	0.0	31.9	0.0
11-KPh + Fwl	140.5	118.7	83.1	34.3	1.3	0.0	21.8	0.0
12-KPh + SAver	138.3	116.2	109.4	6.7	0.0	0.0	22.1	0.0
13-KB + SAver	120.3	90.0	64.1	15.3	10.5	0.0	30.3	0.0
14-KB + Act	160.4	117.0	91.3	19.0	6.7	0.0	43.4	0.0
15-Renew	169.4	132.5	89.5	36.2	6.8	0.0	36.9	0.0
Average	170.5	129.7	97.7	26.3	5.7	0.0	40.8	0.0
L.S.D. (.05)	32.6	30.2	19.6	11.6	ns	ns	15.1	ns

The judicious use of insecticides in combination with plant tolerance or resistance, and other practices such as the ones that may come out of this and future studies, management of Zebra chip could be attained using an integrated control approach for disease management.

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POPULATION GENETICS OF THE POTATO PSYLLID: IMPACTS ON ZEBRA CHIP EPIDEMIOLOGY

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Summary

Monitoring populations of potato psyllids (*Bactericera cockerelli*), and their migrations throughout the growing season, especially populations connected with the incidence of zebra chip, will result in more efficient pesticide use, reducing costs and environmental exposure. Potato psyllids have two biotypes, which can be distinguished with a single nucleotide polymorphism in the mitochondrial COI gene. Potato psyllid biotypes can be determined quickly and inexpensively using high resolution melt temperature analysis after QRT-PCR. Populations can be tracked through their migrations using inter-simple sequence repeat (ISSR) polymorphism patterns. These techniques may be used to target populations causing zebra chip complex (ZC) in potato crops.

Introduction

In 2007 the presence of the insect, *Bactericera cockerelli*, the potato psyllid, was correlated with the incidence of Zebra Chip (ZC) in potato crops (4). The first signs of ZC were noted in 1994 near Saltillo, Mexico. The first recorded incidence of ZC in the United States was in 2000 near Pearsall, Texas. Since then, ZC has been reported in Guatemala, California, Colorado, Kansas, Nebraska, Nevada, and New Mexico (10).

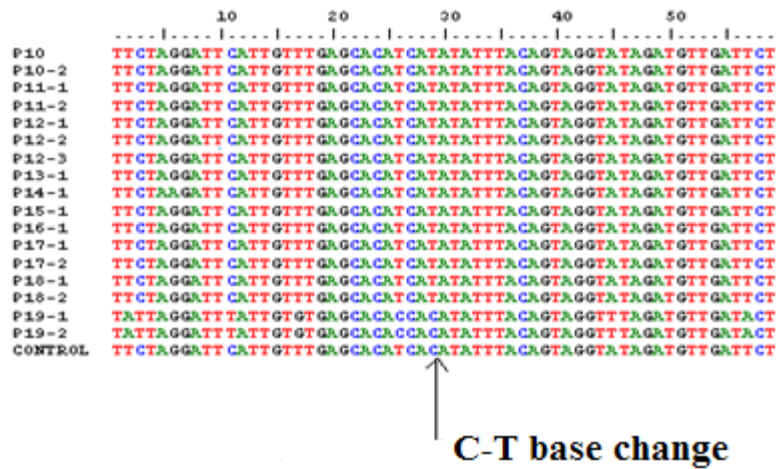
Several studies over the course of the last century have shown that populations of *B. cockerelli* tend to migrate, overwintering in locations with warm climates and traveling northward over the course of the growing season with the aid of monsoon winds (2), (6), (9), (12) (Figure 1). It was reported by Liu, in 2006, that two distinct potato psyllid biotypes were present in the United States (3). Using molecular techniques, it was determined that a single nucleotide polymorphism (SNP) within the cytochrome oxidase I (COI) gene of the mitochondria could be used to distinguish between the two biotypes of the potato psyllid (3) (Figure 2). Theoretically, the biotypes



Figure 1. Potato Psyllids migrate from South to North during the growing season. Populations are thought to move through the central portion of the U.S., or along the West Coast.

may have resulted from geographic isolation by mountainous areas or stretches of water.

Figure 2. The single-nucleotide polymorphism in the COI gene. The COI primers were used to align the sequences, and a C-T base change was present.



High resolution melt peak analysis was used to differentiate between the SNPs, which pulled apart at different temperatures. The melting temperature (T_m) can be used to tell apart closely related DNA segments (2) (8) by releasing a molecule that fluoresces when the strands of DNA pull apart (5). The result of the fluorescence is plotted on a curve, which creates a consistent profile for the amplicon (7) (11) (Figure 3).

Inter-simple sequence repeat (ISSR) markers were used to further characterize potato psyllid populations collected in the United States and Mexico, with the goal of determining the original location of US potato psyllids (3). Further research by Jackson et al. (1) showed that even though populations in Mexico and the Lower Rio Grande Valley of Texas were considered geographically isolated, there was psyllid gene flow from Mexico into Texas.

Potato psyllids were collected from locations in North Dakota, Colorado, Nebraska, Kansas, and Texas in an attempt to track population movements within the mid-continental United States.

Materials and Methods

Potato psyllids were collected from multiple locations and extracted by crushing the insects in PBS buffer, followed by DNA extraction with the DNeasy blood and tissue kit (Qiagen, Valencia, CA).

Real-time polymerase chain reaction (RT-PCR) was performed with SYBR® Green PCR kit (Qiagen, Valencia, CA) and primers BB bc melt COI forward (GGATTCATTGTTTGAGCACATC) and BB bc melt COI reverse (TGAAATAGGCACGAGAATCAA) in 25 μ L reactions. The biotype was determined using high resolution melt curve analysis. Melt curve analysis was performed in duplicate using BB bc melt COI forward and reverse primers. The following protocol was performed in a Bio-Rad iCycler (Hercules, CA): 95°C for 5m, 95°C for 5s and 60°C for 10s for 40 cycles, 72°C for 5m, 70°C for 20s for 51 cycles, and an infinite hold at 15°C.

Population similarity was determined using ISSR. PCR was performed in duplicate using the primer ISSR-847 (FCACACACACACACARC) (3) with the following protocol: initial denature at 95°C for 10m, and 35 cycles of denature at 95°C for 30s, anneal

at 51°C for 30s, extend at 72°C for 60s, final extension at 72°C for 7m, and infinite hold at 4°C. The PCR product was purified using a Qiaquick PCR purification kit (Qiagen, Valencia, CA). Gel electrophoresis was run through 1% agarose gel and DNA fragments were compared to a 100kbp ladder.

Results and Discussion

Determination of the potato psyllid biotypes using high resolution melt curve analysis was a relatively simple and accurate method (Figure 3). Melt curve peaks consistent with 73.0° Celsius were correlated with the mid-continental biotype, and melt curve peaks at 75.0° Celsius were correlated with the west coast biotype. The samples from North Dakota had melt peaks at 73.0° C, and the samples from Colorado were of mixed biotype, with melt peaks at 73.0° and 75.0° C (Figure 3).

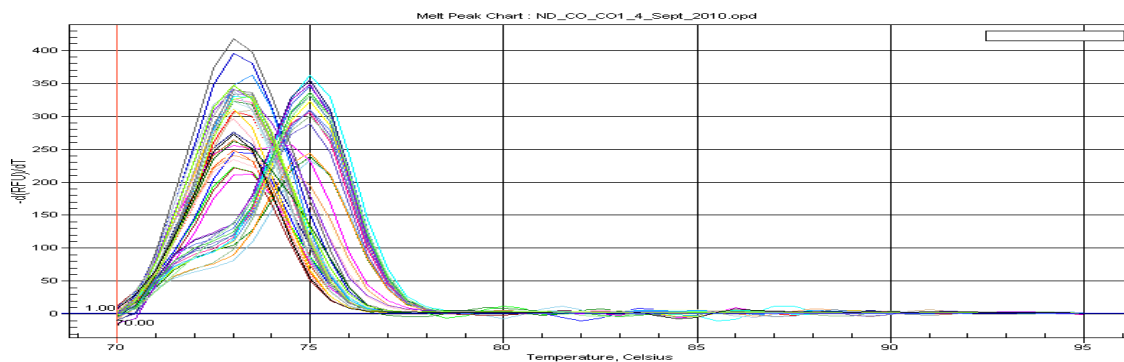


Figure 3. High resolution melt curve peaks, in duplicate, from potato psyllids collected in North Dakota and Colorado in 2010. All samples from North Dakota had melt peaks at 73.0° Celsius. The Colorado samples had melt peaks at both 73.0° and 75.0° Celsius.

Populations were determined using ISSR-PCR, and gel electrophoresis yielded similar banding patterns in samples from North Dakota and Colorado, with little variability. The patterns from Kansas showed some variation, and the Nebraska samples showed no variation. However, samples from locations in Texas yielded highly variable banding patterns (Figure 4).

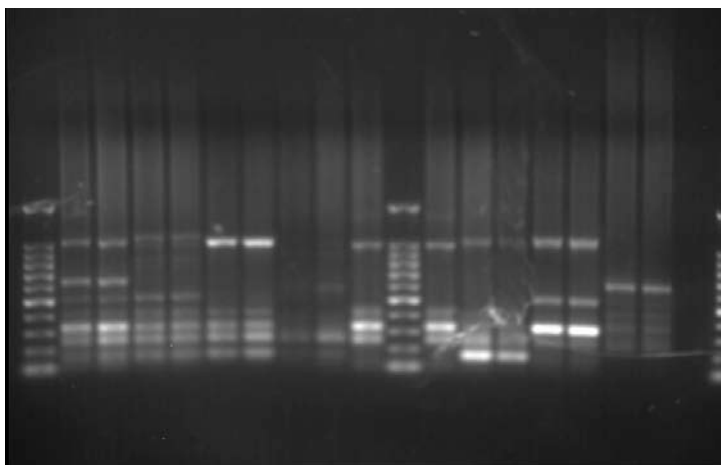


Figure 4. The ISSR-PCR results from samples from Pearsall and Dalhart, Texas yielded high variability in banding patterns.

Acknowledgements

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ALTERNATIVE STRATEGIES: PLANT RESISTANCE AND BIOLOGICAL CONTROL

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Summary

The use of insecticides for potato psyllid management is both a preventative and therapeutic response to this pest. However, the development of alternative strategies to maximize efficient use of insecticides can reduce pesticide applications and production costs. In this study we investigated the potential use of host plant resistance and biological control for potato psyllid management. Potato plant lines were tested against adult potato psyllids and showed a variety of effects on psyllid behaviors. Most notably ‘463-4’ and ‘NY138’ were effective at significantly lowering feeding duration and significantly increased the amount of time psyllids spent off the potato leaflet. For the biological control studies, surveys of natural enemies indicated that 90% of the natural enemy species were from seven taxonomic groups. Laboratory tests indicated the groups tested were indeed predators of the potato psyllid. Cage studies further indicated that natural enemies can significantly lower survival of potato psyllid nymphs in potato fields and the adjacent weed hosts as well.

Introduction

Insecticides dominate the management strategies used to control the potato psyllid and the “zebra chip” (ZC) pathogen [*Candidatus* Liberibacter psyllaeus/solanacearum (LPS)] its vectors (Liu and Trumble 2004, 2005; Goolsby 2007b; Vega-Gutierrez et al. 2008; Gharalari et al. 2009). However, a basic principle of insect pest management is that long-lasting solutions depend on several harmonious tactics (Pedigo and Rice 2006). Two strategies we have investigated are the potential for use of host plant resistance and biological control to integrate and augment with the current measures such as insecticides for potato psyllid management. The use of pesticides can be reduced by knowing which lines can tolerate or even resist insect pests (Smith 2005), which could lower pesticide applications and production costs. Plant resistance also has many advantages and can serve as an excellent component tactic that may be integrated with other combinations of pest control such as biological control. Understanding the effects of plant lines and biological control can lead to the effective selection tactics for new opportunities of potato psyllid management. Limiting infestation can be key to preventing yield loss by preventing potato psyllid feeding and subsequent LPS transmission.

Materials and Methods

Host plant resistance:

Behavioral assays. *Bactericera cockerelli* were originally obtained from field collections in Texas on potatoes and the population was reared in our laboratory on tomatoes (*Solanum*

lycopersicum L. cv. 'Yellow Pear'). Adult females were used for all behavioral tests. A total of twenty-two potato (*Solanum tuberosum* L.) lines were tested to examine the effects of these lines on adult potato psyllid behaviors. These lines were either from Idaho: 'Atlantic', 'GemStar Russet', '463-4', 'P2-3', 'P2-4', 'Etb 5-31-3', 'Etb 6-21-3', 'Etb 6-21-5', 'A00ETB12-2', 'A00ETB12-3', 'A05379-69', 'A05379-211'; or from Texas: 'Norkotah', 'King Harry', 'NY138', 'BTX1749-1W/Y', 'NDTX731-1R', 'TX05249-10W', 'ATX85404-8W', 'ATTX98500-3PW/Y', 'BTX1544-2W/Y', and 'AOTX95295-1W'. Plants were used for tests once they reached the 'vegetative growth' stage (Growth Stage II), which is marked by the plant producing 8-12 leaves (Strand 2006). Plant leaves used as substrates for the behavioral assays were standardized by selecting the uppermost fully expanded leaf. All assays were based on the protocols of Liu and Trumble (2004). Assays were monitored in arenas made by layering the following components: a Plexiglass rectangle (9 by 11.5 cm) serving as the base, a 9-cm-diameter Whatman® filter paper on the Plexiglass, the test leaflet (psyllid was placed on abaxial surface), foam (0.5 x 8 x 9 cm) with a 2.5 cm² hole, and an additional 12.5-cm-diameter glass plate that covered the arena. An adult female was placed into the arena and allowed to adjust for 5 min before initiating behavioral recording. An observation period lasted 15 min. Preliminary studies (Liu and Trumble, unpublished data) indicated that the 15-min observation period was sufficient for the psyllids to exhibit most of the behaviors. The observations were recorded using the Noldus Observer program (Noldus, Wageningen, The Netherlands).

Specific behaviors recorded included cleaning (using legs to cleanse or wipe antennae, appendages or abdomen), feeding (stylet penetration into leaflet), jumping (leaping from one point to another on the leaflet), off-leaflet (exiting or abandoning the leaf surface), tasting (tapping the mouthparts on the leaf surface sporadically), resting (no activity on the leaflet and mouthparts not in contact with the leaflet), and walking (walking on the leaf surface). Jumping occurs so rapidly that accurately recording duration time was not possible, so only numbers of occurrences were recorded. The behavioral observations were replicated 20 times with different psyllids for each of the plant lines.

LPS transmission. To examine if plant lines had an effect on transmission of LPS, ten psyllids (subsequently determined to be infected, see below) were caged on a 7 x 7.5 cm cage on the terminal leaflet of one of the fully expanded potato leaves for a 24-h inoculation access period for each of the plant lines. After 24 h, the psyllids were removed from the leaflet and placed in 100% ethanol and stored at -20°C until real-time PCR analysis. The plants were held for 2 wk after potato psyllid exposure to allow disease development. The potato leaf was then removed from the plant and placed in a Ziploc® bag and stored at -80°C until real-time PCR analysis. A Taqman-based real time PCR assay was employed in detection of LPS by using a protocol modified from Li et al. (2006) and Manjunath et al. (2008).

Biological control:

Our objectives of this research were to determine the identity of natural enemies that attack the potato psyllid in southern California, and determine the impact natural enemies have on potato psyllid population dynamics. To determine the identity of natural enemies, surveys

were conducted across southern California in Riverside, Orange and Ventura counties in potato, tomato, and bell pepper crops for the 2009 and 2010 growing seasons. Visual counts and sweep net samples were used in agricultural fields to detect the presence of natural enemies. Offering potato psyllids to possible predators in laboratory tests further confirmed that natural enemies observed in the field would feed on specific psyllid stages.

To determine the impact natural enemies have on psyllid population dynamics, exclusion cages tests in the potato crop and in the adjacent weed nightshade were performed. Cages were placed over leaflets on plants and were closed, open, or uncaged (Van Driesche et al. 2008). Closed cages excluded natural enemies. Open cages allowed natural enemies to reach the potato psyllids, and be exposed to the same microclimate (i.e., temperature and humidity) as the closed cage. If psyllid densities and survival are similar between the open and uncaged treatments, then it suggests that there were no important cage effects. The difference between the closed and open cages can then be considered to reflect the effect of natural enemies (Van Driesche et al. 2008). Fields of potatoes (cultivar ‘Atlantic’ in 2009 and ‘Cal White’ in 2010) were established using standard agronomic practices (Strand 2006) at the UC field station in Orange County. The experiment consisted of a 2 x 2 factorial design in a randomized complete block design with cages (closed, open and uncaged) and habitat (potato crop and a predominant weed, nightshade [*Solanum americanum* Mill.]). Ten-fifteen lab-reared potato psyllids second-third instar nymphs were established per cage. All natural enemies were removed before cages were closed. We used two replicates of the six treatments per block. Cages covered the terminal leaflet on one of the uppermost fully expanded leaves, and counts were made of all psyllids and natural enemies on each plant until psyllids were no longer found in the open cages.

Results and Discussion

Host plant resistance:

Behavioral assays. Potato lines had a variety of effects on potato psyllid behaviors. There were significant differences between the plant lines for the number of occurrences of feeding ($F = 1.86$; $df = 21, 431$; $P = 0.0125$). Psyllids exhibited at least one feeding occurrence on the lines ‘P2-4’ and ‘TX05249-10W’, which were significantly lower than the lines ‘Norkotah’, ‘A00ETB12-3’, ‘NDTX731-1R’, ‘Etb 5-31-3’, ‘A05379-69’, ‘NY138’, ‘Atlantic’, and ‘BTX1544-2W/Y’. The numbers of occurrences of selected behaviors such as tasting, cleaning, resting, walking, and number of occurrences off the potato leaflet were not significantly different between the lines tested. Potato lines had a significant effect on the feeding duration of psyllids ($F = 1.96$; $df = 21, 431$; $P = 0.0074$). The two lines in which psyllids spent significantly less time feeding were ‘463-4’ and ‘NY138’. The feeding duration for psyllids on ‘463-4’ was reduced by nearly 62% and by nearly 49% on ‘NY138’ compared to ‘Norkotah’. Psyllids spent the most time cleaning on the varieties ‘AOTX95295-1W’ and ‘King Harry’ compared to ‘A05379-211’, ‘P2-4’, ‘A00ETB12-3’, ‘NDTX731-1R’, ‘P2-3’, ‘Etb 6-21-5’, ‘GemStar Russet’, ‘BTX1544-2W/Y’, ‘Atlantic’, ‘Etb 5-31-3’, ‘TX05249-10W’, ‘463-4’, and ‘NY138’ ($F = 1.89$; $df = 21, 431$; $P = 0.0104$). Psyllids spent the most time resting of the varieties ‘BTX1749-1W/Y’ and ‘TX05249-10W’ compared to ‘Norkotah’, ‘A05379-69’, ‘P2-3’, ‘P2-4’, and ‘A00ETB12-2’ ($F = 1.81$; $df = 21$,

431; $P = 0.0164$). Psyllids spent the most times off the potato lines '463-4' and 'NY138' compared to 'Norkotah' ($F = 2.00$; $df = 21, 431$; $P = 0.0057$).

LPS transmission. While not statistically analyzed yet, it appears that the potato lines lowered transmission of LPS. The positive controls of 'Atlantic' and 'Norkotah' had 80% of the potato lines infected with LPS. The five lowest levels of infection occurred on the lines 'A00ETB12-3', 'ETB 6-21-3', 'P2-4', 'BTX1544-2W/Y', and 'TX05295-10W', which all had an average infection rate of 30%.

Biological control: Greater than 90% of the observed natural enemies in potato, tomato and bell pepper fields were from seven groups. These groups included lacewing species (*Chrysopa* spp.), spiders (Araneae), species of Miridae, parasitoids such as *Tamarixia triozae* (Hymenoptera: Eulophidae) and *Metaphycus psyllidis* (Hymenoptera: Encyrtidae), *Orius tristicolor* (Hemiptera: Anthocoridae), *Geocoris* spp., and Coccinellidae. These natural enemies occurred in different abundances in the different crops. Taking field collected natural enemies back to the laboratory and testing if they would attack potato psyllids revealed that natural enemies can attack a significant number of psyllids. The hemipterans such as mirids, and *O. tristicolor* proved to be significant predators of nymphal psyllids. The coccinellids tested such as *Hippodamia convergens*, *Coccinella septempunctata*, and *Harmonia axyridis*, the hemipteran *Nabis* spp. and lacewing larvae were both predators of nymphs and adults of the potato psyllid. The egg stage was also tested but was not vulnerable to predation by all of the predators tested. Cages studies also indicated that natural enemies can have an effect on potato psyllid survival in the field. Over the two year study, the exclusion of natural enemies resulted in significantly greater survival of nymphs compared to nymphs exposed to natural enemies. Dynamics were similar in the potato crop and adjacent nightshade suggesting that natural enemies can work effectively in both plant types and those natural enemies can provide valued ecosystem services by lowering the number of psyllids that can invade agricultural fields.

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ENTOMOPATHOGENIC FUNGI (HYPOCREALES) FOR CONTROL OF POTATO PSYLLID

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Summary

Laboratory and field trials of the four isolates of entomopathogenic fungi, *Isaria fumosorosea* and *Metarhizium anisopliae* were conducted to evaluate their potential for control of the potato psyllid. In the laboratory, 2 ml aqueous suspension of 10⁷ conidia/ml in a of each isolate applied in a Potter spray tower provided effective control of adults and nymphs of the psyllid. Maximum mortality of adult potato psyllid occurred 3 days after exposure to *I. fumosorosea* and *M. anisopliae*, whereas maximum mortality in nymphs was observed 4 days after exposure. Field trials in Weslaco, TX in 2009 and 2010 were conducted with two commercially produced fungi, *I. fumosorosea* (Pfr 97, Certis USA) and *M. anisopliae* (F 52, Novozymes, Biologicals), abamectin (AgriMek, Syngenta) and neem oil (Trilogy, Certis, USA). Each fungus product provided control of nymphs that was comparable to abamectin. Season long control using the recommended concentrations of F 52 and Pfr 97 ranged from 60-78%. Relative to control plots application of both products resulted in improved tuber yield and reduced plant damage.

Introduction

Due to the short life cycle and rapid reproduction of the potato psyllid, *Bactericera cockerelli*, (Cranshaw 1994), high populations often require frequent application of chemical pesticides. Broad-spectrum insecticides can have deleterious effects on beneficial insects including predators and parasitoids of the psyllid leading to outbreaks of insect pests of potato. In order to minimize environmental concerns, an integrated pest management (IPM) strategy is being increasingly used for sustainable control of several insect pests of potato (Strand 2006). Due to their specificity for insects, entomopathogens are ready made components for IPM due to their safety for applicators, the food supply and the environment. Examples of the use of entomopathogens, including fungi for control of insect pests of potato are presented by Lacey et al. (2009b) and Wraight et al. (2009). Due to the piercing and sucking mouthparts of the Hemiptera, fungi are the predominant pathogens of these pests because of their ability to penetrate the insect's integument (*i.e.* not requiring an oral route). Fungi have been shown to be especially important natural enemies of aphids and whiteflies under warm and humid conditions (Steinkraus 2007, Lacey et al. 2008). However, there are no reports of naturally occurring fungal pathogens of *B. cockerelli*. The objectives of our studies were to evaluate isolates of the fungi, *Isaria fumosorosea* and *Metarhizium anisopliae*

(Hypocreales), by developing an effective laboratory bioassay and field testing methods that would enable testing of their efficacy for psyllid control. Both species of fungi are commercially produced in the USA.

Materials, Methods and Results

Laboratory evaluations. Four isolates of fungi, and two *Metarhizium anisopliae* and two *Isaria fumosorosea*, were bioassayed against *B. cockerelli* on potato leaves under ideal conditions for the fungi. Conidia used in the research were grown on SDAY agar. All applications were made with a Potter spray tower. With the exception of concentration-effect studies, all other applications were made using 10^7 conidia/ml in a 2 ml aqueous suspension.

All isolates produced 95-99% mortality, corrected for control mortality, in adults 2-3 days after application of conidia and 91-99% in nymphs 4 days after application. *I. fumosorosea* Pfr 97 produced 95% corrected mortality in both 1st and late 3rd instar nymphs. *M. anisopliae* (F 52) produced 96% corrected mortality in 1st and 3rd instar nymphs. Pfr 97 and F 52 were evaluated for insecticidal activity against 3rd instar *B. cockerelli* using 10^5 , 10^6 , and 10^7 conidia per ml. Mortality produced by *I. fumosorosea* Pfr 97 ranged from 83 to 97% and that of *M. anisopliae* F 52 was 88 to 95% at these concentrations.

Three field trials of commercial formulations of *Metarhizium anisopliae* (F 52[®], Novozymes Biologicals) and *Isaria fumosorosea* (Pfr 97[®], Certis USA) and abamectin (Agri-Mek[®], Syngenta USA) were conducted in Weslaco, Texas one of the regions severely affected by zebra chip. F 52 applied at 0.51, 1.1, and 2.2 l/ha and Agri-Mek applied at 584 ml/ha produced reductions of *B. cockerelli* eggs and nymphs of 45, 59, 67, and 63% respectively. Only Agri-Mek significantly reduced plant damage. Pfr 97 at 1.1 kg/ha with and without 1% Trilogy[®] (neem oil, Certis, USA), and Agri-Mek at 584 ml/ha resulted in psyllid reductions of 78, 76, and 84%, respectively. Significantly decreased plant damage and ZC incidence were observed for all treatments. Tuber yields for Pfr plus Trilogy and Agri-Mek were significantly different from the control. F 52 applied at 1.1, and 2.2 l/ha and Pfr 97 at 1.1 and 2.2 kg/ha produced 62, 62, 66, and 65% reduction, respectively. Tuber yield for both rates of Pfr and the high rate of F 52 were significantly higher than the control. All fungal treatments significantly reduced plant damage.

Discussion

Our research on the susceptibility of *B. cockerelli* adults and nymphs to *I. fumosorosea* and *M. anisopliae* demonstrate the potential utility of these fungi for augmentative control of the psyllid within an IPM program. Both commercially produced fungal formulations evaluated in the study reduced *B. cockerelli* nymphs foliar damage and increased yields that are comparable to that of Agri-Mek at the recommended rates. Although effective control of other Hemiptera (whiteflies, aphids, scales, stink bugs) with inundatively applied or conserved EPF have been reported by several authors (Lacey et al. 2008, Wraight et al. 2009, and others) only limited field trials of EPF have been conducted on psyllids. Liu et al. (1990) reported 82% reduction of the psyllid *Heteropsylla cubana* after applying *B. bassiana*. Puterka (1999) evaluated *I. fumosorosea* and *B. bassiana* against the pear psyllid, *Cacopsylla pyricola* in a pear orchard at an application rate of 5.4×10^{13} conidia/ha but only produced 18-37% mortality of the psyllid. In contrast, the *I. fumosorosea* and *M. anisopliae*

commercial products evaluated in our studies demonstrated considerably higher potential for the reduction of *B. cockerelli* in potato fields in southern Texas.

Temperature, humidity, and solar radiation can have profound effects on growth, sporulation, infectivity and survival of EPF. Despite nighttime temperatures that were generally outside of the optimal range for infection by EPF, a substantial portion of the days during the field trials were well within the ranges of temperature and RH for infection of *B. cockerelli* by Hypocreales fungi. Rain, irrigation and transpiration effects within the potato canopy most likely sustain optimal humidity even when ambient RH outside of the canopy is lower. The principal factor limiting residual activity of EPFs is inactivation of conidia by ultraviolet radiation.

The timing of EPF applications for control of *B. cockerelli* is an important consideration. Because fungus-infected adults die in 2-3 days (Lacey et al. 2009a) oviposition and disease transmission could be reduced early in the growing season if applications were timed for invading adults. Applications to larger fields will likely produce greater reductions in *B. cockerelli* populations due to reduced influx of untreated adult psyllids. This effect could be further enhanced if the treated fields were isolated from other infested fields or bordered by different crops.

Improvements in control of *B. cockerelli* with EPF can be expected with the testing of additional species and strains of EPF, more effective delivery systems and new formulations. Studies are underway in our laboratory on the efficacy of other Hypocreales fungi (*B. bassiana* and *Lecanicillium muscarium*) for control of *B. cockerelli*. The utility of *B. bassiana* for a wide range of pest insects, including other Hemiptera has been reviewed by Wraight et al. (2009). Although no naturally occurring fungal infections have been reported for *B. cockerelli*, prospection for EPF in this and other Hemiptera could yield other species and strains with potential for microbial control of psyllids. Integration of insect pathogens in the integrated pest management (IPM) strategy for control of the psyllid could reduce reliance on synthetic insecticides and increase the levels of control especially against early season psyllids.

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SPRAY COVERAGE AND INSECTICIDE PERFORMANCE

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Summary

The observed performance of an insecticide applied under commercial standards is affected by a wide range of factors, including weather conditions, application equipment, and crop canopy density. In this study, we highlight the importance of optimizing spray coverage to increase application efficiency and to reduce risk of resistance development in target pest populations. A large study was conducted in three commercial potato fields in Texas, and we examined the correlation between spray coverage (quantified on the basis of water sensitive spray cards) and a series of explanatory variables (including: weather data, plant height, and application method). Analyzing data from 14 commercial sprays, we developed a multi-regression model, which has been made available as a user-friendly decision-support tool that can be accessed via the internet (<http://pilcc.tamu.edu:8080/PILCC>). This decision-support tool shows how even small variations in abiotic conditions can cause a dramatic difference in spray coverage. A controlled field experiment was conducted in which a wide range of spray coverages were applied to individual potato leaflets. Subsequently, the leaflets were bioassayed to determine oviposition, probing and adult mortality of potato psyllids [*Bactericera cockerelli* (Sulc)]. The main conclusions from this study are that actual spray coverage in commercial fields often varies 30-100-fold within fields and rarely exceeds 30% - especially when applied with airplane. Consequently, it is possible that some commercial insecticide spray applications have limited effect on psyllid populations.

Introduction

In economic analyses of row crop productions, it has been shown that insecticide applications constitute only 2-10% of overall yield value (Naranjo et al 1996, Trumble et al 1997, Wold and Hutchison 2003, Antwi et al 2007, Reay-Jones et al 2007), and there are still many pest-crop systems without accurate and reliable scouting programs and pest action thresholds. As a consequence, and despite very well-described examples of how low-input pest management strategies can be economically more profitable than conventional management strategies (Trumble et al 1997, Reitz et al 1999), many insecticide applications may be of a “better safe than sorry” nature rather than the result of a decision based on extensive monitoring data. And when pest populations flourish shortly after a given insecticide application, it has been our experience that many growers tend to blame the insecticide, while there seems to be less acknowledgement of how application conditions may have affected the outcome. In this study, we collected data from three commercial potato fields and evaluated spray deposition during 14 commercial insecticide applications. Multi-regression analysis was used to correlate spray deposition data with a series of explanatory variables including: weather data, plant height, and application method. An experimental study was conducted with potato

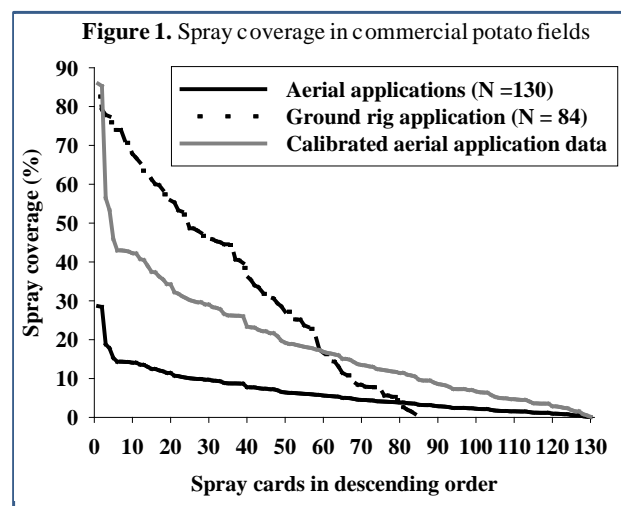
psyllids [*Bactericera cockerelli* (Sulc) (Hemiptera: Trioizidae)] and potato plants treated with Agri-Mek (abamectin) to further examine the importance of spray deposition on their efficacy.

Materials and Methods

Evaluation of commercial insecticide spray applications. A total of 14 insecticide spray applications were evaluated in three commercial potato fields in three of the principal potato growing regions in Texas [Edinburg (south) = 6 sprays, Pearsall (north central) = 4 sprays, Olton (north west) = 4 sprays]. In each field, we flagged 10 points about 30 m apart, and all data from each field were collected from these points. At each point, we placed one water sensitive spray card (5.1 cm × 7.6 cm, Syngenta, Wilmington, DE, USA) centered horizontally 10 cm from the top of the canopy (yellow side facing upwards) when plants were lower than 30 cm, and when plant grew larger we placed one additional card centered horizontally 10 cm from the bottom of the canopy (also facing upwards). A few water sensitive spray cards were lost, but a total of 215 spray cards were obtained. In all three commercial fields, a weather station was deployed to record the following variables in 5-min time intervals: solar radiation (W/m^2), average wind speed and wind gust (km), average temperature and dew point ($^{\circ}\text{C}$), relative humidity (%). Each spray application lasted about 90 min, and average weather variables for that time period were determined.

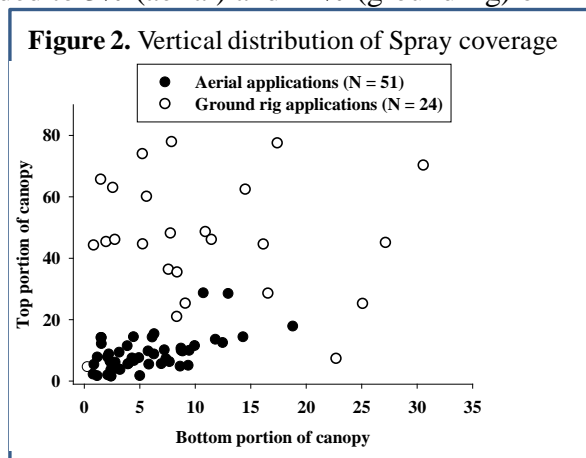
Field spraying test. An insecticide formulation of 710 g/ha of Agri-Mek (abamectin) in the equivalent to 190 L water was prepared and applied with a backpack sprayer to single potato leaflets with a water sensitive spray card placed immediately adjacent to the sprayed leaflet. A total of 25 leaflets were sprayed with each insecticide, and we varied the distance between spray nozzle and leaflets to obtain a wide range of spray deposition (5-70%). Immediately after each spray application, the leaflets were placed with the petiole in a plastic vial with water and bioassayed as described above. After 72 hours, we counted eggs, recorded status of adult psyllids, and conducted McBryde staining to count number of probing events on each leaflet.

Statistical analysis. All data analysis was conducted using PC-SAS 9.1 (SAS Institute, Cary, NC). PROC REG with linear and quadratic responses was used to examine effects of the following explanatory variables and spray deposition (%) as response variable: plant height (cm), liters of water as carrier, top portion of potato plant canopy = 1 or bottom portion = 0, solar radiation (W/m^2), average wind speed and wind gust (km), average temperature and dew point ($^{\circ}\text{C}$), relative humidity (%). For the final decision support tool presented on a website, explanatory variables were in converted into American units, so different coefficients were obtained.



Results and Discussion

Evaluation of commercial spray applications. Of the 14 commercial spray applications included in this study, 8 were applied with airplane and 6 with ground rig, and it is seen that (Fig. 1): 1) the highest spray deposition obtained with aerial application was about 30% and the median was about 8%, 2) the highest spray deposition obtained with ground rig application was 87% and the median spray deposition was about 40%. However, it is important to highlight that aerial applications were based on 3-4 times small spray volume, so spray depositions obtained with aerial applications were tentatively multiplied by 3 for direct comparison with ground rig applications. Even after “calibration”, spray deposition with aerial application (average = 18.78%) was found to be significantly lower than with ground rig (average = 37.35%) ($df = 1,213$, $F\text{-value} = 41.19$, $P\text{-value} < 0.001$), which is most likely attributed to higher level of insecticide drift with aerial applications. Using water sensitive spray cards, Nansen et al (2010) analyzed spray depositions obtained after ground rig and aerial applications of abamectin in a commercial potato field in northern Texas and showed that estimated application rates corresponded to 3% (aerial) and 21% (ground rig) of the optimum for each application method. In other words, these two studies demonstrate that proportionally more formulation is being lost due to drift with aerial compared to ground rig applications. In nine of the 14 commercial spray applications, we obtained spray deposition data from two vertical positions within the canopy and could therefore assess the level of canopy penetration, and it was seen that (Fig. 2): 1) regarding aerial applications, there was a highly significant correlation ($df = 1,50$, $F\text{-value} = 24.984$, $P\text{-value} < 0.001$), and 2) there was random relationship between spray deposition in top and bottom portions of the canopy when applied with ground rig. It should also be noted that eight of the 24 ground rig spray cards from the bottom portion of the canopy received $>15\%$ deposition, while only one aerial spray card from the bottom portion of the canopy received that amount. Based on Tukey analysis, spray deposition was significantly highest in the top portion of the canopy when sprayed with ground rig, while spraying with aerial applicator did not cause a significant vertical effect. These vertical distribution data strongly suggests that larger droplet size (ground rig applications) provides considerably better canopy penetration, so higher overall level of deposition with ground rig applications is due to a combination of less drift and better canopy penetration. Regarding consistency of spray depositions, we calculated standard deviations / average for all 14 data sets from the top portion of the potato canopy, and we found that it was significantly higher for aerial compared to ground rig applications ($df = 1,13$, $F\text{-value} = 11.87$, $P\text{-value} = 0.005$). Low canopy penetration, especially with conventional aerial applications has been reported in other studies (Derksen et al 2008).



Regression analysis of commercial spray deposition. Based on forward stepwise multi-regression analysis of observed spray deposition on water sensitive spray cards with linear,

Figure 3. Decision-support tool

Potato Insecticide Leaf Coverage Calculator

Application Type:

Canopy Portion:

Plant Height(inches):

Dew Point:

Temperature:

Wind Speed:

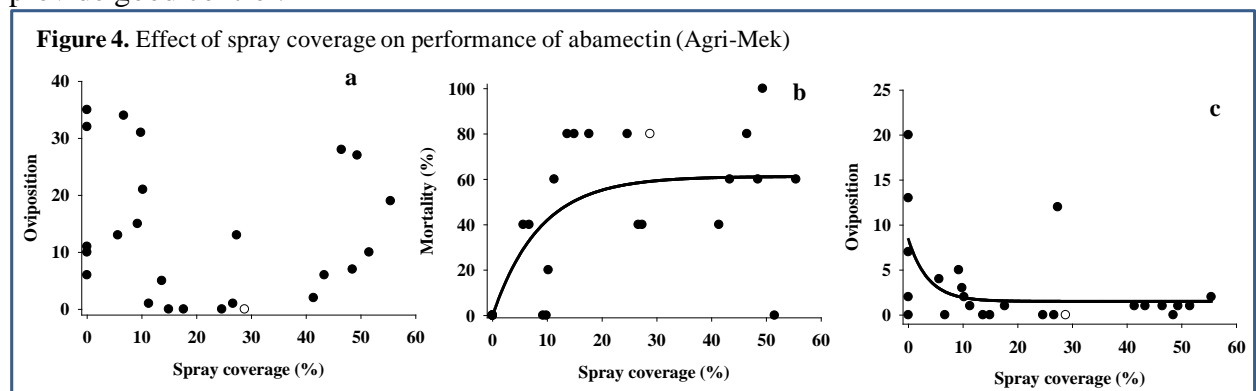
Gust:

Percentage Leaf Coverage:

quadratic and cubic responses, a highly significant regression fit with 11 parameters was obtained ($df = 10,214$, adjusted R^2 -value = 0.690, F-value = 48.71, P-value < 0.001). Fig. 3 shows the web site (<http://pilcc.tamu.edu:8080/PILCC/>) with the decision-support tool, and by changing one variable at a time it is possible to show that: 1) ground rig applications provide much higher spray deposition than aerial applications, 2) a wind speed of 12 km compared to 15 km can markedly reduce spray deposition, 3) top portion of the canopy receives 10-25% higher coverage than the bottom portion.

Importance of spray coverage. Using a backpack sprayer, we sprayed individual

leaflets under field conditions with a water sensitive spray card placed immediately adjacent to each potato leaflet. These sprayed potato leaflets were bioassayed individually with five unsexed adults potato psyllids, and we found that: 1) there was no apparent effect of spray deposition on oviposition (Fig. 4a), 2) adult psyllid mortality increased significantly ($df = 2,24$, adjusted R^2 -value = 0.489, F-value = 21.328, P-value < 0.001) towards an asymptote which was reached when spray deposition approached 35% (Fig. 4b), and 3) number of probing events decreased exponentially ($df = 2,24$, adjusted R^2 -value = 0.249, F-value = 4.785, P-value = 0.019) and leveled off when spray deposition approached 20% (Fig. 4c). The sensitivity of psyllid adults to abamectin was confirmed, and due to the translaminar characteristic of abamectin, it is not surprising that a fairly low spray deposition appears to provide good control.



Final remarks. We have demonstrated that environmental factors can have marked impact on the observed spray deposition in commercial potato fields and that particularly low-volume aerial applications can lead to low and inconsistent spray deposition. Without sufficient knowledge about how to obtain consistently effective insecticide applications, it is understandable that growers may be “over-spraying” to be safe rather than sorry. In

conventional and most IPM programs, application of insecticides continues to be the most important responsive pest control tactic. For both immediate and long-term optimization and sustainability of insecticide applications, it is paramount to study the factors affecting the performance of insecticides when applied in commercial fields. Regarding our analysis of abamectin, it appears that a 20-30% leaf coverage is sufficient for adequate psyllid control, so the decision-support tool should be used to decide when environmental conditions allow to obtain this level of coverage.

Acknowledgement

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EFFICACY OF SEVEN CHEMICAL PROGRAMS TO CONTROL POTATO PSYLLIDS IN THE TEXAS PANHANDLE

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Summary

Potato production is among the leading agricultural industries throughout the state of Texas. In recent years, growers and producers throughout the state have been faced with an increase in potato psyllid (*Bactericerca cockerelli*) populations as well as high incidence of zebra chip (*Candidatus Liberibacter*). One of the strategies in which growers have relied upon to control psyllid populations as well as reduce the incidence of zebra chip has been the use of chemical applications. However, the effectiveness of commonly utilized chemicals is an area of focus to many regional growers. Therefore, a pesticide efficacy trial was developed in Dalhart, TX in order to determine the efficacy of seven season-long chemical programs to treat psyllids. This trial was also designed to provide an insight into the occurrence of psyllid yellow and zebra chip affected plants resulting from psyllid feeding as well as yield losses and zebra chip defects in raw tubers and “fry tests”.

This study may have a significant impact on the ability of regional growers to produce high quality potatoes at acceptable yields. This study utilizes commercial potato production equipment to replicate chemical applications throughout a growing season to compare which treatments and chemical strategies work best for growers in the Northern High Plains. As similar research progresses, growers will be able to utilize these findings in order to reduce psyllid populations in commercial potato fields as well as zebra chip, ultimately resulting in a superior product.

Introduction

Potato production has been valued at approximately \$84 million the last three years throughout the state of Texas (Rosson 2009). Of this, the Northern High Plains has been responsible for 36%, making this region the largest area of production in the state (Rosson 2009). Since 2000, growers and producers throughout this region have been subject to substantial economic losses due to increased potato psyllid pressure as well as their associated pathogen, zebra chip (Crosslin et al. 2010). Psyllids can be problematic in the region due to some overwintering populations as well as large adult migrations that have the potential to move into the Northern High Plains via large storm systems and strong winds from the Lower Rio Grande Valley and south Texas. Some of the IPM practices growers have utilized to control potato psyllids in recent years have been altering planting dates, selecting for potato varieties that are less attractive to psyllids, and chemical applications (Texas A&M Agrilife 2010). However, due to the fact that research regarding chemical applications for psyllids is relatively new, a study was designed in order to determine the efficacy of the most commonly utilized chemicals. This study was also intended to explore the effects of psyllids on potato yields and zebra chip in raw tubers as well as fried samples.

Materials and Methods

The study took place on a half pivot approximately four miles northwest of Dalhart, TX. Wooden stakes were used to mark forty eight 50'x50' plots throughout the field. Each plot was randomly assigned one of the seven chemical programs to be tested or utilized as an untreated check (UTC). Therefore, the study consisted of eight different treatments replicated six times. After all plots were appropriately marked, they were planted on May 26th with FL1833s. After planting, wooden stakes were used once again to mark 50'x30' subplots within each plot. All foliar applications were applied within the 50'x30' subplots in order to create buffer zones between each plot. Therefore, spray drift or psyllid migration between plots was minimal. Chemicals were provided by Bayer Crop Science and Syngenta and applied using a "ground rig" with flat-fan nozzles spaced at 20". Chemicals were applied with 20 gallons of water per acre at 25-30 psi. The chemical programs evaluated in the study are listed below:

1. Untreated Check (UTC)
2. Positive Control: Admire In-Furrow, then Agrimek + Movento every week starting 45 days after planting
3. Standard: Admire In-Furrow, 2 applications of Fulfill, 2 applications of Movento, 2 applications of Agrimek, 2 applications of Beleaf
4. Standard with Oberon at End: Admire In-Furrow, 2 applications of Fulfill, 2 applications of Movento, 2 applications of Agrimek, 2 applications of Oberon
5. Standard Stopping Early: Admire In-Furrow, 2 applications of Fulfill, 2 applications of Movento, 2 applications of Agrimek, then nothing
6. Standard with Platinum instead of Admire: Platinum In-Furrow, 2 applications of Fulfill, 2 applications of Movento, 2 applications of Agrimek, 2 applications of Beleaf, 2 applications of Rimon
7. Standard with Nothing In-Furrow: 2 applications of Fulfill, 2 applications of Movento, 2 applications of Agrimek, 2 applications of Beleaf, 2 applications of Rimon
8. Standard with Agrimek Added Based on a Threshold: Admire In-Furrow, 2 applications of Fulfill, 2 applications of Movento, 2 applications of Beleaf, 2 applications of Oberon, 2 applications of Rimon

* The threshold was reached when the presence of any form of the immature life stage was detected or an average of 5+ psyllid adults were captured on yellow sticky traps placed throughout the field

Psyllid counts for eggs, small nymphs, and large nymphs were conducted 24-48 hours post application by sampling 15 leaves per plot on a weekly basis. An evaluation of the number of plants per plot that displayed psyllid yellow or zebra chip symptoms was also conducted four times throughout the growing season in order to determine which plots contained more symptomatic plants. Plants displaying psyllid yellow or zebra chip symptoms were then lab tested to determine if they tested positive for zebra chip. All plots were vine killed on September 28th and a 10'x10' square within each subplot was harvested on October 7th in order to determine differences in yield. All harvested tubers were then cut and evaluated for zebra chip symptoms in the raw tuber as well as fried in order to determine zebra chip defects in a "fry test".

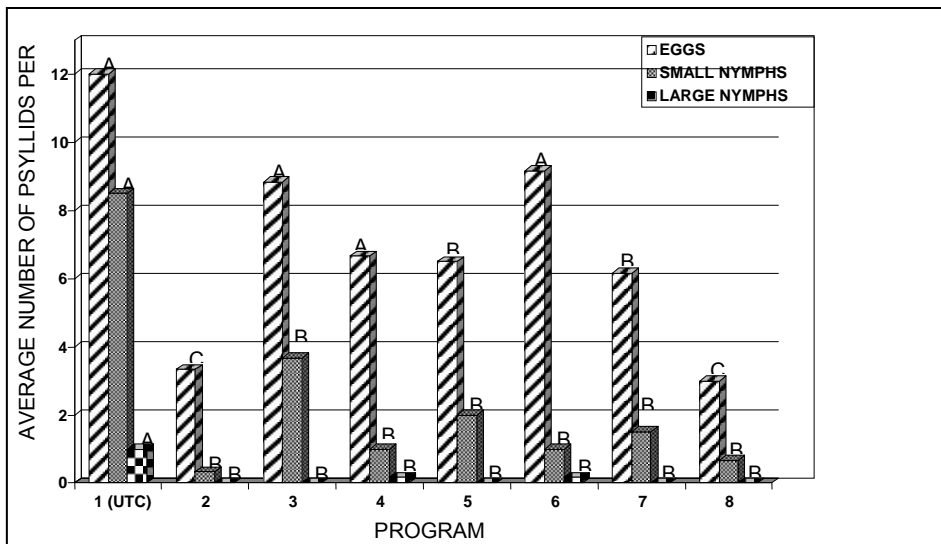
Results and Discussion

Psyllid pressure was relatively light throughout the month of July on the test pivot. As a result, there were no significant differences between the UTC and any of the chemical programs ($p = .9393$, $f = .43$, $N = 144$). However, as the growing season progressed, psyllid

pressure slightly increased and differences were detected early in August. On August 6th, the UTC plots contained significantly more eggs when compared to all other programs ($p=.0123$, $f=2.65$, $N=48$). On August 13th, it became evident that egg development and nymph survival was hindered in the plots that received chemical treatments, while the UTC plots had significantly more eggs ($p=.0075$, $f=2.86$, $N=48$), small nymphs ($p=.0176$, $f=2.49$, $N=48$), and large nymphs ($p=.0085$, $f=2.81$, $N=48$). This trend was continued on August 20th, when significantly more small nymphs ($p=.0214$, $f=2.41$, $N=48$) and large nymphs ($p=.0012$, $f=3.71$, $N=48$) were found in the UTC plots when compared to all other programs. However, differences between plots that received chemical applications were not detected until August 26th.

Results for August 26th are shown in Figure 1. Programs 2, 5, 7, and 8 all had significantly less eggs when compared to the UTC ($p=.0238$, $f=2.36$, $N=48$). More importantly, program 2, which received an application of Agrimek + Movento, and program 8, which received an application of Oberon + Agrimek, had significantly less eggs when compared to programs 3 and 6, which both received applications of Beleaf.

Figure 1. The average number of eggs, small nymphs, and large nymphs per plot on August 26th.



This trend continued into early September, as we found that programs 2, 5, and 8 had significantly less small nymphs when compared to the UTC as well as program 3 ($p=.0008$, $f=3.90$, $N=48$), shown in Figure 2. If we look at chemical applications for this time period, we find that program 2 received another application of Agrimek + Movento, while program 8 received an application of Rimon + Agrimek. If we look at Figure 3, displaying the last time in which psyllid counts were conducted, we find that the trend continues. Program 2, which received its last application of Agrimek + Movento, and program 8, which received another application of Rimon + Agrimek, had significantly less small nymphs when compared to programs 1, 3, and 4, which did not receive chemical applications during the last week of testing ($p=.0088$, $f=2.80$, $N=48$).

Figure 2. The average number of eggs, small nymphs, and large nymphs per plot on September 2nd.

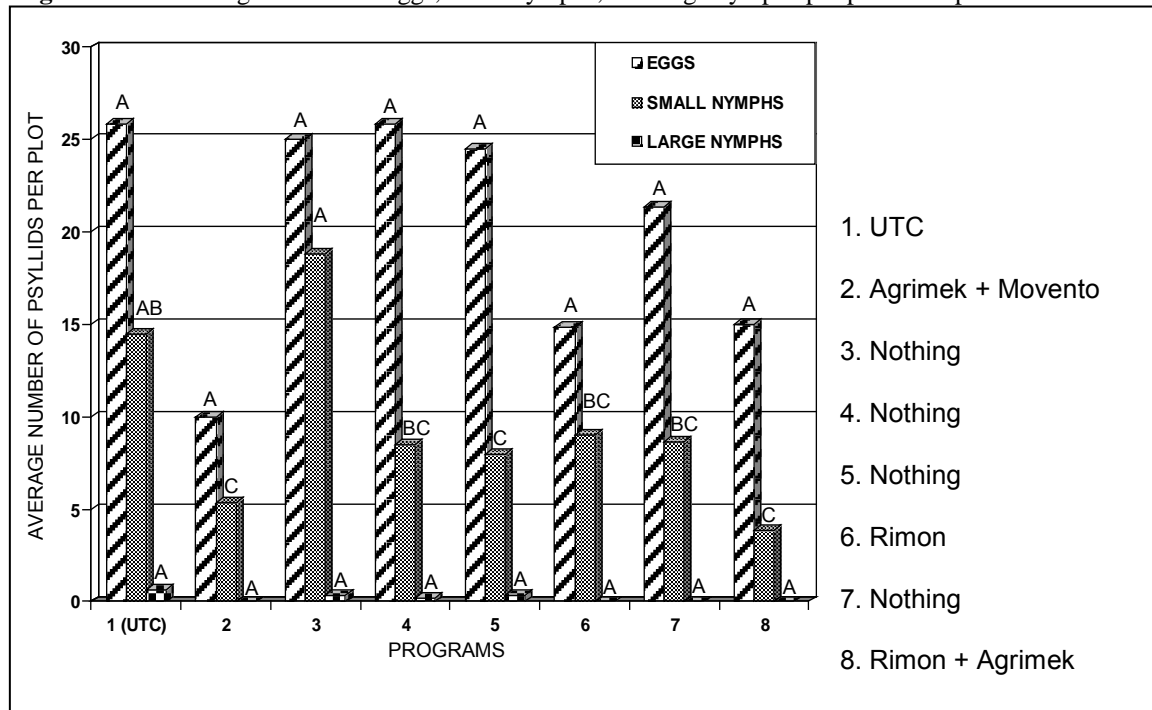
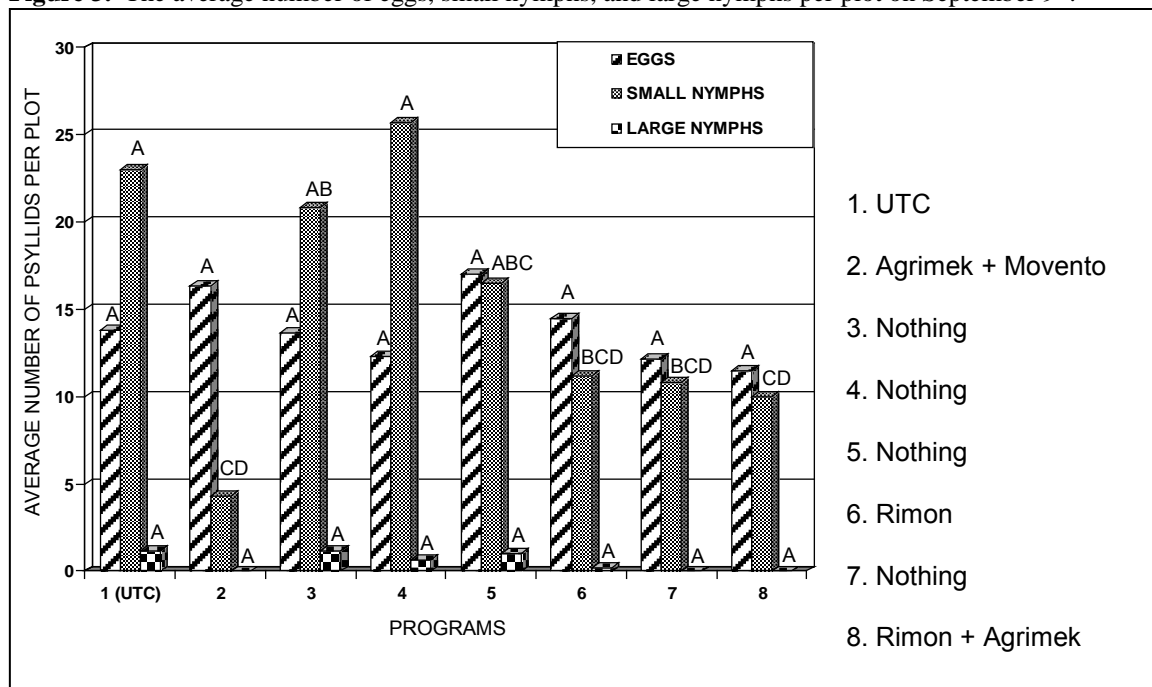


Figure 3. The average number of eggs, small nymphs, and large nymphs per plot on September 9th.

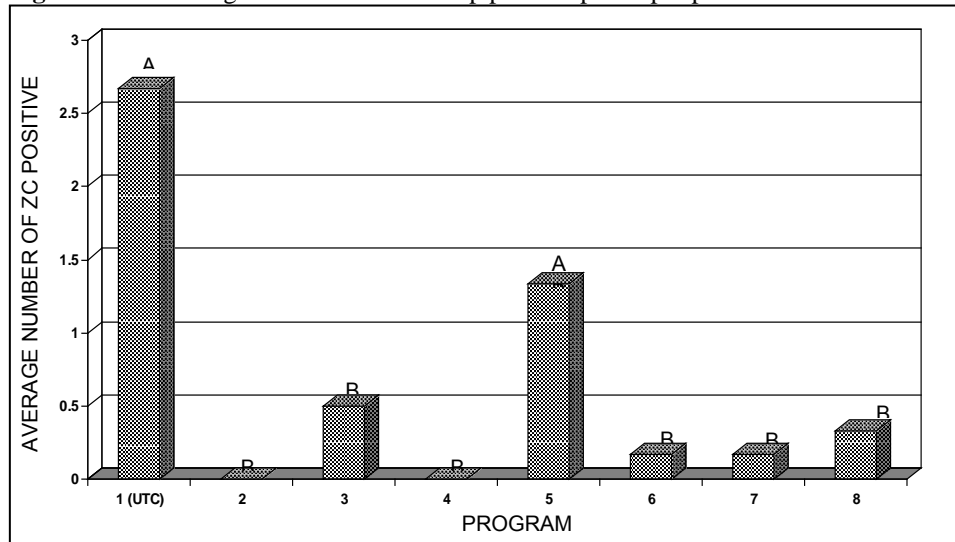


Focusing on the number of plants that showed either psyllid yellow or zebra chip symptoms in the field, we found that there were no significant differences between programs early in August ($p=.6000$, $f=.84$, $N=48$). However, on August 12th, significant differences between the number of psyllid yellow/zebra chip symptomatic plants was detected ($p=.0035$, $f=3.21$, $N=48$). While programs 2, 3, 6, 7, and 8 had significantly less symptomatic plants

when compared to the UTC, program 8 had significantly less than programs 4 and 5 as well. The UTC plots had significantly more symptomatic plants when compared to all other chemical programs on August 19th ($p=.0486$, $f=2.05$, $N=48$) and August 26th ($p=.0006$, $f=4.0$, $N=48$). However, there were no differences between chemical plots later in the season.

Figure 4 displays the differences between the number of psyllid yellow or zebra chip symptomatic plants that lab tested positive for *Candidatus Liberibacter*. As shown, with the exception of program 5, which ended chemical applications early, all other programs had significantly less zebra chip positive plants per plot when compared to the UTC ($p=.0393$, $f=2.14$, $N=48$).

Figure 4. The average number of zebra chip positive plants per plot



Looking at yield data between plots, we found that the UTC averaged 296 cwt/A, which was significantly less than programs 2 (375 cwt/A), 5 (353 cwt/A), 6 (364 cwt/A), 7 (359 cwt/A), and 8 (353 cwt/A) ($p=.0001$, $f=4.82$, $N=48$).

Once all of the tubers were harvested, they were cut to determine if they displayed zebra chip symptoms in the raw tuber as well as fry tests. Although there were no statistical differences between the number of tubers that showed zebra chip symptoms in the raw tubers ($p=.0897$, $f=1.79$, $N=48$) or the fry tests ($p=.0609$, $f=1.96$, $N=48$), the UTC plots averaged 14.7 tubers per plot with zebra chip symptoms in the raw tubers and 14% zebra chip defects. All other programs averaged less than three tubers per plot with zebra chip defects in the raw tuber and less than 3% defects in the fry tests.

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EVALUATION OF SPRAY APPLICATIONS FOR POTATO PSYLLID AND ZC MANAGEMENT

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Summary

Producers use a lot of insecticides and applications to control psyllid populations during the growing season. These applications are very expensive, but all of them may not be needed unless populations are at damaging levels. This study was designed to evaluate different control strategies for determining when to spray for managing potato psyllid and Zebra Chip (ZC) disease.

Psyllid populations (eggs and nymphs) and the incidence of ZC symptomatic tubers were very low in all of the treatments. The number of psyllid adults caught on yellow sticky traps only exceeded 3 per 5 traps on one week during the season. This meant there was only one time when insecticide applications were initiated in the 3 or more psyllid treatment. None of the trapped psyllids tested positive for *Liberibacter*. So no insecticide applications were made to the *Liberibacter* treatment. There were some differences in the average tuber weight among treatments, but overall there were no treatment differences for yield (lbs per acre). A simple comparison for only the total cost of insecticide applied during the season showed the season long commercial treatment was the most expensive (range of \$306.12 to \$426.56/A), followed by the 3 or more psyllid treatment (range \$99.20 to \$104.46/A), and the *Liberibacter* treatment and untreated were the least expensive (\$0.00/A). The study shows that under some conditions spray applications are not always needed during the growing season. Also, the study illustrates the importance of developing IPM management strategies that are based on action thresholds to help producers and crop consultants in making their spray decisions.

Introduction

The potato psyllid, *Bactericera cockerelli* (Sulc.), has been a pest of economic importance for potato growers because of damages associated with psyllid yellows (Richards 1928 Wallis 1955). But, the recent association of potato psyllid as the vector of the *Liberibacter* pathogen (Munyaneza et al. 2007) has completely changed how commercial growers manage psyllid infestations in order to reduce the incidence of ZC symptomatic tubers. Producers have resorted to multiple insecticide applications for season long protection against possible ZC incidence. Spray programs by producers do rotate insecticides from different classification groupings (pyrethriod, neonicotinoid, spinosyns, avermectins, and feeding blockers) to reduce selection pressure for insecticide resistance management (IRM). Still, the numerous applications during a season are exposing psyllid populations to heavy insecticide pressures and disrupting control by natural enemies.

Goolsby et al. (2007) reported that yellow sticky cards were especially an effective tool for detecting low densities of potato psyllids in both potato fields and stands of native host plants. They also stated the use of the sticky cards could be used effectively for “IPM programs” of season long multiple insecticide control. The extensive survey program from the Lower Rio Grande Valley to Nebraska from 2007 to 2010 has shown the incidence of ZC is low when potato psyllids are at low densities for the entire season. The producers’ approach of applying multiple applications to maintain low psyllid densities is extremely costly, as producers spend up to \$300 per acre for insecticide costs alone.

Devising IPM strategies based on action thresholds for determining when to treat could substantially reduce the number of insecticide applications while reducing the incidence of ZC disease. In the present study, we investigated the effectiveness of timing foliar applications based on 1) when 3 or more psyllid adults were caught weekly on yellow sticky traps or 2) timing foliar applications based on when any weekly trapped psyllid adult tested positive for the *Liberibacter* disease and these treatments were compared to 3) season long commercial applications for managing psyllid infestations to reduce ZC disease infection. An additional objective was to evaluate the impact of controlling psyllid infestations on potato before and after flower initiation.

Materials and Methods

The experiment was conducted at the Texas AgriLife Research farm at Bushland, TX. On April 29 the potato variety FL 1867 was planted on 30 inch row spacings in the field plots with a commercial planter. The seed potatoes were spaced 9 inches apart. Irrigations of 0.5 inch to 2 inches were applied weekly with a LESA center pivot irrigation system.

Experimental plots were arranged in a randomized complete block design with 5 replications around the outer perimeter of the field. Plots were 12 rows wide by 20ft. long. The six experimental treatments were 1) untreated control, 2) season long commercial spray schedule (Commercial), 3) applications timed when ≥ 3 psyllid adults are trapped each week (Psyllid), 4) applications timed when any trapped psyllid adults test positive for *Liberibacter*, 5) foliar sprays before flower initiation, and 6) foliar sprays after flower initiation. Data were analyzed using SAS Proc GLM and means were separated using Duncan’s multiple range test ($P=0.05$).

Insecticide selection was based on insecticide products used by commercial growers for control of potato psyllids. These products were Agri-Mek 0.15 EC (Syngenta), Beleaf 50SG (FMC), Fulfill 50 WG (Syngenta), Movento (Bayer CropScience), and Platinum 75 SG (Syngenta).

The foliar applied insecticides were mixed with a 1% v/v rate of a crop oil concentrate. A pre-emergence application of Platinum 75 SG at 6 fl. oz/A was applied to the commercial treatment plots on May 18 with a 6 row tractor mounted sprayer that delivered a total spray volume of 20 gpa. This sprayer was also used to apply a mixture of Agri-mek (10 fl. oz/A), Fulfill 50 WG (5.5 oz/A), and 1% crop oil concentrate to the before flower initiation plots on May 26. All other insecticide applications were made at a total spray volume of 10 gpa with

a hand carried CO₂ pressurized spray boom that was equipped with 5 (XR8001VS) nozzles on 20 inch centers. The insecticide products used and application dates for each treatment are listed in table 1.

Table 1. Insecticides applied and application dates for each application treatment.						
Treatment	5/18	5/26	6/5	6/9	6/15	6/23
Commercial	Platinum – Pre-emergence		Agri-Mek Movento	Fulfill	Fulfill	Movento
Psyllid			Movento Fulfill	Fulfill		
Liberibacter						
Before FI		Agri-Mek Fulfill	Movento Fulfill			
After FI				Agri-Mek Fulfill Movento	Agri-Mek Fulfill Movento	Agri-Mek Fulfill Movento
Treatment	6/30	7/7	7/14	7/20	7/27	8/3
Commercial	Movento	Beleaf	Agri-Mek	Beleaf	Agri-Mek	Beleaf
Psyllid						
Liberibacter						
Before FI						
After FI	Agri-Mek Movento	Agri-Mek Beleaf Movento	Agri-Mek Movento	Agri-Mek Beleaf Movento	Agri-Mek Movento	Agri-Mek Beleaf Movento

Five yellow sticky traps were positioned around the southwest corner of the field beginning on May 18. Traps were replaced weekly until June 7 and then replaced twice weekly until harvest. Traps were counted under a dissecting microscope and adults that were found were removed with forceps, immersed in vegetable oil for 24 hrs, and then placed separately in 70% ethyl alcohol (EtOH) until assayed for *Liberibacter* bacterium. Each trapped psyllid adult was tested for presence of *Liberibacter* using conventional PCR techniques. The results were available within 48 hrs from the time traps were collected.

The number of nymphs and eggs were counted and recorded weekly to monitor density differences among treatments. Five potato leaves were collected from each plot, placed in a cooler, and returned to the lab. Each leaf was then examined under a dissecting microscope. Leaf sampling was discontinued after August 2 due to leaf senescence and deterioration of vines which made finding viable leaves difficult.

On August 19, two center rows were harvested with a potato digger from Dr. Miller's lab. The center 10 feet of both rows per plot were bagged and taken to the lab for processing. Potatoes were separated by size (< 1 inch, 1-2 inch, 2-3 inch, and > 3 inch), counted and weighed (grams). Twenty tubers were randomly selected from a bag, sliced and visually

inspected for Zebra Chip disease symptoms. Any tuber exhibiting any abnormal symptom was then sliced (10 slices) and the slices were deep fried for detection of characteristic ZC disease discoloration.

Results and Discussion

Initially, the number of psyllid eggs and nymphs were highest during the first two weeks of June, but densities crashed and remained very low until numbers began increasing again after July 19 (Fig. 1). The increase from July 19 to August 2 was at a time when potato plants were naturally senescing or dying from other causes. The only time nymph numbers differed statistically was on August 2 when it was difficult to find leaves to sample.

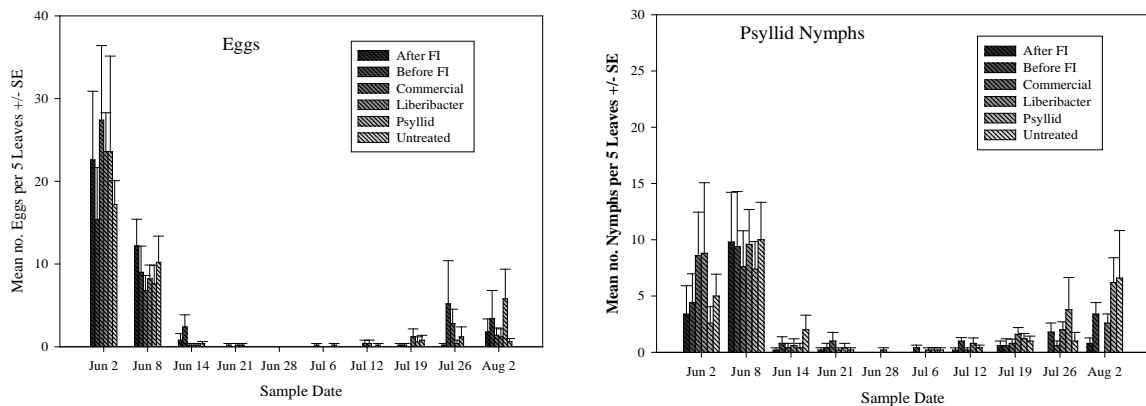


Figure 1. Mean densities of psyllid eggs and nymphs among the treatments during the growing

From the time yellow sticky traps were placed out in the field very few psyllid adults were captured each week. For most weeks no psyllids were trapped, but one psyllid was captured at each of the sample dates June 14 –June 17, June 21–June 27, and July 2 to July 1. The only time ≥ 3 psyllids were trapped was from June 2 to June 7. And, none of these psyllids tested positive for Liberibacter.

With the infrequent occurrence of trapped psyllid adults there were vast differences in the number of insecticide applications made to the season long commercial application treatment compared to the 3 or more psyllid trapped treatment and the Liberibacter positive treatment. There was only one week (June 2 to June 7) when psyllid numbers reached the level for making insecticide applications to the 3 or more psyllid treatment (Table 2). Applications to this treatment were made on June 5 and June 9 to provide control of any hatching nymphs. Since none of the psyllid adults tested positive for Liberibacter, no insecticides were applied to the Liberibacter treatment. Based on local insecticide dealer prices the differences in the cost of insecticides ranged from \$306.12 to \$426.56 for the season long commercial treatment, \$99.20 to \$104.46 for the 3 or more psyllid treatment, and nothing for the Liberibacter and untreated treatments.

The potato yields (lbs/A) and tuber samples for showed no difference among treatments regardless of treatment used to prevent the incidence of ZC infection (Fig. 2). Only one of 600 tubers evaluated for ZC tested positive.

The results indicate season long applications for protection from *Liberibacter* infection were not needed under the psyllid and *Liberibacter* pressure encountered for this field. Further studies are needed so the criteria used in this test or possible other criteria can be used as action thresholds for managing psyllid populations and the prevention of ZC infection.

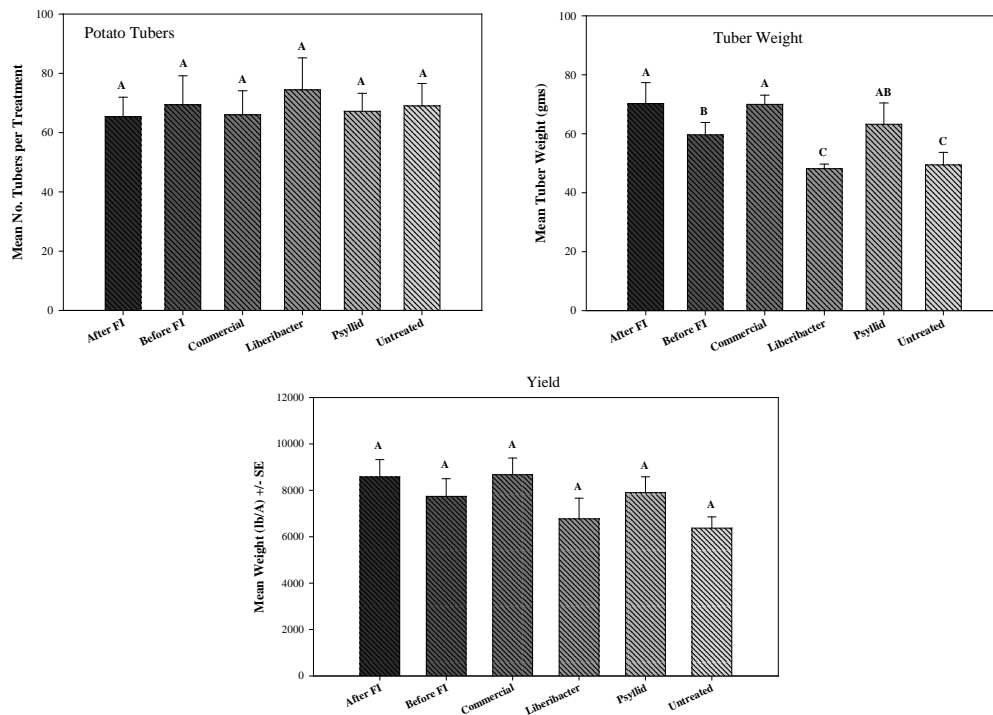


Figure 2. Comparison of mean number of tubers, mean tuber weight, and mean yield per acre for the different treatments.

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ZEBRA CHIP ECONOMICS

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Summary

The focus of the economic analysis has been on estimating grower losses due to zebra chip (ZC). Preliminary estimates suggest that control costs range from \$170 to \$590 per acre for 6 to 10 applications of insecticides. Grower costs due to reduced yield and quality are also expected to be high, with some growers reporting losses of up to 50%, despite application of the recommended insecticides.

Impact Statement

The impact of the economic analysis will be to provide potato growers with information to make profitable pest control decisions. Financially-healthy potato growers will enhance the economic well-being of businesses and people in rural communities.

Introduction

The economic analysis part of the project focuses on the project's primary goal of the development of a comprehensive, environmentally responsible ZC disease management program. The three specific objectives of the economic analysis part are:

- 5.1. Estimate grower losses due to costs of current ZC control practices
- 5.1. Estimate ZC losses due to poor tuber quality
- 5.3. Evaluate the economics of alternative disease management strategies.

The focus of the work through fall 2010 has been on Objective 5.1.

Materials and Methods

Much of the economic analysis relies on data collected from experts. We have contacted ZC researchers and potato growers to estimate current costs of ZC control practices. Many provided anecdotal evidence regarding costs, yield loss, and quality loss for the 2010 crop. More specific data was provided by Dr John Goolsby with USDA ARS in Weslaco, TX. Dr Goolsby's data includes insecticide applications for 32 fields in Texas, Kansas and Nebraska. We obtained insecticide price information from the following sources:

1. University of Idaho (2010)
2. North Dakota State University (2010)
3. Phone calls to agricultural chemical dealers

The geographic areas of analysis will include those states where ZC has been detected as a serious grower problem. To date those states include Texas, Colorado, Nebraska, Kansas, New Mexico and California. We will contact industry experts to collect information about ZC control practices in the states not covered by Goolsby.

After developing ZC control cost estimates for each region the next step is to obtain expert opinion about what would be different if the ZC/psyllid problem did not exist. Comparison with the current control cost estimates will provide a grower-level estimate of the input costs of ZC. We will follow that with an analysis of the yield and quality impacts, again by seeking expert opinion.

Results and Discussion

Preliminary results show that 2010 ZC control costs ranged from \$170 per acre to \$590 per acre for 14 potato fields in Texas, Kansas and Nebraska. The number of insecticide applications ranged from six to ten (Table 1). Although some of those applications might also control other insects, it is likely that many growers face hundreds of additional dollars of control costs.

Table 1. Preliminary Cost Estimates for Zebra Chip and Psyllid Control

Grower	Region	Insecticide Applications	Costs/acre
1	McAllen, TX	6	\$260
2	McAllen, TX	8	\$370
3	McAllen, TX	7	\$310
4	McAllen, TX	7	\$310
5	McAllen, TX	8	\$590
6	Pearsall, TX	6	\$220
7	Pearsall, TX	6	\$180
8	Pearsall, TX	5	\$170
9	Olton, TX	7	\$240
10	Garden City, KS	9	\$280
11	Garden City, KS	7	\$230
12	Imperial, NE	10	\$390
13	Imperial, NE	10	\$280
14	Imperial, NE	6	\$260
Averages		7	\$292
<i>Sources:</i>			
<i>Applications: Dr John Goolsby, USDA, Weslaco, TX</i>			
<i>Prices: various pesticide dealers</i>			

Additional grower-level costs include yield and quality losses. Although we plan to collect expert opinion about those costs in the next year of the project, we have some anecdotal evidence. One grower said that he spent \$500 per acre to control ZC/psyllids and still lost half his crop. That crop was a russet variety for the fresh market. Since impacts in the processing sector might differ from the fresh sector we plan to analyze both.

Previous research conducted by Rosson (2009) looked into ZC yield and quality losses. He contacted industry experts who suggested that ZC infested 35 - 40% of potato acreage in Texas. Rosson assumed that the infected potatoes would either be left in the field or sold for starch at a 90% price discount and estimated a grower loss in Texas of \$33.4 million. When evaluating the impact of different potato psyllid populations on ZC disease incidence, severity, and yield, Munyzneza et al. (2008), found that under the caged conditions of their field experiment commercial yield loss ranged from 48% to 93%. According to a University of California website (2009) ZC yield losses ranges from 20% to 50% percent. Previous work by Guenthner, Michael and Nolte (2001) analyzed the economic impact of another potato disease – late blight. They found that late blight costs were about \$200 per acre across the US. Although ZC is not as widespread as potato late blight, the costs per acre could be higher for ZC.

Since insecticide application is the primary ZC control practice, the value of insecticides could be quite high. Guenthner, Wiese, Pavlista, Sieczka and Wyman (1999) analyzed pesticide use and value in the US potato industry. They found that one insecticide, methamidophos, had a value of \$281 million to US growers during the early 1990s. It could be that the value of the insecticides used to control ZC could have a value of that magnitude, on a per acre basis.

Acknowledgements

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PROGRESS IN IDENTIFYING HOST PLANT TOLERANCE/RESISTANCE TO ZC IN POTATO GERMPLASM

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Summary

ZC screening for tolerance/resistance was continued in 2010, with 168 additional selections from all market classes evaluated. In addition, some 250 entries in the new National Breeders' Chip Trial were also evaluated for ZC, yield, and chip quality. We continue to cooperate and facilitate the research of several other ZC related programs throughout the state.

Impact

The Texas program has gained access to the most advanced public chip selections from virtually all of the public breeding programs in the US for our ZC evaluation. This represents millions of dollars in research funding toward the identification/development of ZC resistant/tolerant varieties. This has long-term, positive implications for the national ZC research effort.

Introduction

The objective of our program is to identify and/or develop potato varieties with tolerance/resistance to the ZC complex. In 2010, host plant screening was continued, as well as verification of previously screened material. A major new aspect to our program was added, involving early generation evaluation of chip selections from 11 public breeding programs throughout the U.S.

Methods and Results

Screening and evaluation. Our program has cooperated with a number of others at both the state and national levels. In Texas, we cooperated with Drs. T.X. Liu and John Jifon in Weslaco. The results of this verification trial are reported elsewhere in these proceedings by Dr. Elizabeth Pierson. At College Station, we cooperated with Drs. Cecilia Tamborindéguy (Entomology), Veria Alvarado and Dennis Gross Plant Pathology and Microbiology). At

Lubbock and Bushland, we cooperated with Dr. Christian Nansen, Kathy Vaughn, and Dr. Charlie Rush. At Halfway, we had cooperative trials with Dr. Pat Porter and Dr. John Goolsby. At Springlake, we had cooperative trials with Dr. Ron French. In 2010, we conducted major trials at Springlake and Dalhart. We also had cooperative studies with Dr. John Trumble and Casey Butler at Riverside, CA, Dr. Joe Munyaneza at Wapato, WA, and Dr. Rich Novy at Aberdeen, ID.

In 2010, we continued our ZC screening for host plant tolerance/resistance. At Weslaco, we conducted replicated caged trials with 22 entries. In previous years, we had evaluated 441 advanced selections for their ZC expression under field conditions. The 22 entries had not expressed ZC symptoms in earlier trials. Under caged conditions, the entries were overwhelmed with the hot psyllid treatments. However, in adjacent uncaged plantings, several of the entries expressed no ZC symptoms under field conditions with high psyllid infestation. These included NY138, TX05249-10W, and TX05249-11W. Other entries showed only mild expression, while the check variety Atlantic exhibited severe ZC symptoms.

At Springlake, some 193 entries were planted in replicated trials. Of these, 60, which had not been previously evaluated, were scored for ZC both in fresh cut and chip evaluations. At Dalhart, some 452 entries were planted, of which 108 were similarly evaluated for ZC expression.

National Breeders' Chip Trial. At the request of the National Potato Promotion Board, breeders from 11 of the 13 U.S. public breeding programs met in Chicago, IL on December 15, 2009 to discuss the possibility of accelerating chip variety development in the U.S. The objective of the meeting was to devise a strategy for rapid evaluation of advanced chip selections, with the goal of developing new varieties which bulk faster or as fast as Atlantic (without the problems of Atlantic, such as heat necrosis, etc.) and store longer than Snowden. These are the most popular public varieties currently in use. A major constraint in current chip variety development has been the availability of disease-free seed in sufficient quantities to test advanced selections. Current regional and national chip trial protocol requires from 100 to 550 lbs of seed per entry. This goal has proven very difficult for the various programs to meet and has greatly restricted the number of entries, resulting in limited progress in the release of new chip varieties.

The group determined that an alternative strategy might be early generation evaluation using a very limited quantity of seed. It was decided that there would be nine sites. There would be a Southern tier (California, North Carolina, Florida, and Texas) and a Northern tier (New York, Michigan, Wisconsin, North Dakota, and Minnesota). Each site would require enough seed to plant 15 hills, requiring a total of only 20 lbs per entry. Each breeding program could submit 10 or more entries, with no more than 250 total entries.

Unique opportunity now presented for the ZC screening program:

Having the most advanced chip selections made available for ZC screening presented an outstanding opportunity, which we wanted to use to our advantage. In Texas, we actually

conducted two trials - a two-hill trial in Springlake and the standard 15-hill trial in Dalhart. The rationale was a concern for hail and the possibility of different levels of psyllid pressure. Some 265 chip entries were planted in the two-hill trial at Springlake and 245 entries were planted in the 15-hill Dalhart trial. A number of the entries looked promising as potential new varieties based on yield and quality (including ZC tolerance) evaluations. The breeders are scheduled to meet again in Chicago on December 14, 2010 to review the data from these multi-state trials and make trial and data collection adjustments, including the addition of new entries, for 2011. It is anticipated that those entries which exhibit ZC tolerance/resistance will be re-evaluated in caged confirmation screening trials.

Acknowledgements

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INVESTIGATIONS ON PUTATIVE ZEBRA CHIP (ZC) TOLERANT ADVANCED SELECTIONS

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Summary

Twenty-two selections comprising 18 putative ZC-tolerant advanced selections and controls were evaluated for ZC tolerance under heavy (caged) and field (uncaged) insect feeding pressures. In cages under high insect pressure where insects were forced to feed on all plants, all selections developed moderate to severe ZC symptoms. This finding demonstrates that even in the ZC-tolerant advanced selections, there is no resistance to heavy infestation. In contrast, under moderate insect pressure in the field, several selections were completely free of ZC in all replicates. Data presented here and in other talks at this symposium support the notion that this lack of symptom development may be due to insect preference, plant tolerance, or both. If insect preference is a major contributor to tolerance, this should be factored into field planting design. Future work will be directed at studying how best to utilize selections that have demonstrated repeated potential for ZC-tolerance in small trials.

Impact

Information obtained in this study can provide a basis for more effective pesticide utilization and deployment of putative ZC tolerant selections/varieties.

Introduction

Here we describe a ZC-screening experiment conducted at the Texas AgriLife Research Center, Weslaco, TX, as part of the winter 2009 ZC-tolerant advanced selection trials directed by Dr. Creighton Miller. Twenty-two selections comprising 18 putative ZC-tolerant advanced selections and controls were tested. The objective of this field experiment was to verify ZC tolerance of field- evaluated advanced selections under heavy (caged) and field (uncaged) insect feeding pressures. The goal was to determine the extent of ZC tolerance in the advanced selections under situations where insects were not given a choice on what to eat (caged treatment/heavy insect pressure) versus were given a choice on which cultivar to feed (uncaged/field pressure).

Materials and Methods

Advanced Selections: Eighteen selections that were tentatively identified as ZC tolerant were included in this study. As reported last year (1, 2), these selections were based on evaluation of more than 32,000 individual tubers (fresh cut and chipped) for ZC tolerance as part of the Texas Potato Breeding Program. To date, 441 selections/named varieties have been evaluated one or more times. In addition to the 18 selections identified as potentially ZC tolerant, Prince Hairy and King Harry also were included because of their characteristic trichomes.

Atlantic and Russet Norkotah were included as ZC positive controls. All market classes were represented including red, chip, russet, and specialty potatoes.

Insect material: Potato psyllids (*Bactericera cockerelli*) were obtained from one of two different populations of insects: a “hot” colony (~70% of the insects harbor the bacterium “*Candidatus Liberibacter*” CLs) or a “cold” colony (~0% of the insects harbor the bacterium). However, the insects in the “cold” colony later turned out to be ZC-infectious.

Field design: The trial consisted of two treatments: caged and uncaged plantings. There were a total of eight cages with each cage having one plant of each of the 22 entries spaced 1.5 ft apart. On March 7-8, 2010, 10 “hot” psyllids were released onto each plant in cages 1-7. Cage 8 was to be inoculated with 10 “cold” psyllids per plant. However, these insects also turned out to be “hot”. The insects and their progeny were not removed and remained on the plants until the end of the experiment. The uncaged treatments were located in the center three rows (between sets of cages) and consisted of four replicate blocks containing the 22 entries (Fig. 1). The treatments were planted only in the outer rows (Rows 1 and 3) and consisted of two plants of each entry within a 4 ft plot with 9 in spacing between seed pieces. Atlantic was planted in the center row (Row2) to verify the presence of natural psyllid infestation. Only three of the four replicates of the uncaged treatment were harvested for ZC evaluation.

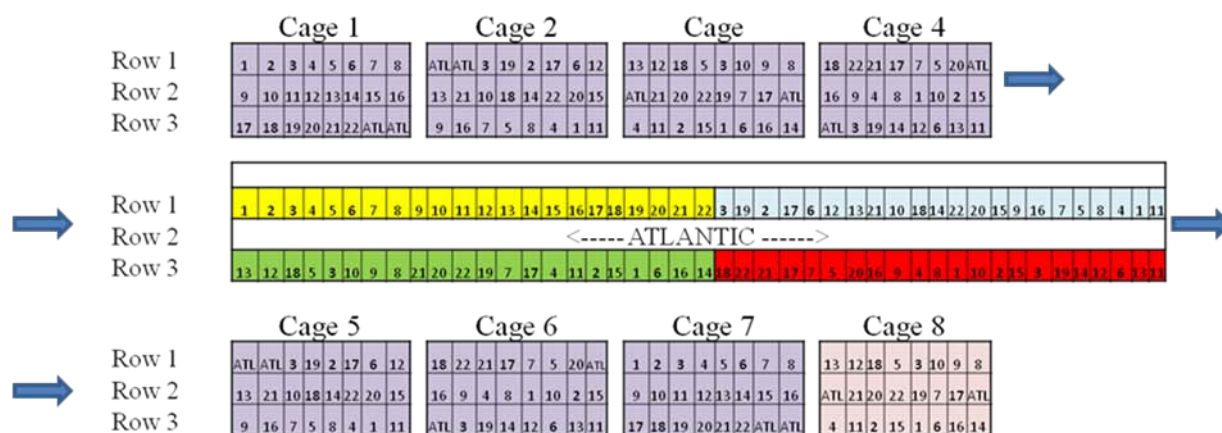


Figure 1 shows three planting rows consisting of eight caged and four uncaged replicates each containing 22 different potato selections. All caged replicates were exposed to heavy insect pressure by “hot” psyllids. Psyllids used to inoculate plants in cage eight came from a different insect colony than those used to inoculate cages 1-7. Uncaged replicates are planted in the outer rows with an inner row of Atlantic.

Field measurements: The trial was planted December 6, 2009. Midseason observations were made on March 11, 2010 after psyllid inoculation. Tubers were harvested by hand on April

14, 2010. Tubers were brought to College Station, washed and graded (size and count). On April 16, 2010 a single slice from each tuber was chipped (fried in vegetable oil, 360°F, ~1 minute). Chip evaluation criteria included number of bad/good chips and severity of ZC.

Results: At the time of harvest, all plants in the cages were dead and severely infested with aphids. Based on the fresh cut and chip evaluations, all of the entries in the eight cages were positive for ZC with varying degrees of severity. Some plants outside the cages were alive and visually evaluated for ZC. All of the susceptible Atlantic within replicates and most plants in the middle row showed ZC symptoms. There were three entries from the three replications outside of the cages where there was no ZC based on the chip evaluations. These entries were NY138, TX05249-10W, and TX99194-3Ru. For TX05249-11W, five out of six plants had no ZC symptoms. Other entries with light ZC were BTX1749-1W/Y, NDTX049265-3WRS_p/Y, NDTX059828-2W, TX03196-1W, TX1674-1W/Y, and King Harry.

Discussion

As has been observed by many growers and researchers, when insect pressure is high such that insects are forced to feed on all plants, they will develop moderate to severe ZC symptoms. This finding unfortunately underscores the current lack of ZC resistance even in the advanced ZC-tolerant selections. However, in the uncaged field trials, some entries had no ZC symptoms despite the presence of infective psyllids, which caused significant disease on Atlantic. Was this lack of symptom development due to insect preference, plant tolerance, or both? Other data presented at this meeting suggests that both insect preference and pathogen tolerance may be playing a role. For example, Casey Butler (this symposium) reported that in trials in California, insects spent significantly less time feeding on the ZC-tolerant variety NY138 and more time off leaves of this variety than ZC-susceptible Russet Norkotah. Julien Levy (this symposium) reported that although rates of pathogen translocation did not differ between ZC-tolerant NY138 and ZC-susceptible Russet Norkotah, the symptoms developed on average two weeks later in the infested stems of NY138 than in Russet Norkotah. Texas selection TX05249-10W, also was evaluated in insect preference tests by Casey Butler (this symposium). Insects fed less frequently (lower number of feeding occurrences) and spent more time resting on this variety than Russet Norkotah. This selection also developed only slight ZC symptoms when evaluated by Joseph Munyaneza (this symposium) in field trials in Washington State.

We believe that data from repeated field trials throughout Texas and extending north into Washington support the notion that certain advanced selections are ZC-tolerant, at least in small scale trials, and that both insect preference and other unidentified mechanisms of tolerance are playing a role. Now the question becomes: what is the next step for these selections? Are we ready to plant these selections in full-scale field trials? And if insect preference is a major contributor to ZC-tolerance, how should field plantings be designed to take advantage of this phenomenon? For example, should plots be sown with a trap row or refuge of non-tolerant varieties to maintain a “choice” for insects? Could such a trap be used for better insect management?

Future field trials will consider how to best make use of putative ZC-tolerant advanced selections.

Acknowledgements

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UNIQUE TRI-SPECIES GERMPLASM WITH MULTIPLE INSECT RESISTANCES AND ITS USE IN BREEDING FOR RESISTANCE TO RESISTANCE TO PSYLLID/ZC

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Summary

Unique germplasm derived from the wild potato species *S. etuberosum* and *S. berthaultii* has been shown to exhibit resistance to many insect pests of potato including green peach aphid, Colorado potato beetle, and wireworm. Based on the multiple insect resistances, this germplasm would seem to be a good candidate for resistance to potato psyllid, the insect vector of “*Candidatus Liberibacter solanacearum*” (syn. *Ca. L. psyllaureus*) associated with zebra chip (ZC). The haploid *tuberosum* x *S. berthaultii* parent and four generations of progeny from backcrossing to cultivated potato were screened for resistance to psyllid and to *Liberibacter* both in field (cage) and greenhouse evaluations. Psyllid behavioral assays suggest this unique germplasm is resistant to psyllid which may contribute to reduced transmission of *Liberibacter* and subsequent incidence of ZC. Resistance to infection by *Liberibacter* may also be present, with a breeding clone not noted as having psyllid resistance displaying a decreased incidence of infection by *Liberibacter*. Field screening for ZC resistance was less conclusive, with two BC₄ progeny displaying light to moderate ZC symptoms in freshly cut tubers, but displaying strong ZC symptoms following frying. Further evaluation of this unique germplasm for potato psyllid and ZC resistance appears warranted.

Impact Statement

Assessment of Tri-Species germplasm suggests resistance to potato psyllid and to the *Liberibacter* responsible for ZC may be present. Host plant resistance to *Liberibacter* and its insect vector can be a component of an integrated approach for controlling 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 ZC. However, to date, it appears that all cultivars currently being used in potato production are susceptible to ZC, although there are varying levels of susceptibility. Unique germplasm derived from the wild species *S. etuberosum* and *S. berthaultii* which has been shown to exhibit resistance to many insect pests of potato including green peach aphid (Novy et al. 2002), Colorado potato

beetle (Juan Alvarez, unpublished data), and wireworm (Alvarez et al., accepted with revisions). The myriad of resistances to other insect pests, suggests that this germplasm may express resistance to potato psyllid as well, thereby contributing to reduced transmission of the *Liberibacter* associated with ZC.

Materials and Methods

A haploid *tuberosum* x *S. berthaultii* parent (designated 463-4) and four generations of progeny from backcrossing (BC) to cultivated potato were screened for resistance to psyllid and ZC in greenhouse and field (cage) assays as described below:

Psyllid Behavioral Assay: Based on the published protocols of Liu and Trumble (2004), the behaviors of psyllids for cleaning, feeding, jumping, resting, off-leaflet, walking, and tasting are observed and data recorded over a 15 minute interval after being placed on plants of submitted entries.

Liberibacter Transmission: Ten bacteriliferous psyllids were caged on plants of submitted entries for 24 hours and then removed. Plants were retained for a two weeks and then were tested for the presence of *Liberibacter* using Taqman-based real time PCR. The mean percentage of plants of each entry infected with *Liberibacter* was then calculated and compared to the control cultivar, Atlantic.

Field Cage Screening: Details of the evaluation are provided in the 2010 Proceedings of the ZC Reporting Session in the report of Munyaneza et al., *Potato variety screening trial for Zebra Chip resistance under controlled field cage conditions*.

Results and Discussion

Psyllid Behavioral Assay: Of the eleven clones evaluated, five had a significantly reduced number of feeding occurrences relative to the potato cultivar, Atlantic. Parental clone 463-4 and BC₁ progeny P2-4 had the fewest number of psyllid feeding occurrences, with two BC₂ clones, and the potato cultivar, GemStar Russet, also having a reduced number of feeding occurrences. GemStar Russet was included in this evaluation based on previous observations of possible psyllid resistance.

Average resting duration of psyllids also was significantly reduced relative to Atlantic for four breeding clones that represented BC generations 1, 3, and 4. Average duration off the potato leaflet was significantly increased relative to Atlantic only for parental clone, 463-4, possibly indicating a non-preference relative to other entries.

No significant differences relative to Atlantic were observed among entries for average feeding duration, and no breeding clones were identified with significantly reduced cleaning durations relative to Atlantic.

Liberibacter Transmission: At the time this report was compiled, no statistical analyses had yet been completed, but the percentage of infected plants appeared to be dramatically reduced relative to Atlantic for three clones representing BC generations 1 to 3. Infection for these three clones was 30%, whereas the infection rate of Atlantic was 80%—indicative of possible reduced transmission/ resistance to *Liberibacter*. Of interest, was the observation clone A00ETB12-3, with no apparent psyllid resistance, was among the three clones

identified as having the lowest *Liberibacter* infection. This observation suggests possible resistance to the *Liberibacter*, and not just the psyllid vector, may be contributing to reduced *Liberibacter* infection.

Field Cage Screening: Two BC₄ progeny displayed light to moderate ZC symptoms in freshly cut tubers, but displayed strong ZC symptoms following frying.

Acknowledgement

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IMPACT OF POTATO PLANTING TIMING ON ZEBRA CHIP INCIDENCE IN TEXAS

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Summary

Zebra Chip (ZC), an emerging and damaging disease of potato in southwestern United States, Mexico, Central America, and New Zealand is causing losses of millions of dollars to the potato industry, in Texas in particular. The disease has recently been associated with “*Candidatus Liberibacter solanacearum*” (syn. *Ca. L. psyllauros*), a previously undescribed bacterium species vectored by the potato psyllid, *Bactericera cockerelli* (Munyaneza et al. 2007a,b; 2008; Hansen et al. 2008; Liefting et al. 2008, 2009; Secor et al. 2009; Crosslin et al. 2010). Effective management of ZC will not be realized until knowledge of the interactions between the potato psyllid, liberibacter, potato, and environmental factors is developed. A study was conducted in Weslaco and Pearsall, Texas to assess the impact of potato planting timing on ZC incidence. Experimental plots of untreated potatoes were planted several different times at each location from November to March for three years and ZC incidence was estimated at the end of each of the growing seasons. Results indicated that, in southern Texas, early-planted potatoes were more affected by ZC than those planted later in the season. The trend was not observed for potatoes planted in Pearsall. Further studies are needed to conclusively determine the impact of potato planting timing on ZC incidence.

Results from these field experiments will increase the understanding of the impact of potato planting timing on ZC incidence and provide information on when potatoes are the most vulnerable to the disease. This information will help potato growers affected by ZC to quickly minimize losses due to this damaging disease by timely planting and appropriately protecting vulnerable potatoes with insecticides. This approach will lead to the development and adoption of better and sustainable management strategies for ZC.

Introduction

Zebra Chip (ZC), an emerging and damaging disease of potato in southwestern United States, Mexico, Central America, and most recently in New Zealand is causing losses of millions of dollars to the potato industry, in Texas in particular. The disease has recently been associated with “*Candidatus Liberibacter solanacearum*” (syn. *Ca. L. psyllauros*), a previously undescribed bacterium species transmitted by the potato psyllid, *Bactericera cockerelli* (Munyaneza et al. 2007a,b; 2008; Hansen et al. 2008; Liefting et al. 2008, 2009; Secor et al. 2009; Crosslin et al. 2010). Despite this important discovery associating the potato psyllid and liberibacter with ZC, effective management strategies for the disease are still lacking. Ultimately, effective management of ZC will not be realized until knowledge of the interactions between the potato psyllid, liberibacter, potato, and environmental factors is

developed. The objective of the present research was to assess the impact of potato planting timing and potato psyllid exposure on ZC incidence.

Materials and Methods

Field trials were conducted at the USDA-ARS Research Farm in Weslaco in the lower Rio Grande valley (LRGV) of Texas in 2008, 2009, and 2010 to assess the impact of potato planting timing on ZC incidence and severity. An additional trial was planted at Black Gold Farms in Pearsall, TX in 2010. At each site, potatoes were planted in mid-November, December, January, February, and/or March, depending on the year. At each planting date, the potatoes were planted in a large plot that consisted of 4 rows of 300-ft long each. No insecticides were applied to the potatoes throughout the study to allow potato psyllids to colonize the plots. Potato psyllids were monitored weekly at each research site by deploying yellow sticky traps and using a D-VAC machine for adults and counting psyllid eggs and nymphs on 100 leaves collected from the plots. ZC incidence in the plots at each site was estimated at the end of the experiment by hand-harvesting each potato plant and making a cutting near the stem end of raw tubers. A plant was considered infected with ZC if at least one tuber exhibited ZC symptoms.

Results and Discussion

Potato psyllids were observed in potatoes at both locations throughout the duration of the experiments. In Weslaco, potatoes planted in December had higher ZC incidence compared to those planted in January and February (Fig. 1). ZC incidence was lowest in February-planted potatoes and the trend was consistently observed in 2008, 2009, and 2010 (Fig. 1). In contrast, no significant difference was observed in ZC incidence in potatoes planted in December, January, February, and March in Pearsall trial conducted in 2010 (Fig. 2). The reasons behind high ZC incidence in potatoes planted early in the season in southern Texas are unknown. However, these differences in ZC incidence in potatoes in Texas may be due to yearly and seasonal fluctuations in *Liberibacter* infection rate in overwintering and migrating psyllids. The potato psyllids are believed to overwinter along the Rio Grande River between Texas and Mexico and migrate northward with increasing high temperatures (Pletsch 1947; Wallis 1955). It is possible that overwintering potato psyllid populations invading early-planted potatoes in the LRGV of Texas are more infected with the bacterium compared to those colonizing potatoes late or migrating north. Further studies are needed to increase understanding and the impact of population dynamics and *liberibacter*-infection rates of potato psyllids from their overwintering sites/hosts to their colonization of potatoes in different parts of Texas.

Results from these studies will increase the understanding of the impact of potato planting timing on ZC incidence and provide information on when potatoes are the most vulnerable to the disease. This information will help potato growers affected by ZC to quickly minimize losses due to this damaging disease by timely planting and protecting vulnerable potatoes with insecticides. This approach will lead to the development and adoption of better and sustainable management strategies for zebra chip.

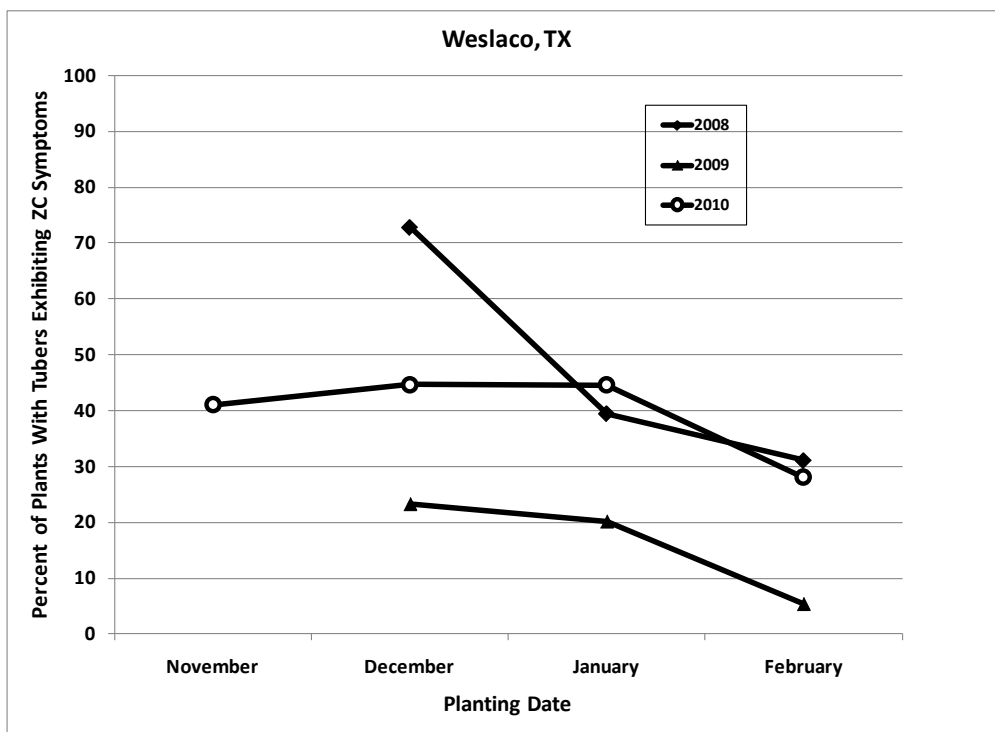


Figure 1. Incidence of zebra chip in untreated potatoes planted in Weslaco, TX at different times in 2008, 2009, and 2010.

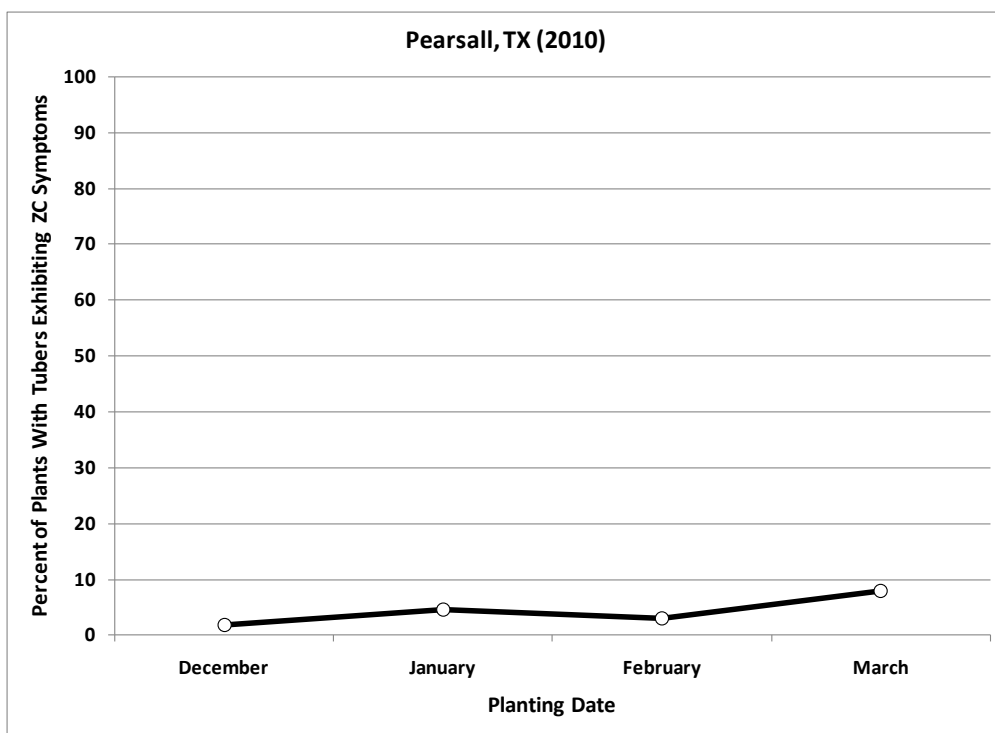


Figure 2. Incidence of zebra chip in untreated potatoes planted in Pearsall, TX at different times in 2010.

Acknowledgements

We are grateful to Andy Cruz, Venkat Sengoda, Millie Heidt, and Black Gold Farms crew for their invaluable technical assistance. Financial support for this work was partially provided by Frito Lay, Inc., USDA-ARS State Cooperative Potato Research Program, Texas Department of Agriculture/Texas AgriLife, USDA-CSREES-RAMP (Project # 2009-51101-05892) and USDA-CSREES-SCRI (Project #2009-51181-20176).

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AN EVALUATION OF PLANT-DERIVED ANTIMICROBIAL AND ANTI-INSECT GENES ON REDUCING ZEBRA CHIP DISEASE IN TRANSGENIC POTATO

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Summary

We have generated transgenic plants of the potato chipping var. ‘Atlantic’ by *Agrobacterium*-mediated transformation carrying either plant-derived antimicrobial or anti-insect genes. Preliminary experiments have shown that expressing antimicrobial genes A or B can delay the onset of *Candidatus Liberibacter solanacearum* (Lso) invasion into potato from psyllid feeding and reduce the level of bacterial inoculum in plant tissues as demonstrated by PCR analysis. In terms of plant phenotype, plants expressing antimicrobial gene A at moderately high levels showed lodging 28 days from infestation along with wildtype plants but the transformed line A-1 showed more green shoots compared to wildtype. Similarly, the transformed line exhibited less necrosis inside the tubers, prior and after frying, compared to wildtype. These observations suggest that antimicrobial genes may have value in reducing the level of ZC in potato.

Impact Statement

We expect that potato plants which exhibit high levels of transgene expression to show effective economic control.

Introduction

It is now presumed that the agent for causing zebra chip (ZC) disease in potato is *Candidatus Liberibacter solanacearum* (Lso) as demonstrated by graft and psyllid transmission, electron microscopy and PCR (Secor et al. 2009). This disease was first identified in commercial potato fields of Mexico in 1994 and subsequently spread into Texas and Nebraska in 2000 and now in many of the major potato growing areas of the USA, New Zealand and Guatemala. The major diagnostic symptom which separates ZC from other known potato diseases is that the tubers show extensive dark and light striped patterns, in vascular ring and medullary rays, of tubers and becomes more enhanced when fried as chip potatoes. At present, there have been no reports of any natural resistance to ZC in commercial potato and so alternative strategies are required to help control the incidence of this problematic disease. In this study, we have investigated the effectiveness of introducing plant-derived antimicrobial and anti-insect genes into an important commercial potato chipping var. ‘Atlantic’ by *Agrobacterium*-mediated transformation. Selected high expressing potato lines for the transgene were infected with Lso using the potato psyllid as vector in cages and their phenotype in terms of tolerance to ZC disease was measured using PCR for the 16S rDNA gene for the bacterium and by frying tubers from infected plants.

Materials and Methods

Agrobacterium and plasmids

The binary vector pBin34SGUS (Yang et al. 2000) carrying the plant selectable marker neomycin phosphotransferase II (nptII) gene and the β -glucuronidase (gusA) reporter gene located between left and right border T-DNA sequences respectively was used in all transformation studies. Anti-insect or antimicrobial genes (A or B) were inserted between the two marker genes.

Plant transformation

Several explants from tissue culture-derived plants were used for *Agrobacterium*-inoculation. Explants were floated in an overnight culture of *Agrobacterium*. Plant tissues were transferred to cocultivation medium for 2 days and then onto callus induction medium containing kanamycin for the positive selection of transformed cells. After 2-3 weeks, explants are transferred to shoot regeneration medium to promote organogenesis from potato callus. Shoots approx. 1 cm in length were excised from parent tissue and rooted on culture medium containing kanamycin. To confirm GUS expression in such shoots, pieces of leaf, stem and root were stained histochemically.

Molecular analysis of transgenic plants

Southern hybridization was used to confirm the integration of the agronomic useful gene into the nuclear genome of each transgenic event. Approx. 10 μ g of genomic DNA was extracted from leaf tissue using a CTAB extraction method (Dellaporta et al. 1983) and digested with a restriction enzyme that cut once at the border of the agronomic gene so that the number of bands seen would represent the number of copies integrated into the plant genome. Digested DNA was electrophoresed in a 0.8% agarose gel, blotted onto a nitrocellulose membrane and hybridized using a P^{32} -labeled probe using standard procedures (Sambrook et al. 1989). Northern blotting was performed to study the expression levels of our agronomic genes. Fifteen μ g of total RNA was extracted from leaf tissue according to the method described by Verwoerd et al. (1989). Electrophoresis, blotting and hybridizations were performed using standard methods (Sambrook et al. 1989). Western blotting was performed to detect the expression of our agronomic gene at the protein level. Approx. 40-50 μ g of total protein from each plant was separated on a SDS-PAGE gel and the amount of protein was visualized by colorimetric analysis using the method described by Yang et al. (2000).

PCR for screening the presence of Lso in potato

Four-week-old plants of antimicrobialA-1 (event 1) and antimicrobialB-1 (moderately high expressers) were transferred to insect cages from a growth chamber and allowed to acclimatize under 16 h photoperiod at 22°C for 10 days. Ten ‘hot’ psyllids (carrying Lso) were added to one cage containing one pot (4 plants/pot), and likewise, 10 ‘cold’ psyllids (without Lso) were added to another cage for comparison. At regular intervals, apical shoot tips were harvested from each set of plants from which genomic DNA was extracted using Power Plant DNA Kit (MoBio, Carlsbad, CA). Five hundred ng from each sample preparation was used as a template for detecting the presence of *Liberibacter* using OA2/OI2C primers by PCR. At the end of the experiment (approx. 35 days from infestation),

tubers were harvested, sliced (2 mm thick) and the presence of ZC disease observed before and after frying (350°C, 2 min in vegetable oil).

Results and Discussion

Molecular characterization of transgenic plants

Southern blotting was performed on all antimicrobial A and B plants and anti-insect potato plants to determine the number of integrations of the foreign gene into the genome. The number of insertions varied for each gene construct with 1-7 copies being detected for anti-insect and antimicrobial A genes and 1-6 copies being found for antimicrobial B gene. The P³² – labeled probes failed to hybridize to wildtype genomic DNA. In terms of level of transcript for these genes in transgenic potato, a range of high, medium and low expressers were found as determined by northern blotting (Fig. 1). There appeared to be a link between low copy number and high level of expression of the anti-insect gene. For example, the highest expressers for the anti-insect gene had 1 or 2 insertions and the weak expressers had up to 7 integrations. This suggests that a high copy number of this gene may provoke a gene silencing mechanism, which hinders the expression of the transgene. However, such a relationship did not exist for the antimicrobial gene constructs with the highest expressers having a range of integrations from 3-7 copies. To determine the level of expression of the transgene at the protein level for the anti-insect gene, as demonstrated by western blotting, plants which exhibited high transcript levels as shown by northern hybridization also gave the highest accumulation of protein. This suggests there is a good correlation between transcript level and protein accumulation for the anti-insect gene. At present, work is continuing to see whether such a relationship exists for the antimicrobial genes.

Evaluation of antimicrobial genes on ZC disease in transgenic plants

Transgenic lines antimicrobial A-1 (gene A, event 1) and antimicrobial B-1 were selected for this analysis as they demonstrated relatively high levels of expression of the transgene. In the case of event A-1, apical shoots were harvested at 0, 14 and 28 days after ‘hot’ psyllid infestation and 0, 7, 14, 21, 28 and 35 days for event B-1 for determining the presence of Lso in these plants by PCR (Fig. 2). For event A-1, Lso was detected by day 14 in both wildtype and transgenic line but the level of inoculum was much reduced in the transgenic line compared to wildtype at days 14 and 28. In terms of transgenic line B-1, Lso was detected as early as 7 days from infestation in wildtype but not in the transformed plant. It was not until day 21 that line B-1 showed a clear sign of inoculum, at which time, the wildtype showed substantially more inoculum compared to B-1. However, by days 28 and 35 of the experiment, the level of bacteria in the wildtype and B-1 line were comparable. These preliminary PCR results suggest that both transgenic lines appear to have delayed the onset of inoculum accumulation in the plant and that expression of these antimicrobial genes may be the contributing factor. To study the effects of antimicrobial gene A-1 on plant phenotype, both transgenic line and wildtype were photographed at days 3 and 28 after infestation (Fig. 3). At day 3, both the transgenic line and wildtype showed no impairment in growth. However at day 28, both A-1 and wildtype showed lodging and shoot necrosis with the transformed line showing more green shoots following infestation with ‘hot’ psyllids. At day 28, tubers were harvested from both A-1 and wildtype to see whether typical ZC symptoms could be detected in both uncooked and fried tuber slices (Fig. 3). As expected, plants

infested with ‘cold’ psyllids showed no necrotic lesions in both transgenic and wildtype tubers, before and after frying. However, those plants infested with ‘hot’ psyllids showed typical brown lesions indicative of ZC disease but the level of necrosis was more intense in the wildtype compared to line A-1. Overall, the PCR and the phenotypic data suggest that the antimicrobial genes used may have value in not only delaying the onset of ZC disease but also reduce the level of Lso in potato.

Figure 1. Northern analysis of transformed potato lines carrying antimicrobial genes A or B or an anti-insect gene.

Antimicrobial
gene A

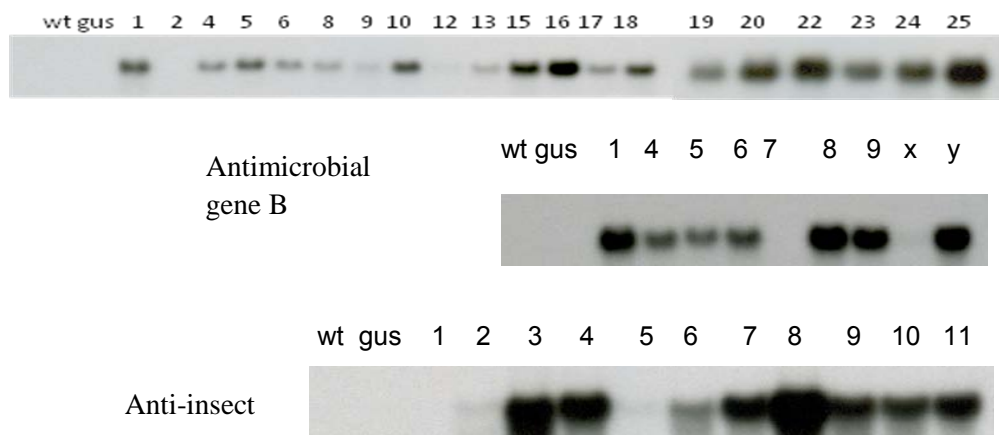
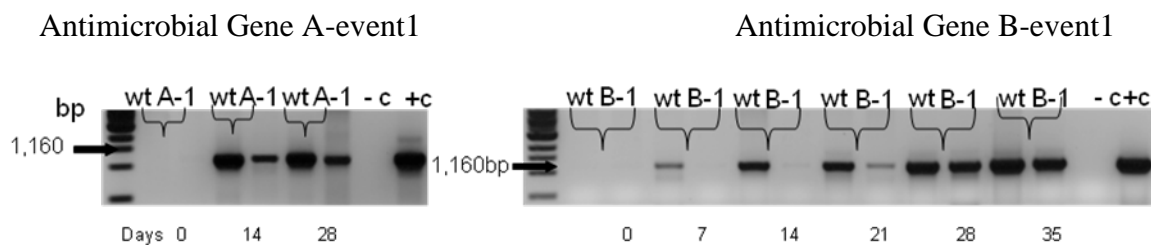
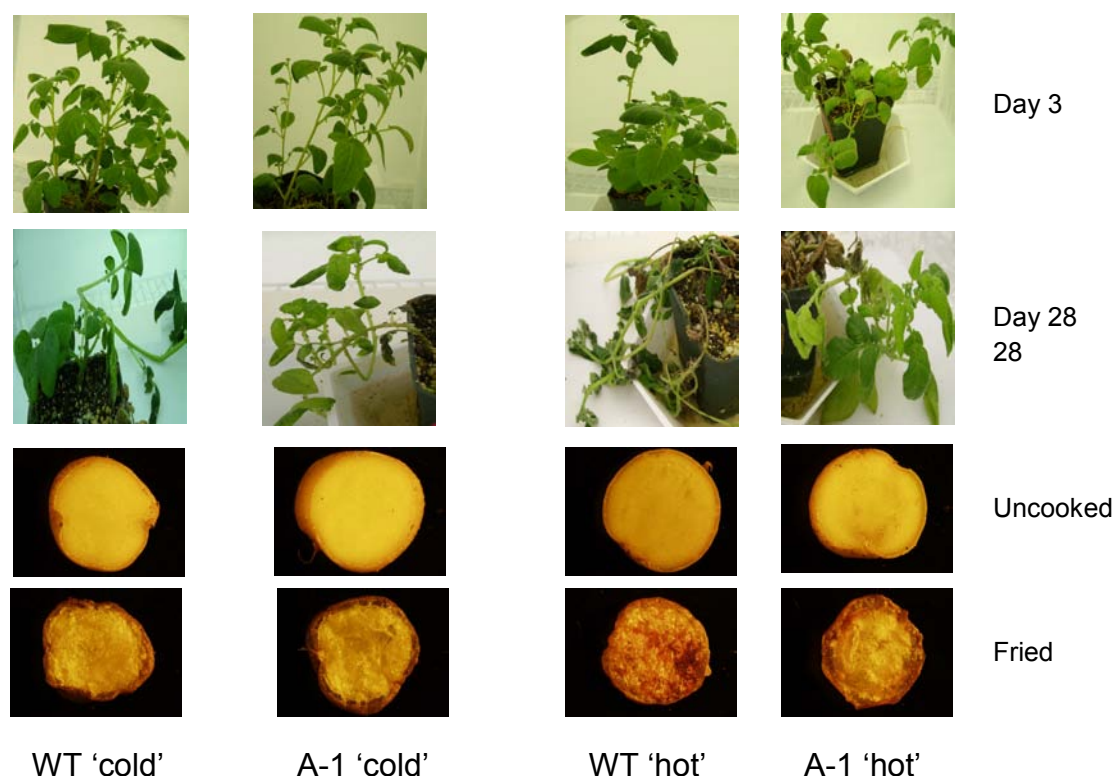


Figure 2. Detection of Lso in apical shoots of potato after set time intervals from infestation using PCR



wt = wildtype; A-1, B-1 = transgenic lines; -c = water control; +c = ‘hot’ psyllid

Figure 3. Phenotypes of potato plants infested with ‘cold’ or ‘hot’ psyllids



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ESTABLISHING AND FINE-TUNING AN *IN PLANTA* SYSTEM FOR POTATO ZEBRA CHIP DISEASE RESEARCH IN CALIFORNIA

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Summary

We have acquired an APHIS-PPQ permit to work on “*Candidatus Liberibacter solanacearum*” at the USDA-ARS San Joaquin Valley Agricultural Sciences Center in Parlier, California. “*Ca. L. solanacearum*”, associated with Zebra chip disease (ZC) of potato. This bacterium is currently unculturable *in vitro*. Attempts have also been made to develop media to culture “*Ca. L. solanacearum*” *in vitro* following a published procedure for culturing citrus HLB-associated liberibacters with no success. Meanwhile, we are also working on culturing and / maintaining “*Ca. L. solanacearum*” *in planta* based on the currently available tissue culture procedures through collaboration. In another development, we have identified a location in California to collect “*Ca. L. solanacearum*”-infected potato. ZC materials are maintained in a greenhouse. Grafting ZC-affected potato to tomato plants has been successful. Unlike potato plants, affected tomato plants showed symptoms of leaf marginal chlorosis and stunting of young shoots, but did not succumb to the bacterial infection.

Impact Statement: “*Candidatus Liberibacter solanacearum*” is currently regarded as a pathogen involved in the etiology of potato zebra chip disease and the information is used for disease control. However, the pathogen status is only established by strong association. Cultivation of “*Ca. L. solanacearum*” is a critical step to fulfill Koch’s postulates that will firmly establish the disease etiology. Culture of “*Ca. L. solanacearum*” is also needed to obtain high quality DNA for bacterial genome sequencing that could provide useful biological information of the bacterium for zebra chip disease research and control.

Introduction

“*Candidatus Liberibacter solanacearum*” has been regarded as the putative pathogen of potato zebra chip disease (ZC), an economically important disease in potato production (Munyaneza et al., 2007). However, the etiological role of “*Ca. L. solanacearum*” in ZC has not been conclusively established. Because the bacterium is currently not culturable *in vitro*, research on many perspectives of this bacterium, including pathogenicity, has been difficult. Our current knowledge about “*Ca. L. solanacearum*” is mostly derived from DNA sequence data. Indeed, the bacterium is defined and identified by signature DNA sequences. The physiological and biochemical characteristics of the bacterium are not known. Morphological and cellular structure information remained to be confirmed. With little information available about the bacterium, options for ZC control strategies are limited.

This research project was initiated to develop methods or techniques to culture “*Ca. L. solanacearum*”. Traditionally, bacteria are cultured *in vitro*, i.e. in artificial media, which

would lead to pure cultures. *In vitro* culture has an advantage of separating the target bacterium from other non-target micro-organisms. This is a critical step in establishment of bacterial etiology or pathogen confirmation. The second rule in Koch's postulates demands *in vitro* culture of a candidate pathogen. In the genomics era, pure culture also guarantees the quality of bacterial DNA for sequencing accuracy. A less stringent technique for bacterial cultivation is *in vivo* or *in planta* culture. In this case, the target bacterium is cultured along with live cells, usually from hosts. Although the target bacterium is still mixed with other live materials, the titer of the target bacterium is significantly increased, facilitating bacterial characterization and manipulation.

There are no reports of successful cultivation of "*Ca. L. solanacearum*" *in vitro*. A report on *in vitro* cultivation of "*Ca. L. asiaticus*", a phylogenetically related bacterium associated with citrus Huanglongbing (HLB) has been published (Sechler et al., 2009). However, the reported results have not been validated so far. Nevertheless, one conclusion is that *in vitro* cultivation of "*Ca. L. spp.*" is a highly challenging task. Therefore, while the ultimate goal of this project is *in vitro* culture of "*Ca. L. solanacearum*", an alternate goal is also *in planta* cultivation of "*Ca. L. solanacearum*", which in turn, may be necessary for achieving the ultimate goal. The current research is focused on establishing and fine-tuning an *in planta* system for potato zebra chip disease research in California.

Materials and Methods

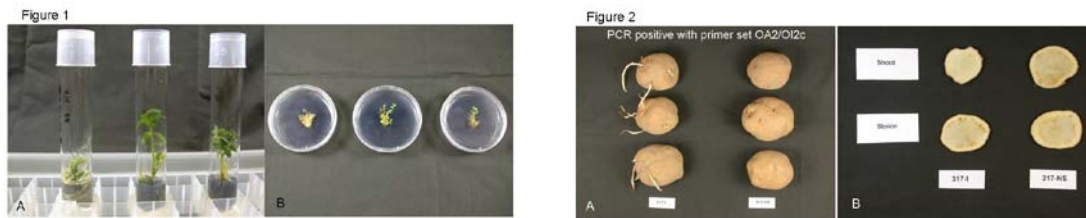
Sources of ZC-potato materials. APHIS-PPQ permit was acquired for research work on "*Ca. L. solanacearum*" at the USDA-ARS San Joaquin Valley Agricultural Sciences Center in Parlier, California. To satisfy the PPQ permit conditions, ZC materials received from outside California were used for lab manipulation only. We received ZC-affected potato tubers from Washington and Texas, kindly provided by Joe Munyaneza, USDA-ARS, Wapato, WA, to begin the bacterial cultivation experiments. A collaboration plan was developed with Jim Crosslin, USDA-ARS, Prosser, WA to culture and maintain "*Ca. L. solanacearum*" *in planta* based on the available tissue culture procedure (Crosslin and Munyaneza, 2009). In addition, efforts were made to identify a ZC potato source in California. Potato tubers and plants with ZC symptoms were collected in Santa Maria, CA.

PCR detection of "Ca. L. solanacearum". DNA was extracted from ZC-affected potato tuber or leaf petiole tissues using a commercially available DNA extraction kit. Primer set OA2/OI2c (Liefting et al., 2009) was used for PCR detection of "*Ca. L. solanacearum*" following a previously described procedure (Chen et al., 2005). Briefly, PCR (25 µl) was carried out using the Takara Taq (Hot Start Version) kit (Takara Bio Inc., Otsu, Japan). The reaction mixture contained 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM MgCl₂; 100 µM each dNTPs; 400 mM of each primer; 1 unit of Taq DNA polymerase; and 1 µl of cell suspension. Amplification was conducted in a thermocycler (Model PTC-200; MJ Research, Waltham, MA) with an initial denature at 96°C for 10 min followed by 30 cycles consisting of denaturing at 96°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. The amplification products were then stored at 4°C. The amplified DNAs were resolved through 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining.

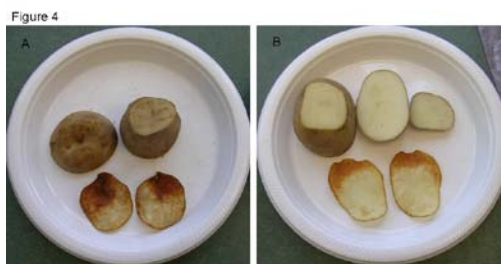
In vitro and in planta cultivation. For *in vitro* culture of “*Ca. L. solanacearum*”, ZC-affected potato tubers from Texas were used and the procedure of Sechler et al. (2009) for cultivation of “*Ca. L. asiaticus*” was followed with a modification of replacing citrus juice extract with tomato juice extract. For *in planta* culture, both tissue culture and whole plant culture in greenhouse were performed. ZC-affected potato plant tissue cultures were carried out in a tissue culture facility. Growth of “*Ca. L. solanacearum*” inside plants was monitored by PCR. In the greenhouse experiment, potato tubers with ZC symptoms collected in California were germinated and maintained in a greenhouse for observation and PCR testing. Since ZC-affected potato plants were often found severely stunted and difficult to grow, or “*Ca. L. solanacearum*” became not detectable in new growth that developed, attempts were made to transfer “*Ca. L. solanacearum*” by grafting potato stem pieces to young tomato seedlings. Symptom development in tomato was recorded and presence of the bacterium was monitored by PCR.

Results and Discussion

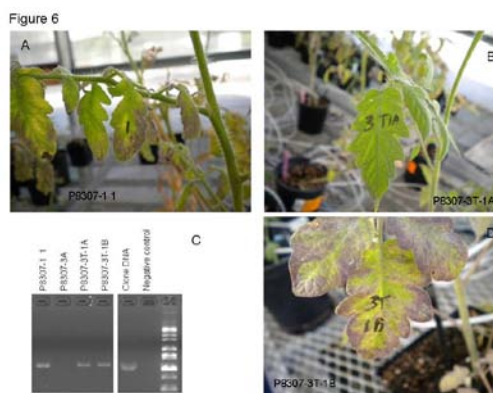
Efforts to culture “*Ca. L. solanacearum*” *in vitro* have not been successful. In most cases, no bacterial growth was observed. Occasional growths of bacterial colonies from ZC-affected tissues were all identified as non-liberibacter bacteria based on 16S rDNA analyses. We have successfully maintained “*Ca. L. solanacearum*” in potato plant tissue culture (Fig. 1, A and B). However, the plantlets with “*Ca. L. solanacearum*” remain stunted and the growth is slow. Occasionally, new and normal shoots develop on infected plants. Yet, “*Ca. L. solanacearum*” has not been detected in these tissues by PCR. This phenomenon was reported previously (Crosslin and Munyaneza, 2009). Ways to facilitate the growth of ZC affected potato plants and to reach to a high bacterial titer remain to be determined.



A source of naturally-occurring ZC-affected potatoes in California was identified in Santa Maria, Ca. Early in March, 2010, we observed that some potato tubers harvested in 2009 did not sprout or sprouted poorly compared to others from the same plot (Fig. 2, panel A). In addition, poorly and non-sprouted tubers failed to develop into healthy potato plants under greenhouse conditions (Figure 3, plants in bottom row). Cross-sections of potato tubers showed that non-germinated tubers often (but not always) had necrotic vascular lesions. Interestingly, stolon end sections were apparently more symptomatic than the shoot end sections (Figure 2, panel B). Fried chips from tubers with vascular discoloration or necrotic lesions showed uneven distribution of browning compared to those from asymptomatic tubers (Fig. 4. Left plate-symptomatic, right plate-asymptomatic). PCR tests from many of the symptomatic potatoes were positive in “*Ca. L. solanacearum*”. All these meet the general criteria of potato ZC.



Beginning in August, 2010, potato plants with typical ZC symptoms were found in field. Symptoms on affected plants included stunting, chlorosis, swollen internodes, proliferation of axillary buds and aerial tubers, browning of the vascular system in belowground portions of stems, and leaf scorching (Fig. 5, panel B) as described by Munyaneza et al. (2007). Field surveys along with sample collections were performed in August, September and October. “*Ca. L. solanacearum*” was detected in the collected symptomatic samples, confirming the presence of ZC in this California location.



Using symptomatic potato shoot as scions, grafted tomato plants appeared to be able to host “*Ca. L. solanacearum*” as evidenced based on PCR analyses (Fig. 6, panel C). It is noted that no potato scions were able to develop into normal growth on tomato. Instead, the grafted potato shoots survived for 1-3 weeks and then died. Inoculated tomato plants developed symptoms of leaf marginal purpling (necrosis in a severe case) and stunting of new growth (Fig. 6, panel A and D). “*Ca. L. solanacearum*” was also detected by PCR in apparently non-symptomatic leaves (Fig. 6, panel B). Overall, the inoculated tomato plants did not succumb to infection of “*Ca. L. solanacearum*”. This suggests that the bacterium can be enriched in tomato.

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GENOME BASED BIOMARKERS FOR IMPROVED MOLECULAR DIAGNOSIS AND GENETIC ANALYSIS OF *CANDIDATUS LIBERIBACTER SOLANACEARUM*, THE BACTERIUM ASSOCIATED WITH POTATO ZEBRA CHIP DISEASE

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Summary

Two genome based biomarker systems were developed for improved ZC-associated “*Candidatus Liberibacter solanacearum* (Lso)” detection and for population genetic analysis. The first system is an Lso specific diagnostic system. The procedure involves two steps of PCR using Lso species specific outer and inner primer pairs. This new detection system can reliably detect as low as single digital copies of target DNA. The sensitivity of this dual primer PCR is comparable to the two-tube nested PCR but the whole process is completed in a single closed tube, greatly eliminating the potential risk of cross contaminations commonly associated with conventional two tube nested PCR. The second marker system is a multi-locus simple sequence repeat (SSR) genotyping system. The highly polymorphic nature of SSR loci makes this marker system an ideal tool for the genotyping and population analysis. The new biomarker systems developed in this study provide powerful tools for unambiguous detection, strains identification and the molecular epidemiological study of Lso populations which will provide critical information and facilitate the development of effective potato zebra chip disease management.

Introduction

Zebra chip (ZC) is a new and economically important disease of potato (*Solanum tuberosum* L.). The disease has led to multi-million dollar losses for potato growers in the central and western United States over the past decade and impacted the livelihood of potato farmers in Mexico and New Zealand. However, the causal agent had been an enigma for years. Recently, molecular and phylogenetic evidence based on cloned 16S rRNA gene sequence from ZC symptomatic potato tubers and the associated potato psyllid (*Bactericera cockerelli* Sulc) suggests the presence of a new species of *Candidatus Liberibacter* (Hansen *et. al.* 2008; Liefting *et. al.* 2009; and Lin, *et. al.* 2009). The other three species of *Ca. Liberibacter* known to date are associated with huanglongbing (HLB) disease in citrus. However, due to the fastidious nature of the bacterium, until now, Koch’s postulate has not been fulfilled and therefore information regarding the etiology and pathogenesis of ZC associated causative agent is lacking. To gain important insights into this uncharacterized new pathogen, to decipher its pathogenesis, function, and to understand how it interacts with its host and how it causes disease, the genomic information about this causal agent is needed. Using metagenomic approach, we have successfully obtained the whole genome sequences of Lso. The availability of the genomic information has provided a valuable resource for developing genome based applications such as new Lso diagnostic tools to improve sensitivity and specificity of Lso detection and multilocus biomarkers for genotyping and genetic analyses of Lso populations. Here we describe our research progress in developing a new diagnostic

system (single tube dual primer (STDP) TaqMan PCR) and a simple sequence repeat (SSR) marker system for Lso population genetic analyses.

Materials and Methods

ZC-infected materials: Fourteen ZC-symptomatic potato (vars. FL1867 and Atlantic) plants and tubers were collected from Garden City, KS, and Dalhart, TX (Provided by Jon Gilley). Psyllid nymphs and adults were obtained from laboratory-reared colonies maintained at the USDA-ARS in Wapato, WA (provided by Dr. Joseph Munyaneza). In addition, sixty eight Lso-positive samples were provided by Dr. Neil Gudmestad. These samples were collected from petiole, midvein, aboveground stem, stolon, and tuber tissue of the potato, tomato and pepper hosts. This set of samples representing eleven potato growing regions were used for genetic diversity study with SSR molecular markers.

Total genomic DNA was extracted using either DNeasy Plant Mini Kit (Qiagen, Valencia, CA) or the CTAB method as previously described (Lin et al, 2008). The quality of DNA samples was checked by electrophoresis in 1.2 % agarose gels. DNA concentrations were determined spectrophotometrically and adjusted to 50ng/μl. Three other “*Candidatus Liberibacter*” species, asiaticus, africanus and americanus were included in this study as negative controls. Due to the select agent status of the citrus “*Candidatus Liberibacter*” species, HLB-infected DNA samples from São Paulo, Brazil, South African and China were extracted in their respective origins and sent to California as microbially-sterile, non-infectious DNA samples. Healthy potato plants and HLB-free citrus DNA samples were prepared at the USDA-ARS, San Joaquin Valley Agricultural Sciences Center in Parlier, CA. All samples were verified using a published Lso real time PCR detection system (Li et al., 2009).

New diagnostic primers and probes design: “*Ca. Liberibacter solanacearum*” sequences representing a single copy in the outer membrane protein (*opm*) gene region were selected for the designing of diagnostic primers and TaqMan® probe. This region has 2,694 bps (NCBI accession FJ914617). The optimum TaqMan® probe and inner primers were designed with the following criteria: GC% ≥ 40-50, T_m = 55 °C ± 2, primer length = 18-22 bp with amplicon size ranging from 120-200 bp. The melt temperature (T_m) for the TaqMan® probe was set 10°C higher than the T_m for inner primer. To ensure amplification efficiency, the primers and probes were designed in a region where no secondary structures have been 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 T_m of 65 °C (10°C higher than inner primer T_m) 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 combination 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). These sequence analyses resulted in the identification of two sets of inner/outer primers and TaqMan® probes. 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).

Design SSR markers for genotyping and genetic analysis of Lso: Genome-wide sequence search was conducted to identify simple sequence repeat (SSR) loci using complete Lso genomic sequences. The search included mono, di-, tri-, tetra- or any types of perfects, in-perfect and compound types of simple repeat motifs. The criteria to identify SSR loci for designing primer were as follows: 1) for each type of repeat, we selected loci containing at least 5 or more of SSR motifs. 2) each locus has one copy per genome. The results led to the identification of 85 loci that met the criteria and were potentially useful to design SSR markers. To ensure that sequence loci selected for SSR marker development were unique to Lso, *in-silico* sequencing analyses were carried out using BLAST analysis to compare candidate loci with all the available microbe sequences in the NCBI databases. Next, selected SSR sequences were aligned by the CAP3 program to remove any duplicate loci. The final set of SSR loci were used for SSR primer designs using Molecular Beacon software (V. 7.0).

Data analysis: For SSR analyses, a genotypic profile consisted of 8 SSR primers with 68 Lso-infected samples was generated. GenAlEx Version 6.3 (Peakall et al. 2006) was used to calculate the number of alleles per locus (N_a), the number of effective alleles per locus (N_e), and the number of private alleles (N_p) per locus. A global test (Fisher's method) implemented in GENEPOP web version 4.0.10 (Raymond et al., 1995) was used to test for the genotyping linkage disequilibrium at each pair of loci across all samples. Next, the program STRUCTURE 2.3.1 (Pritchard et al., 2000) was used as a clustering algorithm based on a Bayesian model to assign individuals to a specified number of clusters (K). To estimate the number of clusters (K), 10 independent runs of $K=1-10$ were carried out without any prior information as to the origin (location or host) of individual samples. For each run, a burn-in period of 50,000 iterations was used followed by a run length of 100,000 Markov chain Monte Carlo iterations, and a model with correlated allele frequencies and admixture among populations. The optimal probabilities for all individuals were estimated from 20 replicate runs at $K=2$ with permutation analysis using CLUMPP version 1.1.2, and the output of genetic clustering was visualized using software DISTRUCT version 1.1 (Rosenberg et al., 2004).

Results and Discussion

Molecular Diagnosis: Sensitive and accurate detection is critical for efficient disease management and regulatory responses to prevent the introduction to, and spread of ZC-associated Lso in unaffected areas. Since ZC-infected plants are usually present in low titer in aboveground tissues and often distribute unevenly in host plants, an improved new method for more reliable and sensitive detection is needed. The current Lso detection methods have relied primarily on PCR amplification of a portion of the ribosomal RNA genes. Although these methods generally work well for the detection of potato tuber samples with high Lso titers, it has often been observed that they are not reliable for consistently detecting “*Ca. Liberibacter*”-infected, but asymptomatic leaf / stem tissues or low titer samples. More importantly, molecular diagnostic assays designed in ribosomal RNA gene region are lack of adequate specificity and frequently produce non-specific products due to highly conserved

sequences. To ensure reliable detection, other species-specific sequencing loci should be used to avoid ambiguous PCR results (Lin et al., 2010). We developed a new sensitive detection system, STDP TaqMan® PCR for molecular diagnosis of ZC. This new diagnostic assay is designed in a non-ribosomal region, which has been proved to be highly specific to Lso. The system is conceptually analogous to the conventional nested PCR, but the whole process is completed in a single closed tube. The procedure involves two rounds of PCR using the Lso species-specific outer and inner primer pairs. Because both pairs of primers were designed to work at different temperatures, the interferences between primer pairs were minimized. This new detection system can reliably detect as low as single digital copies of target DNA. Our experimental data showed that the sensitivity of the dual primer PCR system is comparable to the two-tube nested PCR but the new system reduces the potential risk of cross contaminations commonly associated with conventional nested PCR. We compared this new diagnostic system with the standard TaqMan PCR system using field-collected samples. Our results showed that the new detection system can detect 5-10 % more positive, most of them contained 100 or fewer copies of target DNA. This system is versatile. It can also be used for a standard TaqMan® quantitative PCR if only the inner primers and TaqMan® probe are included, or as conventional gel-based PCR if only the outer primers are used. This STDP TaqMan PCR provides gel free, reduced hands-on time and is cost effective while significantly improving sensitivity, reliability and high throughput capability suitable for routine use in large scale epidemiological studies.

SSR Marker Development: This part of research has been collaborated with Dr. Neil Gudmestad's group. Dr. Amine Wen prepared ZC DNA samples and assisted SSR primer screening. Eight SSR DNA markers have been developed and validated for Lso genetic analysis. Using this set of SSR markers, a genotypic profile of 68 Lso isolates was developed. These data represent isolates collected from eleven regions including the US and Mexico. The total allelic numbers from eleven locations range from 3 to 16 indicating polymorphic detection power of these SSR markers (Figure 1). Genetic analysis of Lso populations identified two distinct clonal complexes of Lso associated with US ZC populations. Analysis of genotypic data presented amongst Lso populations allowed us to make inferences about the hypothesis that US's Los populations could be derived from two founders (Figure 2). The panel of markers we introduce here will be useful for future population studies of ZC and may also aid in the identification of segments of the Lso chromosome associated with bacterial virulence.

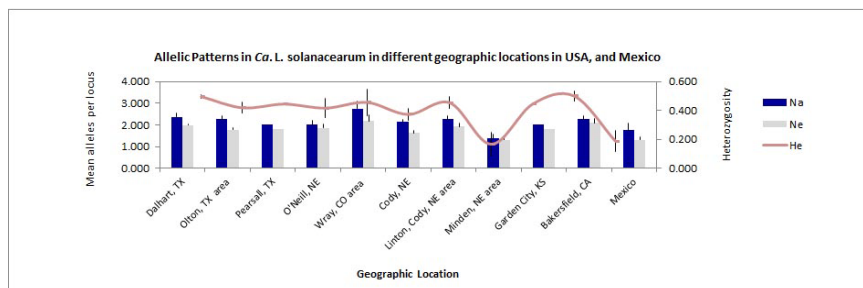


Figure 1 Allelic Patterns of Lso in 11 different geographic locations in the United States and Mexico

* Na, number of alleles per locus; Ne, number of effective alleles per locus; He, genetic diversity

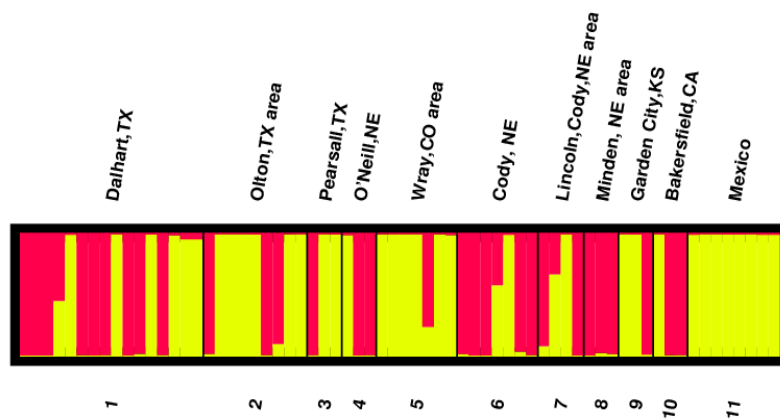


Figure 2 Individual assignments of Lso (collected from 11 different geographic locations in the United States, and Mexico) by STRUCTURE analysis; there were two clusters (K). Black lines within the squares distinguish geographic locations.

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A QUICK AND INEXPENSIVE EXTRACTION METHOD FOR EPIDEMIOLOGICAL AND DIAGNOSTIC TESTING OF *C. LIBERIBACTER*

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Summary

A new method for extraction of “*Candidatus Liberibacter solanacearum*” (Lso) from a single psyllid was created using FTA technology as an inexpensive and convenient alternative to DNA extraction kits. Twenty adult and nymphal psyllids were collected from Lso infected plants and extracted using the standard DNeasy kit from Qiagen (Valencia, CA) and twenty were extracted using a Whatman Indicating FTA Micro Card (Piscataway, NJ). Composite samples of ten adult and ten nymphal psyllids were also extracted and then aliquoted to create ten DNeasy kit extractions and ten FTA extractions to evaluate differences between extraction methods. Extracted samples were tested for the presence and quantification of Lso using absolute quantification real-time PCR. A normal distribution plot and *t*-test were then conducted on the Ct values to detect differences between the kit and FTA extraction methods using SAS software (SAS Institute Inc, Cary, NC). The results of the single adult and nymphal psyllids demonstrated a large amount of variation between single psyllid samples for both the kit and FTA extraction methods. Composite sample Ct values were determined to be lower for the kit extractions when compared to the FTA extractions for both the adult and nymphal stage psyllids. The Ct values for both the kit and FTA extractions showed a normal distribution around the mean when plotted. Lower Ct values for the kit extractions demonstrate that the kit extractions were more efficient than the FTA extraction. However, the FTA method is reliable in extracting Lso DNA from a single infested psyllid. All of the composite samples were determined to be positive and displayed a normal distribution around the mean showing that the FTA extraction method could be used as a convenient and inexpensive extraction technique.

Introduction

Zebra chip (ZC) disease of potato is an important disease affecting potato production. Zebra chip is caused by the putative pathogen “*Candidatus Liberibacter solanacearum*” (Lso), which is vectored by the potato psyllid (*Bactericera cockerelli*). The disease causes leaf proliferation and scorching as well as internal tuber necrosis making them unusable for potato chip production (Munyaneza, et. al. 2007; 2010). Zebra chip diagnosis and its epidemiological studies involve detection of the pathogen not only within its host but also within its vector. Studies conducted on Lso often involve tedious collections of whole plants and tubers to be processed in the laboratory. Vector collections also involve complicated preserving and packaging of numerous bottles and preservatives. Detection of *Liberibacter* sp. within single psyllids has been reported using Qiagen DNA extraction kits and real-time PCR (Inoue, et. al. 2009); however, for diagnostics and epidemiology studies that require large numbers of samples, use of commercial kits can be quite expensive. Therefore, a new inexpensive method of Lso detection within a single psyllid was developed utilizing Whatman FTA technology.

Materials and methods

Psyllid collection and kit extractions. Adult psyllids were collected from a Lso-positive colony on potato plants in the greenhouse and placed in 75% ethanol and stored at -20C until processing. Before DNA extraction twenty single adult psyllids were removed from the 75% ethanol and allowed to dry, then placed in individual tubes and frozen in liquid nitrogen for processing. Twenty single nymphs were also collected and placed directly into individual 2.0 ml centrifuge tubes and frozen with liquid nitrogen and then placed at -20C. Both the adult and nymphal psyllids were homogenized with a 5mm stainless steel bead for 30 sec. using a Talboys High Throughput Homogenizer (Thorofare, NJ). Total DNA extraction was conducted using a DNeasy® Blood and Tissue Kit from Qiagen (Valencia, CA) following the manufactures standard protocol. Both adult and nymphal psyllids were also collected from a Lso-negative colony and extracted using the same protocols as above.

Single Psyllid FTA extractions. Twenty single adult psyllids were removed from the 75% ethanol and allowed to dry. Individual psyllids were then placed on a Whatman Indicating FTA Micro Card (Piscataway, NJ) covered with parafilm and then gently pressed into the FTA matrix using a pipette tip. The parafilm was then removed from the card along with the exoskeleton of the psyllid and dried at room temperature for 1 hour before processing. The same procedure was also employed in pressing twenty individual nymphal stage psyllids collected directly from infected leaf tissue. The sample area was then removed from the matrix card using a Whatman 2.0 mm Harris Punch and placed in a 1.7 ml centrifuge tube. Sample disks were then washed twice with 200µl of Whatman FTA Purification Reagent and incubated for 5 min at room temperature. The FTA purification reagent was then removed and the disks were then washed twice with TE⁻¹ buffer (10mM, Tris-HCl, 0.1M EDTA, pH 8.0) by incubation at room temperature for 5 min. The TE⁻¹ buffer was then removed and the disks were dried using a Savant DNA Speed Vac. (Thermo electron corp., Waltham, MA) for 15 min. DNA was then eluted from the disks by adding 35µl of (0.1N NaOH, 0.3mM EDTA, pH=13.0) and incubating at room temperature for 5 min. After incubation 65µl of (0.1M Tris-HCL, pH=7.0) was added. The solution was flash vortexed three times to mix and then incubated at room temperature for 10 min. After incubation the sample was flash vortexed to finish elution of genomic DNA. Samples were then stored at -20C until testing by real-time PCR.

Composite sample psyllid extractions. Ten adult and ten nymphal stage psyllids were placed into two separate 2.0 ml centrifuge tubes with a 5 mm stainless steel bead and frozen with liquid nitrogen. The composite psyllid samples were then homogenized as described earlier and 84µl of the first buffer used in the Dneasy® Blood and Tissue Kit (buffer ALT) and 8.9 µl of proteinase K was added to the homogenate. The samples were then incubated at 65C for 2hr. vortexing every 30 min during incubation. The composite sample was then split to create 10 kit extractions and 10 FTA extractions using 4 µl of homogenate for each kit and FTA extraction sample. To complete the kit extractions 176µl of buffer ALT was added to the 4 µl of composite sample homogenate and then the extractions were continued using the standard protocol. For the FTA composite sample extractions 10 samples of 4µl of composite sample ALT mixture was added to the FTA card matrix and allowed to dry for

1hr. The entire area of the card was then removed using a sterile scalpel and the DNA was eluted using the above protocol for FTA extraction.

Real-time PCR for Lso. A Prism 7000 Sequence Detection System from Applied Biosystems (Carlsbad, CA) was used for amplification of Lso. The 16s ribosomal region of *Liberibacter* was amplified using primer ZCF (Wen, et. al. 2009) and primer/probe HLBr/HLBp (Li et al. 2006). Working primer concentrations of 50nM and probe concentrations of 250 nM were used in each reaction. The reaction was conducted using Universal Master Mix Real-time PCR Kit (ABI) and 4µl of sample extraction was added to each reaction totaling 25µl. Absolute quantification was conducted using standards of *Liberibacter* genomes of 3M, 300K, 30K, and 3 thousand genomes. Reaction parameters for detection were set as 95C 10min, and 40 cycles of 95C for 15 s and 58C for 40 sec.

Analysis. Adult and nymphal single psyllids were tested and quantified to determine if the FTA method of extraction could be used as an alternative method to the Dneasy kit extractions. The maximum and minimum quantifications were then determined for each single vector sample set and recorded. Composite samples for the adult and nymphal psyllids were evaluated by determining the mean and standard deviation (STDEV) of the sample Ct values. Ct values were then plotted to determine if the sample values were normally distributed and *t*-test (SAS Institute Inc, Cary, NC) was conducted on the composite sample data to determine significance between the kit and FTA extractions. Composite sample tests were replicated for the experiment.

FTA extraction of Lso positive tuber tissue. A small tuber approximately 3cm wide found to be positive for Lso was squashed on the FTA card matrix. The card was allowed to dry for 1hr and then DNA was eluted from the matrix following the same protocol as shown earlier with the addition of two washes with 100% isopropanol between the FTA purification wash and the TE⁻¹ wash. Sample were then eluted and tested by real-time PCR as described before.

Results and discussion

Single psyllid kit and FTA extraction test. Twelve out of the 20 single adult psyllid samples tested were found to be positive for Lso using the FTA method and 15 out of 20 for the kit extractions. Maximum and minimum values for positive adult psyllids were 4.07×10^5 and 1.07×10^4 and 8.94×10^6 and 1.75×10^4 genomes for the FTA and kit extractions, respectively. The number of samples determined to be positive for the nymphal psyllids were 10 out of the 20 for the FTA and 15 out of 20 for the kit extraction method. Nymphal psyllids tested were

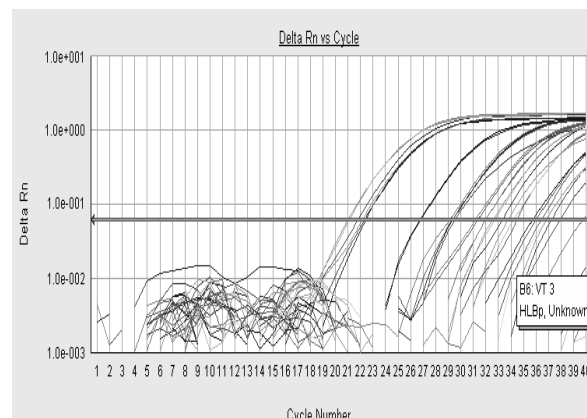


Fig. 1. Single adult psyllid samples tested using real-time PCR

determined to have maximum and minimum values of 4.2×10^3 and 1.28×10^1 for the FTA and 3.94×10^5 and 1.62×10^1 genomes for the kit extractions. A large amount of variation was found between single psyllid samples for both the adult and nymphal stages (Fig. 1). Similar variations were also observed in colonies from which the psyllids and nymphs were collected (Rush, et. al. 2010). It was also found that nymphal stage psyllids had a lower bacterial level than adult psyllids. This was also found during studies involving “*Candidatus Liberibacter asiaticus*” in adult and nymphal stage *Diaphorina citri*, where bacterial quantity was lower in the nymphal stage psyllid and increased with age which also increased transmission efficiency (Inoue, et. al., 2009).

Composite adult and nymphal psyllid extractions. Average Ct values for the first and second rep. were 30.2 STDEV 0.06 and 28.1 STDEV 0.09 for the FTA and kit extractions and 25.4 STDEV .32 and 22.9 STDEV 0.24 respectively for the composite adult psyllids. The composite nymphal psyllids were found to have an average Ct value of 30.9 STDEV 0.09 and 28.8 STDEV 0.05 for the FTA and kit extractions and 28.7 STDEV 0.41 and 26.1 STDEV 0.41 for the first and second rep., respectively. All the composite samples for the adult and nymphal psyllids were found to have normal distributions around the mean Ct value and were significantly different ($P < 0.001$) when comparing the FTA and kit extractions for both reps (Fig. 2). Although, the kit extractions were found to have a lower Ct value than the FTA exactions, all of the composite samples were found to be positive for Lso and displayed a normal distribution around the mean. This shows that even though the kit extraction method is slightly more efficient the FTA method is a reliable an inexpensive alternative. This method was also successfully used for detection of Lso in infected tubers and will decrease the need for packaging and expensive shipment of tubers for diagnostics.

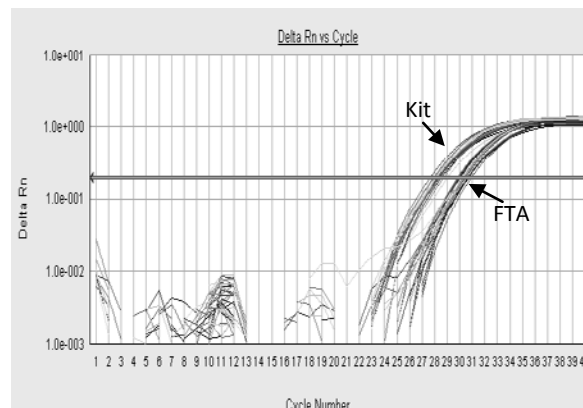


Fig. 2. Composite adult psyllid samples for the kit and FTA extractions.

Concluding remarks

- The FTA extraction method can be used for quick identification of *Liberibacter* titer within a single vector and tuber tissue.
- FTA cards can be used for field diagnostics and collections.
- The use of FTA extractions creates new options for collection strategies dealing with epidemiological studies and diagnostics.

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NEW APPROACHES FOR DEVELOPING AND IMPROVING PCR DIAGNOSTIC METHODS FOR THE ZC PATHOGEN

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Summary

The 16-23S rDNA intergenic region was used to design the Lso TX 16/23 primer set for improved PCR detection of the zebra chip (ZC) pathogen, '*Candidatus Liberibacter solanacearum*' (Lso), in potato and psyllid samples. The Lso TX 16/23 primers were specific for Lso, amplifying a 383 bp DNA band that was not present in DNA isolated from '*Ca. Liberibacter asiaticus*' (Las). Field-grown potato samples from Texas, Kansas, and Nebraska that exhibited ZC disease symptoms were used to evaluate performance of the new Lso TX 16/23 primers as compared to the standard LsoF/OI2c primers used for Lso detection. The Lso TX 16/23 primer set detected the presence of Lso in about 20% more ZC field samples as compared to the LsoF/OI2c primers. Studies on the development of a loop-mediated isothermal amplification procedure (LAMP) for an improved, simplified PCR-based detection system that can be used in the field are in progress. LAMP shows promise as a rapid and less expensive method for detection of the ZC pathogen in the field.

Introduction

Zebra chip disease of potatoes poses a major economic threat to potato production in Texas and is spreading to other potato-growing regions of the USA. Recently, it was demonstrated that ZC is caused by a phloem-limited bacterium identified as Lso that is transmitted by the feeding of the potato psyllid (1, 3, 8, 10). Accurate pathogen detection is essential for developing strategies useful to growers that will help forecast the presence of the pathogen and improve control strategies to reduce costs. The detection of the ZC pathogen in potatoes by existing PCR protocols can be difficult due to low titer and uneven distribution of Lso in plants. The PCR protocols currently available for detection of Lso are based on the use of primers designed from the 16S rDNA sequence of Lso (1, 3, 5, 8, 10). Although the Lso 16S rDNA sequences are useful in identifying *Liberibacter*, they share 96% identity to Las and other species of *Liberibacter* and, therefore, lack specificity. Consequently, PCR primers are needed with improved specificity to distinguish between species and strain populations of Lso associated with potatoes and the psyllid vector.

Another PCR method for pathogen detection is called LAMP, which has the advantage of being both economical in cost and highly sensitive (6, 7). LAMP technology is a relatively new development in diagnosis and has found success in medical laboratories for rapid detection of bacteria in specimens from humans (4). Thus, the development of LAMP technology for Lso detection promises to be more specific than the conventional or real-time PCR methods currently used for pathogen detection. The LAMP technology is isothermal, and this permits LAMP to be used in low-tech laboratories and field stations.

This report describes the development of the Lso TX 16/23 primer set based on the 16-23S rDNA intergenic region of Lso. The primer set was demonstrated to be more reliable and sensitive than existing PCR primer sets based on analysis of ZC potato field samples

from Texas, Kansas, and Nebraska. A progress report is given on the development of LAMP PCR detection of the Lso bacterium in ZC-infected potato plants and tubers.

Materials and Methods

Potato plants showing ZC symptoms were collected from commercial fields in Texas, Kansas, and Nebraska (Table 1). Plant DNA was extracted from the different parts of potato tubers, roots, stems, petioles and leaves of ZC symptomatic and healthy plants. Total DNA was extracted from different plant parts of the sample from the field using a modified method of Doyle and Doyle (2). Samples of Las DNA from citrus were obtained from N. Wang at the University of Florida.

Table 1. Sampling of ZC-infected potato plants collected in Texas, Kansas and Nebraska

Field Locations	Number of Samples Collected Per:					Total
	Tuber	Root	Stem	Petiole	Leaf	
Bushland, Texas	14	0	5	1	5	25
Weslaco, Texas	2	0	9	0	9	20
McAllen, Texas	3	3	3	0	3	12
Spring lake, Texas	6	0	6	0	6	18
Olton, Texas	3	0	3	0	3	9
Dalhart, Texas	10	10	10	0	6	36
Garden City, Kansas	5	0	5	0	5	15
Bridgeport, Nebraska	6	0	2	0	2	10
Greenhouse psyllid-exposed plant	11	2	6	2	5	26
Greenhouse psyllid-free plants	3	3	3	3	3	15
Total	63	18	52	6	47	186

The ‘*Ca. L. solanacearum*’ specific primer was designed based on conserved 16S-23S rDNA of Texas isolates of Lso and the closely related species Las, ‘*Ca. L. americanus*’, and ‘*Ca. L. africanus*’). The nucleotide sequences were compared by multiple alignments using CLUSTAL X (9). The aligned sequences with the variable 16-23S intergenic rDNA region were identified among the isolates of Lso. Those variable regions were used for designing the forward and reverse primers specific for Lso, designated as Lso TX 16/23 F and Lso TX 16/23 R. The designed forward and reverse primers were subjected to *in silico* validation and analysis, including T_m values, percent GC, stem loop formation, and self complementary (primer dimer) within the sequence using the Oligonucleotide Properties Calculator (www.basic.northwestern.edu/biotools/oligocalc.html).

Two primers defined from the sequence of 16-23S rRNA sequence of *Ca. L. solanacearum* were custom synthesized (IDT Incorp, USA) and validated by PCR. They have the following sequences: Forward primer Lso TX 16/23 F (5'-AATTTTAGCAAGTTCTAAGGG-3'); reverse primer Lso TX 16/23 R (5'-GGTACCTCCCATATCGC-3'). PCR amplification was performed in 25-μl reactions containing 1X PCR buffer, 0.5 μM of each of the primers, 200 μM of each of the four dNTPs, 0.5 U of Phusion polymerase (Finnzyme), and 100 ng of DNA extract was used as template in the reaction. The thermocycling conditions consisted of an initial denaturation at 98°C for 30 s, then 35 amplification cycles of 98°C for 10 s, 55°C for 20 s, 72°C for 30 s and a final polymerization step of 72°C for 7 min. The PCR product was resolved in 0.8% agarose gel, excised and purified with a gel purification kit (Promega). DNA sequencing was

performed and sequences were subjected to a BLAST analysis; nucleotide sequence similarities were determined by the closest match within the NCBI database. The LsoF/OI2c primers specific to the 16S rRNA of Li et al. (5) were used in comparisons to the Lso TX 16/23 F/R primer set with the expected product sizes of 1,173 bp and 383 bp, respectively. The DNA extracted from ZC infected plant was quantified and adjusted by serial dilution to 20 ng, 10 ng, 5 ng, 2.5 ng, 1.25 ng, 0.65 ng, 0.33 ng to assess the sensitivity of the PCR primers. Control reactions were DNA from healthy potato stems and sterile distilled water alone.

A set of four primers for LAMP was designed to target the 16-23S rDNA gene Lso. The LAMP reaction was performed in a reaction mixture of 25 µl, containing 40 pM of each of FIP and BIP, 10 pM of each of F3 and B3, 2.8 mM of each dNTP, 1.6 M of betaine, 40 mM of Tris-HCl (pH 8.8), 20 mM of KCl, 20 mM of (NH₄)₂SO₄, 16 mM of MgSO₄, 0.2% Tween 20, 1 µl of 8 U Bst DNA polymerase, and 100 ng DNA. The reaction mixture was incubated at 60°C for 1 h and then at 80°C for 5 min to terminate the reaction. LAMP products were separated by agarose gel electrophoresis and stained with ethidium bromide for visualization.

Results and Discussion

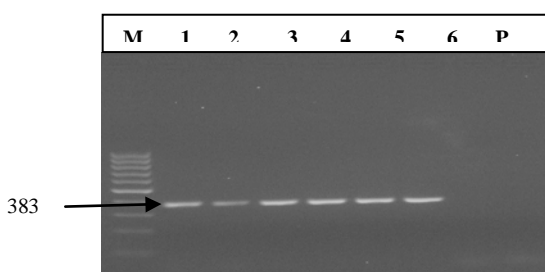


Fig. 1. PCR amplification and detection of a 383 bp band from ZC-infected potato plants using the Lso TX 16/23 primer set. Lanes 1 and 2, tuber samples; lanes 3 and 4, stem; lanes 5 and 6, leaf; lane P, healthy plant DNA; lane N, negative water control.

The primers, Lso TX 16/23 F and Lso TX 16/23 R designed for Lso was used to detect the ZC pathogen and yield an expected amplicon size of 383 bp (Fig. 1). The Lso TX 16/23 F/R species-specific primer set amplified the expected band size of 383 bp only in Lso-infected plants. The Lso TX 16/23 primers amplified only non-specific bands with Las DNA and were much larger than the expected product size (Fig. 2).

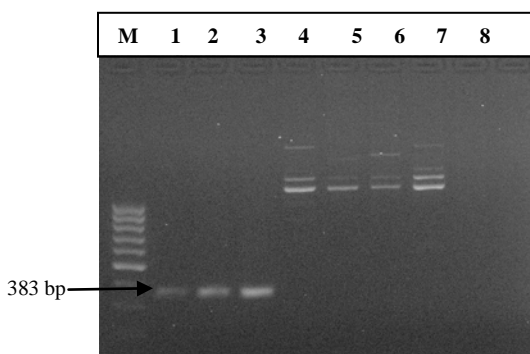


Fig. 2. Specificity of the Lso TX 16/23 species-specific primers for detection of Lso DNA versus Las DNA. Lane 1, ZC infected potato leaf; lane 2, ZC infected potato root; lane 3, ZC infected potato stem; lanes 4 to 7, Las DNA from Florida; lane 8, Las DNA from India; lane N, water control.

The sensitivity of the Lso TX 16/23 F/R and LsoF/OI2c in amplifying Lso DNA differed markedly as shown in Fig. 3. Lso TX 16/23 F/R amplified a 383 bp band at a lowest

DNA concentration of 0.65 ng, whereas the LsoF/OI2c primers using the same DNA samples showed amplification at a threshold of 2.5 ng of DNA.

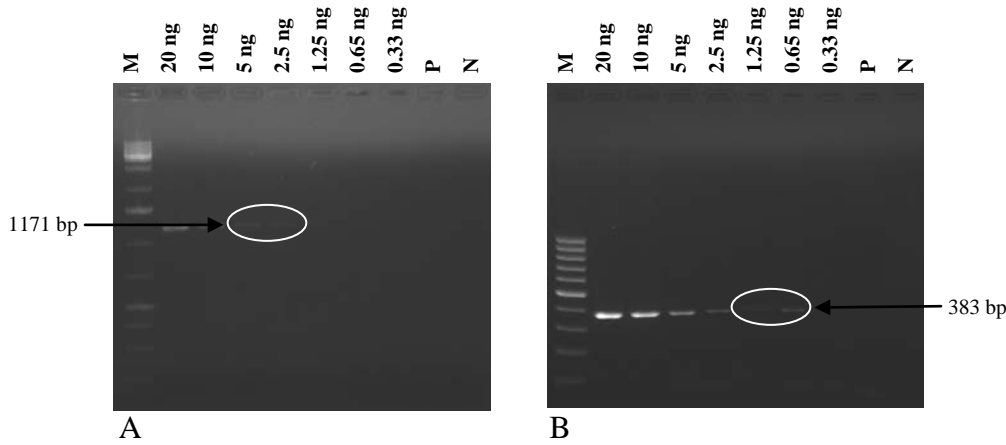


Fig. 3. Evaluation of the sensitivity of the LsoF/OI2c (A) and Lso TX 16/23 F/R (B) primers for PCR detection of Lso DNA diluted from 20 to 0.33 ng.

The Lso TX 16/23 F/R primers showed about 20% more positive results for Lso in plants with ZC symptoms than the LsoF/OI2c primers (Table 2). For example, the Lso bacterium was detected in DNA extracted from 60% of the tuber samples with the LsoF/OI2c primers versus 75% with the Lso TX 16/23 F/R primers; DNA extracts from the greenhouse-grown and psyllid-free plants yielded no products with either primer pair.

Table 2. Comparison of PCR detection of Lso from ZC infected field samples using the standard LsoF/OI2c and the newly developed Lso TX 16/23 F/R primer pairs

Plant Parts Sampled	LsoF/OI2c Primer Set	Lso TX 16/23 F/R Primer Set
Tuber	36/60 (60.0%) ^a	45/60 (75.0%) ^a
Root	7/15 (46.6%)	11/15 (73.3%)
Stem	27/49 (55.1%)	34/49 (69.3%)
Petiole	3/3 (100%)	3/3 (100%)
Leaf	11/44 (22.0%)	26/44 (59.1%)
Lso Detection Total	84/171 (49.1%)	119/171 (69.5%)

^aNumber of positive/total number (percentage)

Application of the LAMP PCR assay with a specific primer set designed to the 16-23S rDNA intergenic region was successful in amplifying the target gene from Lso DNA from ZC potato samples (Fig. 4B). In comparison to conventional PCR (Fig. 4A), the LAMP PCR primers amplified two additional samples of ZC-infected tubers; LAMP PCR was concluded to be as effective as conventional PCR for Lso diagnosis (Fig. 4). The LAMP products are detected as a ladder of multiple bands on the gel due to the formation of a mixture of stem-loop DNAs with various stem lengths (6).

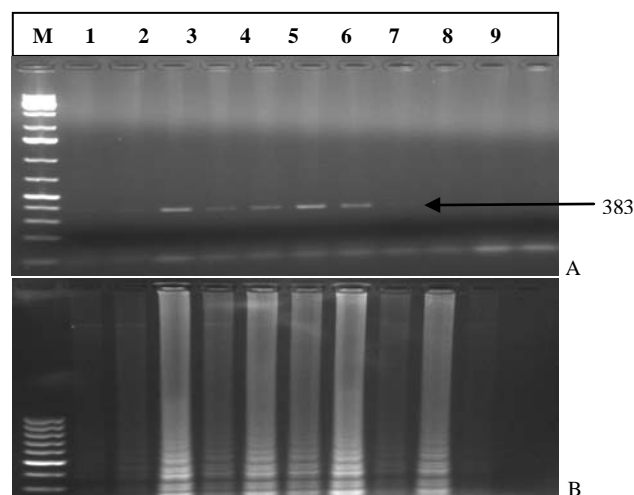


Fig. 4. Comparison of conventional PCR (Lso TX 16/23) (top) and LAMP PCR (bottom) for detection of Lso from ZC-infected potatoes. Lane M, DNA markers; Lanes 1 and 2, leaves; lanes 3 and 4, stems; lanes 5 to 10, tubers; lane N, water control.

A future goal is to develop LAMP primers to Lso housekeeping genes to improve specificity and sensitivity. Improved PCR detection protocols are needed to quantitatively determine differences in Lso populations, and to evaluate their distribution and population size in potato cultivars that vary in ZC tolerance.

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TRANSLOCATION OF *CANDIDATUS LIBERIBACTER SOLANACEARUM*, THE ZEBRA CHIP PATHOGEN, IN POTATO AND TOMATO

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Summary

Understanding the rate and direction of translocation of the zebra chip pathogen “*Candidatus Liberibacter solanacearum*” (Lso) in plants is critical for improving field sampling for early detection as well as understanding differences between tolerant and susceptible potato varieties in disease development. Here we look at translocation patterns of Lso in tomato and potato and examine whether rate and direction of translocation varies by species or even between tolerant and susceptible potato varieties. The goal of this work is to provide growers and researchers with a better understanding of pathogen movement as an aid in the development of detection and variety screening methodologies.

Impact

Information obtained in this study can immediately provide growers and researchers with a better understanding of pathogen movement in potatoes as an aid in the development of improved detection and variety screening methodologies.

Introduction

The work reported here covers some of the first year accomplishments from the project entitled: Development of New Technologies for Detection and Visualization of the zebra chip (ZC) Pathogen and Application for Improved Field Detection and Identification of Selectable Markers for Improved Disease Resistance (PIs: Pierson and Gross). The two primary objectives of this proposal are to: *I. Develop improved technology for detection of “Candidatus Liberibacter solanacearum” (Lso) in potato and psyllids and II. Use Polymerase Chain Reaction (PCR) and Fluorescent In Situ Hybridization (FISH) for the localization and visualization of Lso in potato varieties differing in ZC tolerance.* The ultimate goals of objective II are the improved understanding of disease development with the potential for identifying selectable markers for breeding ZC tolerance.

Understanding the direction and rate of translocation of Lso in plants is critical for knowing when and where to sample plants in the field for early detection. Especially as previous work has demonstrated that PCR detection results are notoriously unreliable and vary depending on the length of time after insect feeding the plant is sampled and the type of tissue sampled. Understanding the direction and rate of translocation of Lso in plants also is critical for knowing whether there are differences between tolerant and susceptible varieties in pathogen movement and when and *in what tissues* to look for differences in plant tissue-microbe interactions that may be the key to understanding disease tolerance.

Translocation patterns of phloem-limited viruses are somewhat well understood. As summarized in Agrios (1, adapted from 2), when a virus infects a plant it moves from cell to

cell via the plasmodesmata. Movement is generally about 1 millimeter/day and thus movement from the site of infection on a leaf just to that leaf's petiole may take 2-5 days. Once the virus enters the phloem, movement may proceed rapidly and the direction of movement follows source to sink patterns of carbohydrate translocation in the plant. For example, after inoculation of a leaf on a lower tier branch, the translocation stream will direct movement of the virus toward the nearest and largest carbohydrate sink, this being the rapidly respiring root system. From there carbohydrates are typically translocated to the top of the plant to provision the newly developing leaves. The virus spreads systemically through the plant following this general pattern of source to sink movement ultimately spreading down from the top leaves to infect mature leaves. It may take up to 25 days for the virus to spread to all mature leaves on the developing plant, however the titer of the virus may vary substantially.

We suggest that if Lso is being translocated in the phloem at a rate and in a manner similar to viruses, this may explain some of the variability in PCR detection results seen when different plant samples (mature leaf, young leaf, stem, root, and tuber) are collected at different time points after insect infestation. A typical sampling strategy might be to observe a plant for insect damage and collect for sampling the closest undamaged mature leaf (often the one just above the damaged leaf). According to what has been observed for virus translocation, this sample is probably the last place you would expect to find the pathogen. The virus may not be present in sufficient titers for detection in a nearby mature leaf for over 3 weeks post inoculation!

Here we ask the questions: Does the Lso pathogen move in a predictable manner? Does the direction and rate of translocation vary by species (e.g. tomatoes vs. tuber forming potatoes)? Does direction and rate of translocation vary by tolerant vs. susceptible potato varieties?

Materials and Methods

Insect material: Potato psyllids (*Bactericera cockerelli*) were maintained on caged tomato or potato. The two different populations of insects: the C1 colony (0% of the insects harbor Lso) and the C2 colony (~70% of the insects harbor Lso) were maintained separately and have been described elsewhere (Nachappa et. al 2010, submitted).

Translocation in tomato: Tomatoes variety "cherry red" were grown from seed in 5 inch pots in Metromix 300 (Sun Gro Horticulture). The plants were grown on light shelves with 24 h light. Approximately four weeks after planting, when the plants had developed multiple (6) tiers of branches, we introduced the psyllids within a small clip cage ('clip cage 1 inch internal diameter foam no thrips', BioQuip, Rancho Dominguez, CA) attached to a single leaf on a *middle tier* branch. For this experiment, we had two treatments: plants infested with two adult psyllids from either a "hot" colony (~70% of the insects harbor Lso), or a "cold" colony (0% of the insects harbor Lso). The cold colony serves as a symptom control to help discriminate symptoms of insect feeding from ZC. The insects were allowed to feed for one week. We then removed the insects and progeny by removing the entire leaf and petiole hosting the insects. Subsequently, at one week intervals, *one* leaf from a top, middle, or bottom branch was sampled. DNA was extracted from the petiole tissue of each leaf. The presence of Lso was examined via PCR using the specific primers LsoTX16/23.

Amplification of β -tubulin was used to verify the quality of the DNA extraction (e.g. if β -tubulin amplified, but the PCR results were negative for the pathogen we would conclude

this negative result was not due to the DNA extraction protocol). DNA extracted from a plant known to have ZC disease and from a plant with no exposure to insects were included as positive and negative controls, respectively.

Translocation in potato: The potatoes were grown from spouting tubers in 2-gallon pots in Metromix 300 on light shelves with 12:12 h dark: light cycles. For each tuber, we allowed two shoots to develop. For this experiment, we had two treatments: susceptible (Norkotah or Atlantic) versus tolerant (NY138 or BTX1749) varieties. Potato varieties were provided by Dr. Creighton Miller's Texas Potato Breeding Program. "Tolerant varieties" were selections that had proven to be ZC tolerant in multiple field trials (Creighton Miller, personal communication). Four to five weeks after planting (before tuber formation), *one shoot* per plant was infested with 2 adult insects from the "hot" colony (~70% of the insects harbor Lso). The insects were maintained on a single leaf within clip cages as described above. This protocol not only forced insects to feed on a leaf from a specific tier of branches, but forced insects to feed on both tolerant and susceptible varieties, removing insect preference from the comparison. As for tomatoes, at one week intervals, *one* leaf from a top, middle, or bottom branch of *both stems* was sampled. DNA was extracted from the petiole tissue of each leaf. The presence of Lso was examined via PCR using the specific primers LsoTX16/23 and amplification of β -tubulin was used to verify the quality of the DNA. Appropriate controls were used.

DNA extraction, PCR assay: A sample of a leaf petiole with central vein tissue about 1.5 cm long was excised from the selected leaves and DNA was extracted as describe previously by Meyerowitz et al.

(http://web.archive.org/web/20030830084603/www.its.caltech.edu/~plantlab/protocols/quick_dna.html). Briefly, samples were homogenized using a sterile mortar and pestle in 500 μ l extraction buffer (100mM Tris pH 8, 50mM EDTA, 500mM NaCl, 10mM β -mercaptoethanol). The extract was transferred to a 1.5 ml tube and 35 μ l of 20% SDS were added before incubating at 65°C for 5 minutes. Following incubation, 130 μ l of 5M potassium acetate was added, mixed thoroughly and incubated on ice for 5 minutes. The mixture was centrifuged at 15,000 g for 10 minutes. The supernatant was recovered and the DNA was precipitated by adding 640 μ l isopropyl alcohol and 60 μ l 3M sodium acetate and held at -20°C for 10 minutes. The DNA was washed by precipitating in 70% ethanol with centrifugation and resuspended in 40 μ l of water supplemented with RNase (Invitrogen) at 1 μ g/ml. The PCR primers for β -tubulin amplification were (F: TGCCACTCACTTGGTGGAGGG; R: TCATGTTGCTCTCGGCTTCAGTG. For Lso amplification we used primers LsoTX16/23 (Aravind et. al, in prep) designed to amplify a 383 bp region between the 16s and 23s rDNA genes. Primer sequences of Lso TX 16/23 F and Lso TX 16/23 R are 5'-AATTTTAGCAAGTTCTAAGGG-3' and 5'-GGTACCTCCCATATCGC-3', respectively. PCR reactions were prepared with GoTaq[®] Colorless Master Mix according to manufacturer recommendations. The PCR was performed using an Eppendorf Thermocycler (95°C for 3min. 40 cycle of 95 °C for 40s, 72 °C for 40s and 72 °C for 1min, 72 °C for 10 min). PCR products were examined by gel electrophoresis and verified as necessary by sequencing.

Results and Discussion

The results presented here are a brief summary of results described in Levy, et al, in prep.

Translocation in Tomato: After one week of insect feeding, we were not able to detect Lso in any of the leaf petiole samples, not even the infested leaf petiole. By week four, none of the tomatoes (n=6) treated with insects from the hot colony (“hot” plants) were showing symptoms of ZC. Despite the lack of symptoms, we detected Lso in all of the hot plants either just in the top or in all parts of the plant and by week 5 we were able to repeatedly find ZC in all leave. We did not detect Lso on the plants treated with insects from the cold colony (“cold” plants). By week eight the upper leaves of “hot” plants showed symptoms of ZC disease (curling and yellowing). Interestingly, by the time the “hot” plants showed symptoms on the upper leaves, they were infected from top to bottom with Lso. The “cold” plant showed no symptoms and did not have the pathogen.

These results demonstrate that direction and rates of translocation of Lso in tomato were predictable. For example, we were not able to detect Lso until 3-4 weeks after infestation. The pathogen was often detected first in the upper leaves suggesting the newly differentiated leaves were the strongest/nearest sink for carbohydrates produced from leaves located on middle tier branched. Interestingly, we did not detect ZC symptoms until week seven and then starting in the upper leaves. These data suggest that symptomology follows the same pattern as pathogen movement but lags several weeks behind the appearance of the pathogen in that tissue. Further titers of the pathogen apparently were high enough in tomato tissue to give consistent PCR results (e.g. samples that tested positive, stayed positive) throughout the multi-week experiment.

Translocation in Potato: As observed in tomato, we were not able to detect Lso using conventional PCR after one week of insect feeding in any of the leaf petiole samples, not even the infested leaf petiole. In both tolerant and susceptible varieties, we first detected Lso 3-4 weeks after infestation and then the first detection was only in the upper or upper and middle leaves of the infested stem. By week 4, we were able to find the pathogen in *leaves on the non-infested stem* and by week 5 we were able to detect the pathogen in all parts of the plant, but not reliably so (e.g. some weeks leaves in a particular tier would be positive and others they would be negative). These findings suggest that rates of pathogen movement are similar in tomato and potato and more importantly in tolerant and susceptible potato varieties. The direction of pathogen translocation appears to follow source to sink movement of carbohydrates toward the newly differentiating leaves. We recognize that this pattern of movement may be defined by the developmental stage of the potato (e.g. flow of carbohydrates may be both upward and downward to provision the newly developing leaves and tubers, respectively). These results also demonstrate that the pathogen is able to move from an infested leaf on one stem to leaves on a second un-infested stem. However, our results using conventional PCR cannot tell us whether titers of the bacteria develop to the same level in different parts of the plants.

As far as disease symptoms, there was a clear difference between tolerant and susceptible varieties in the timing and rate of disease symptom development. The first ZC disease symptom (purpling of upper leaves) were observed at 3 weeks after infestation on the susceptible varieties of potato (Norkotah and Atlantic), approximately the same time we could detect the pathogen and 5 weeks earlier than we observed disease symptoms in tomato. By 4 weeks the entire infested stem of the susceptible plants was symptomatic (yellowing, leaf rolling, leaf death), by 5 weeks was moribund, and by six weeks was totally dead.

Disease symptoms were first observed in the second stem of susceptible plants at 5 weeks (one week after we detected the pathogen) and progressed at the same rate. By Week 8 the second stem of susceptible varieties was typically dead. In contrast, the symptoms developed later in the infested stems of tolerant varieties NY138. For example the symptoms first appeared after 5 weeks as a small region of yellow discoloration on the young upper leaves of the infested stem. At week 6, all plants had striking yellowing patterns symptomatic of ZC on the upper part of the infested stem. By 8 weeks the infested stems of tolerant varieties were dead. Despite the pathogen being observed in the second stems at week 4, symptoms were not apparent until week 7 and for the most part second stems of tolerant plants were still alive 14 weeks after infestation.

These data suggest that although rates of pathogen translocation may not differ between tolerant and susceptible varieties, the rates of symptom development do. Whether this is related to differences between tolerant and susceptible varieties in the titer of bacteria present in different tissues or in their specific response to the presence of the pathogen remains to be determined.

Based on our findings we would recommend that plant sampling for detection of the pathogen include newly developing leaves and that workers keep in mind the fact that it might take up to 3 weeks after insects feed on plants for the bacterial titer to increase to the point where the pathogen can be detected via conventional PCR. We believe that newer methodologies that utilize technologies such as loop-mediated isothermal amplification procedure (LAMP) PCR (Aravind, et al., this symposium) combined with a better understanding of what plant tissues to sample will likely result in significantly better early detection of the pathogen.

Future work will focus on understanding the differences in disease development between tolerant and susceptible potato varieties observed in this study using Fluorescent In Situ Hybridization (FISH) to localize and visualize the pathogen in plant tissues as the pathogen moves through the plant and symptoms develop.

Acknowledgements

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UNDERSTANDING THE WHOLE-PLANT PHYSIOLOGICAL MECHANISMS LINKED TO POSSIBLE TOLERANCE OF ZEBRA CHIP DISEASE

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Summary

This study examined the effects of zebra chip, caused by “*Candidatus Liberibacter solanacearum*” infection, on physiological performance and associated yield parameters of potato grown under varying crop production practices. We evaluated the performance of infected and non-infected potatoes of the cultivar, FL 1867, grown in strip and conventional tillage plots receiving three different levels of irrigation (full irrigation, 70% deficit irrigation, and 70% primed acclimation (70PA), wherein 70% of the full irrigation amount was applied prior to bloom and full irrigation was applied after bloom). Finally, four plant hormone treatments were superimposed upon these other treatments. Two sets of potato plants were infected by caging potato psyllids positive for *Liberibacter*, the first set was infected at full emergence and the second set was infected at bloom. The portion of the study reported on herein, identified and quantified, at the whole-plant level, the physiological changes that occurred after infection, with a focus on carbon assimilation and partitioning for tuber formation and development. Potatoes in the strip tillage plots emerged ca ten days earlier than those in the conventional tillage plots and the strip tillage plots receiving 70% and full irrigation yielded better than those in the conventional tillage. Total tuber weight of potatoes grown under 70PA had greater yields than potatoes receiving full or 70% irrigation. All hormonal treatments improved yields of potatoes grown under 70% irrigation when compared to untreated potatoes and auxins and boron treatments improved yields in the 100% and 70% irrigation treatments. Individual tuber weights were greater for auxins and boron treatments when potatoes were grown under 70% irrigation and individual tuber weights were more uniform in those plots treated with boron. These data suggest that some of these treatments have the potential to improve potato yields while increasing crop water-use efficiency or to optimize uniformity of tuber size.

Introduction

Because of the potential rapid spread of zebra chip disease (ZC) to most potato production regions in the U.S. and the economic devastation associated with it, a complete picture of all aspects of the disease are needed to combat it. The ZC research and extension team assembled at present is providing the needed expertise in most of these areas, but an understanding of the crop’s physiological response under infection could provide excellent information that would complement and support the team. Little is known about how plant physiology, especially related to carbon assimilation, carbon partitioning for tuber formation and development, water-use, and overall plant performance is affected by ZC infection. If physiological mechanisms that maintain plant performance, especially carbon assimilation under infection, could be identified, this information could be used to enhance breeding, crop management tools, and genetic profiling. Symptoms of ZC are seldom observed before flowering, even when infectious psyllids are present in the area, so it is important to

determine if some major change in the plant's physiology after flowering results in a shift from resistance/tolerance to susceptibility. This project investigated whether some agronomic practices might maintain or increase yields of infected potato. The various practices evaluated include conservation tillage, irrigation levels, phytohormones, and timing of ZC infection. Specifically, we attempted to determine if compounding stress factors such as deficit irrigation would affect the rate of infection and the progression of the disease in potato plants. Earlier work of the Texas AgriLife ZC Team indicated a feedback inhibition of photosynthesis in infected plants that was possibly related to decreased phloem transport. Therefore, we also tried to ascertain whether applications of plant hormones and nutrients would have any effect on phloem transport in healthy or infected potato plants and, ultimately, on potato yields. This project identified, at the whole-plant level, the physiological changes that occurred after infection, with a focus on carbon assimilation and partitioning for tuber formation and development.

Materials and Methods

Field-tolerant cultivar FL 1867 potatoes were planted 11 March, 2010 in conventional and strip-till plots measuring 20' long x 13.3' wide (4 rows on 40" spacing) under center pivot irrigation. Three irrigation regimens were used: 70PA (primed acclimation = 70% pre-bloom and 100% after bloom), deficit irrigation (70% season-long) and full irrigation (100% season-long).

Deliberate infection of nine plants per plot was achieved by caging (1467A Sock Enclosure, BioQuip Products) infected psyllids on a single leaf per individual plant for approximately one week followed by psyllid removal using a contact insecticide and cage removal. There were two infection periods for the potatoes, at full emergence (19-26 April) and at bloom (19-24 May).

Four treatments of growth regulators were imposed as foliar sprays on the potatoes subsequent to infection: 1) control – water-only spray, 2) auxins (IBA + NAA) (Dip'n Grow, Inc., Clackamas, OR), 3) boron (Tracite® Liquid Boron, Helena Chemical Company, Collierville, TN), and 4) Gibberellic Acid (GA) (Pro-Gibb 4%, Abbott Laboratories, North Chicago, IL). A single foliar application of two growth regulators, auxins (IBA @ 10 ppm + NAA @ 5 ppm) and boron @ 2,000 ppm, was applied shortly after psyllid cages were removed for the second infection period. Three applications of GA, each at 20 ppm, were applied at 7- to 10-day intervals.

Subsequent to infection the following physiological measurements were conducted on healthy and infected plants: SmartCrop® infra-red canopy temperature, photosynthesis and gas exchange with a LiCor 6400, chlorophyll fluorescence (Opti-Sciences), SPAD chlorophyll content, leaf temperature, Multiplex® leaf pigment content, NDVI (canopy "greenness" - Greenseeker), Sap flow = directly measured stem water flow, on a weekly basis. Due to time constraints imposed by physiological measurements, physiological performance of potato plants was measured only for plants from the two center rows in the conventional tillage plots under 70% and 100% irrigation and treated with GA and water-only spray.

Infected and healthy plants were destructively harvested at INFECTION MAX and infection status for those plants was verified with real time PCR analysis using four tissue types from the harvested plants. Those tissues were upper portion of the tap root, crown tissue just above the soil line, the leaf on which physiological measurements were made and the leaf-petiole tissue immediately below the leaf blade. Potatoes from infected and healthy plants were dug from each plot and potatoes were weighed for estimates of yield. Total tuber weight and mean individual tuber weight measurements were recorded for the various treatments. Visual ratings of plants were made for apparent infection and fry tests for final determination of infection were conducted on a subsample of potatoes from each infected plant and from healthy plants.

Results and Discussion

Potatoes in strip tillage plots emerged approximately ten days earlier than those in the conventional tillage plots. Also, under the 70% and 100% irrigation regimens, potatoes in strip tillage yielded significantly better than the conventional tillage plots, with greater yields achieved in the 70% irrigation than in the 100%. However, potatoes in the strip tillage plots that received 70PA irrigation yielded significantly less than those in the conventional tillage plots. A possible explanation for this reduction is that the 100% and 70PA (= 100% irrigation after bloom) may have experienced overwatered conditions due to a greater water-holding capacity of the soil in those plots.

When evaluating the effects of hormonal sprays on total tuber weights, potatoes under 70PA in the untreated control plots had significantly greater yields than potatoes receiving 100% and 70% irrigation. All hormonal treatments (auxin, boron and GA) improved yields under 70% irrigation when compared to the untreated control; auxin and boron treatments significantly improved yields compared to the untreated control in both the 100% and 70% irrigation treatments while GA improved yields under 70PA when compared to 70% irrigation. These results suggest that plant hormones might offer some yield maintenance or enhancement potential in operations where irrigation water efficiency or water reduction is important.

No adverse effect on individual tuber weight was observed for potatoes grown under 70PA irrigation when compared to 100% irrigation. Auxin and boron significantly improved individual tuber weights for potatoes grown under drought stress (70% irrigation) and there was a large observed numerical increase in individual tuber weight with GA application but the difference was not significant. There also was less variability in individual tuber weight for potatoes treated with boron; this suggests that boron may have the potential to be used in managing for uniformity of tuber size.

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UNDERSTANDING WHOLE-PLANT PHYSIOLOGICAL MECHANISMS OF POTATO RELATED TO ZEBRA CHIP DISEASE

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Summary

This project was focused on identifying, characterizing, and quantifying the physiological responses to zebra chip (ZC) disease and “*Candidatus Liberibacter solanacearum*” infection. This was accomplished by testing the potato variety FL 1867 in a cropping system utilizing strip and conventional tillage with three irrigation levels: full irrigation, 70% deficit irrigation, and a 70% primed acclimation treatment involving 70% application rates until flowering and full irrigation for the remainder of the season. Plants within the conventional tillage and two of the irrigation treatments (70% and 100%) were purposely infested with “hot” psyllids containing *Liberibacter* at two stages: full emergence and at bloom. These treated plants were then measured for physiological function including gas exchange, chlorophyll fluorescence, SPAD chlorophyll reading, infrared canopy temperature, and sap flow. Directly after physiological measurement, plants were collected and divided into four tissue types: root, stem base, leaf base, and first fully expanded leaf and measured with qPCR to quantify ZC infection titre. Infection was significantly different among infection periods and seemed to be related to the amount of water stress the plants experienced. Evidence was found for the movement of the infection from the root crown up the stem, with concomitant decreases in physiological function as the infection progressed. Several of the physiological measurements may serve as detection methods for the progression of ZC in potato.

Introduction

Progress has been made by the Texas AgriLife team and others in elucidating many characteristics of zebra chip (ZC) disease in potato including: vector biology and behavior (Sengoda et al., 2010); epidemiology (Wen et al., 2009); chemical suppression; *Liberibacter* characteristics (Crosslin and Munyaneza, 2009; Munyaneza et al., 2007); and movement of the disease across U.S. regions. However, relatively little research has been conducted examining the physiological responses in the plant to infection by *Liberibacter*. Our project was aimed at quantifying the physiological responses to ZC progression and elucidating possible mechanisms that could impart tolerance to the disease in a potato cultivar. Our specific objectives included: 1) determining what management strategies (conservation tillage, irrigation application, and hormonal sprays) might help increase yield and control ZC in potato; 2) determining if the timing of infection affects the physiological response(s) to the disease including at emergence and flowering; 3) following the progression of the disease through different tissue types from the root system up the main stem; and 4) evaluating remotely sensed methods of physiological function for their ability to detect ZC infection at the field scale. This report will cover the results from objectives 2-4 while a second report (see Troxclair and Rowland) will give the results of the cropping system yields.

Materials and Methods

Please see the second report (Troxclair and Rowland) for a description of the cropping system used. Physiological measurements were taken solely from the conventional tillage treatments for two of the irrigation regimes: fully irrigated (100%) and deficit irrigated (70%); and two of the hormonal applications: control (no spray) and gibberellic acid (GA). Irrigation application was applied based on crop condition and full irrigation involved the application of 1.5 inches at a single time due to the water holding capacity limitations of the soil. At each measurement time period, three plants per plot were measured for a total of 36 plants (2 irrigation X 2 spray X 3 plants X 3 replications).

Physiological measurements were taken on 04/29/10, 05/13/10 and 05/25/10, 06/04/10 for the first infected group (I1- infested at emergence) and second infected group (I2 – infested at bloom), respectively. The first measurement period for each infection group occurred 1-2 days after “hot” psyllids were removed and represented a baseline of physiological performance directly after infection. The second measurement period for each infection group occurred 18 days after “hot” psyllids were removed and at the height of ZC symptom development in the canopy of affected plants. Gas exchange, including photosynthesis, stomatal conductance, and transpiration were measured with a LI-COR 6400 gas analysis portable system (LI-COR Biosciences, Nebraska, U.S.). Chlorophyll fluorescence was measured with an Opti-Sciences OS-30p portable chlorophyll fluorometer (Opti-Sciences, New Hampshire, U.S.). Dark adapted clips were applied to the first fully expanded leaf on the main stem of each measured plant at sunrise and allowed to dark adapt for at least one hour prior to measurement. After chlorophyll fluorescence was measured and the clip removed, SPAD chlorophyll content (SPAD Minolta meter, Spectrum Technologies, Illinois, U.S.) was measured prior to gas exchange.

For the measurements taken at full symptom development, plants were destructively harvested. After completion of the physiological measurements, each plant was dug up and taken back to the laboratory for dissection. Plants were dissected into the following tissue types: main tap root; stem base just above the soil surface; the leaf that was measured for physiological assessment; and the stem section just below the base of this leaf. This tissue was then analyzed using qPCR by Dr. Rush’s lab.

Two other groups of plants were monitored for physiological function. The first group of plants included those infected at very early emergence when stand emergence had not been completed on (4/12/10 – one week prior to the stand emergence infection group). A subset of these plants was chosen for sap flow analysis along with non-infested, healthy appearing plants from the rest of the plot. Sap flow was measured using dynagages (Dynamax, Texas, U.S.) installed on the main stem of plants in the 100% and 70% water application treatments in the no-spray plots on 3 healthy plants and 3 infected plants (12 plants total). Gauges were installed when full plant height had been attained (but prior to flowering) and left in place for the remainder of the season. A second group of healthy and infected plants were monitored for infrared canopy temperature using the SmartCrop®

(SmartField™, Inc., Texas, U.S.) sensor that logs infrared canopy temperature every 5 seconds continually. Sensors were aimed at plants that had been purposely infested and others within the main plot that had not been exposed to “hot” psyllids and appeared healthy. Plants were monitored from 05/04/10 to harvest.

Results and Discussion

ZC infection showed relatively little effect on physiological function in comparison to healthy, non-infected plants during baseline measurements for both 70% and 100% irrigated plants. There was however, in the 100% treatment a trend for up-regulation in infected plants in comparison to healthy plants for both photosynthesis (19.0 infected vs. 16.5 healthy $\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance (0.40 infected vs. 0.31 healthy $\text{mol m}^{-2} \text{s}^{-1}$).

However, water-use (as measured by sap flow) was significantly reduced in mid- and late-season for infected plants in comparison to healthy plants in the 70% irrigation treatment; water-use patterns appeared to be similar between infected and healthy plants under full irrigation. This indicates that the detrimental effects of ZC are exacerbated by water stress.

For infected plants only, infection period (emergence vs. bloom) appeared to have differential effects on the physiology of the plant. Early infected plants were able to maintain both photosynthesis and transpiration to a greater degree in comparison to plants infected at bloom. For example, early infected plants saw approximately a 25% percent decrease in photosynthesis in comparison to their baseline readings across the season while plants infected at bloom saw an almost 75% decrease in their photosynthetic rates. This may be attributable to a more plastic response or to genetic up-regulation of defense compounds early in development because developmental plasticity often varies with plant age (Boege et al., 2007).

Our study found evidence for the directional movement of ZC infection in different plant tissue types and the concomitant effect on physiological function in the plant. When tissue that had been measured physiologically (purposely infected plants) was collected and quantified the ZC titer using qPCR, three distinctive groups of plants emerged: 1) C_T levels greater than 31 (indicative of negative ZC infection) in stem base and root tissue (NEG/NEG); 2) C_T levels indicating negative for ZC infection in stem tissue but positive for infection in root tissue (NEG/POS); and 3) C_T levels less than 31 (indicative of positive ZC infection) in stem base and root tissue (POS/POS). The other two tissue types (leaf base and leaf) showed negative for ZC infection for all plants. Among these three groups, photosynthesis and transpiration were decreased for both NEG/POS and POS/POS plants in comparison to NEG/NEG plants indicating that as infection progressed, physiological function was impacted to a greater degree. When looking at individual plants and the relationship between C_T level and conductance and transpiration, the correlation between increasing titer (lower C_T level) and gas exchange rate became significant in the POS/POS group. This would suggest that as infection progressed through the plant, the relationship to infection titre and physiological function becomes increasingly stronger.

Lastly, there was indication that some measurements of physiological function could possibly be indicating tools for either growers or breeders of ZC infection progression. C_T

level and SPAD chlorophyll reading were correlated such that increasing infection showed decreased levels of leaf chlorophyll. This tool could be easily used in a breeding program for screening large numbers of genotypes because it is fast, simple, and non-destructive. The SmartCrop© system also showed elevated canopy temperatures for infected plants vs. healthy plants for some time periods during the season. This infrared temperature system is commercially available and easily deployed and monitored by growers.

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EVALUATION OF POTATO PSYLLID COLD TOLERANCE, OVERWINTERING SURVIVAL, STICKY TRAP SAMPLING, AND EFFECTS OF LIBERIBACTER ON POTATO PSYLLID ALTERNATE HOST PLANTS

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Summary

Studies of arthropod-vector pathogens that cause plant diseases should include investigations into vector ecology, including temperature tolerances, sampling strategies, and alternate hostplant epidemiology. Here, we examined aspects of potato psyllid ecology that may provide important information for understanding the epidemiology of zebra chip disease in Texas and improve efforts to monitor migrating adults. Potato psyllid adults and nymphs were found to be tolerant of subfreezing temperatures, with nymphs surviving exposure to -15° C, indicating potential overwintering of adults and/or nymphs in areas of the Texas Panhandle. Field studies of potato psyllid attraction to different-colored sticky traps indicated psyllid adults had a preference for neon-green traps. Finally, effects of *Liberibacter* on several alternate hostplants revealed that at least one wild host (silverleaf nightshade) may tolerate *Liberibacter* infection and therefore serve as a possible reservoir host.

Impact Statement

This research addresses several of the priority research topics outlined in the industry and growers feedback document. These results, although preliminary, will not only further understanding the biology of the potato psyllid, but also epidemiology of ZC in Texas.

Introduction

A major objective of the epidemiology of zebra chip (ZC) disease should be an investigation of vector ecology. The vector of ZC, the potato psyllid (*Bactericera cockerelli* Sulc), is a notorious insect pest of potatoes. Losses due to psyllid yellows were especially severe during the 1920s -1940's. In response to major losses incurred by potato farmers in 1938, Wallis (1955) performed ecological studies on *B. cockerelli* from 1939 to 1952 to determine its overwintering hosts, where they were overwintering, and determine environmental conditions that lead to outbreaks. Unfortunately, these surveys did not include the Texas Panhandle, where much of current Texas potato production exists. Potato psyllids are suspected of migrating northward from breeding areas in south Texas (Romney 1939, Janes 1939). However, a psyllid distribution map in Pletsch (1947) designates much of west Texas, including the Panhandle, as 'desert' breeding area for this species. Thus, there is a strong possibility that psyllids are overwintering in parts of the Texas Panhandle.

Wallis (1946) established that *B. cockerelli* was unable to survive the winter in Nebraska. Pletsch (1947) found nymphs of the related *Bactericera maculipennis* feeding on bindweed, *Convolvulus arvensis*, roots several inches below the soil surface, and *B. cockerelli* have been reported as reproducing on bindweed as well. Furthermore, Pletsch (1947) also reported *B. cockerelli* nymphs feeding on bindweed during the fall and surviving exposure to repeated frosts and temperatures as low as 6° F. Therefore, *B. cockerelli* may survive periods of

extreme cold during the nymphal stage by feeding on foliage and subterranean roots of wild host plants. As no overwintering studies have been performed in the Texas Panhandle, it is necessary to determine the possibility that *B. cockerelli* can overwinter in this region.

The number of host plants that psyllids can feed and reproduce upon is extensive (see Knowlton and Thomas 1934; Pletsch 1947; Wallis 1951, 1955), but largely encompasses wild and cultivated Solanaceous plants. List (1939) reported that off season growth of wild solonaceous plants in the overwintering region serve as important hosts of *B. cockerelli*, with movement onto potatoes and tomatoes occurring as these become available. Recent research on epidemiology of ZC in the Texas Panhandle has shown that strong edge effects exist all the way around potato fields, suggesting that *Liberibacter*-infectious psyllids may be entering fields from local wild solanaceous hosts, and not strictly from migrating psyllids.

Prior to making timely and effective insecticide applications for psyllid control, their populations should be sampled. Due to their minute size, potato psyllids of all stages are difficult to observe and employ sampling methods such as sweep netting for adults, counting nymphs and eggs on foliage and trapping adults on yellow sticky cards (Knowlton and Janes (1931), Pletsch (1947), Adams and Los (1989), Al-Jabr (1999), Goolsby et al. (2007)). However, the effectiveness of these approaches to accurately assess psyllid population levels has not been determined, particularly when psyllid populations are very low or otherwise not detectable. Accurate sampling methods are an important prerequisite for effective psyllid detection, epidemiology, monitoring and establishment of psyllid action thresholds.

The objectives of this study were to determine: 1) the potential for overwintering survival of the potato psyllid in the Texas Panhandle, 2) the effect of *Liberibacter* infection on common host plants of the potato psyllid, and 3) evaluate the effectiveness of yellow sticky traps compared to sticky traps of other colors. This information is vital towards understanding the epidemiology of ZC, and employing effective vector and disease management efforts. From an epidemiological perspective, it is important to know if psyllid populations that overwinter in Texas can serve as the initial source of *Liberibacter* inoculum in Texas potato fields.

Materials and Methods

Cold tolerance: Potato psyllid nymphs and adults were collected from a greenhouse colony located near Bushland, TX. Approximately 25-50 adults were aspirated and immediately placed in individual 75 mm x 10 mm petri dishes. Cold tolerance for nymphs was evaluated by placing 25-50 nymphs that were on potato leaves inside individual environmental chambers with temperatures set at 0, -5, -10, and -15° C, and adults at 0, -5, and -10° C. Insects were exposed to these temperatures for the following lengths of time: 30 minutes, 60 minutes, 4 hours and 24 hours. Each temperature and time setting used only one group of insects, and these insects were subjected to these temperatures only once.

Effect of *Liberibacter solonacearum* on wild solanaceous hosts. Preliminary tests for effects of *Liberibacter* were performed on several common weedy hosts of *B. cockerelli*: Wolfberry (*Lycium berlandierii*), Silverleaf nightshade (*Solanum eleagnifolium*), and Bufalobur nightshade (*Solanum rostrum*). Individual potted plants were held in 30 cm x 30 cm x 30 cm BugDorm cages (BioQuip, Rancho Dominguez, CA). Approximately 25 adult *B. cockerelli*

were aspirated from a colony containing *Liberibacter*-infective psyllids, and released into cages containing individual plants. Each test plant had an untreated counterpart inside a different cage for comparison. Infective psyllids fed upon test plants for a minimum of two weeks, after which they were killed with an insecticide. Several plant portions (leaves, stems) were excised and tested for *Liberibacter* using real-time PCR methods.

Sticky trap evaluation: Transects of sticky traps of the following colors were deployed along the edges of potato fields and compared: neon green, neon orange and standard yellow of two different sizes (7.5 cm x 12.5 cm, and 23 cm x 14 cm). Both yellow sticky traps were obtained from commercial sources. The neon-green and neon-orange traps were hand cut to similar dimensions as the standard sticky traps and coated with tangletrap adhesive. Neon-green and neon-orange were found to be highly attractive to adult psyllids in greenhouse studies by Al-Jabr (1999). Traps were attached to 3 m wooden stakes and arranged randomly in groups of the different colors. Each sticky trap was separated by its neighbors by 20 m, and each group of sticky traps was separated by at least 0.25 km. Tests were performed in potato fields located near Olton, Bushland and Dalhart, Texas. Traps were left for one week, after which they were collected and brought to the laboratory where potato psyllids were counted under a dissecting microscope. Psyllid numbers were log-transformed to normalize the data and equalize Poisson-distributed standard errors.

Results and Discussion

Cold tolerance and overwintering survival. Survival of nymphs was 100%, regardless of temperature or duration. This result confirms observations by Pletsch (1947) that *B. cockerelli* nymphs can survive exposure to very cold temperatures. Adult mortality reached 60% after exposure to -10° C for 24 hours. Although temperatures colder than -10° C have not yet been evaluated for adults, it appears that at least a portion of adults may be capable of surviving extended subfreezing temperatures, especially if they are able to locate suitable microclimates. The ability of potato psyllids to successfully overwinter in the high plains would depend on availability of suitable host plants, and/or some behavioral adaptation to escape subfreezing temperatures. Therefore, studies have been initiated to evaluate overwintering survival of *B. cockerelli* in the Texas Panhandle by releasing adults into cages containing either silverleaf nightshade or buffalobur nightshade. Both plant species are naturally growing plants growing outdoors near Bushland, TX. Additionally, adults will be released into sleeve cages that are on branches of Juniper trees, also at Bushland, TX. These cages will be left outdoors to overwinter, and survival will be evaluated in the spring.

Sticky trap evaluation. In general, significant differences were found among the four types of sticky traps evaluated. The small yellow and neon-orange traps performed poorly in comparison to the standard yellow and neon-green traps, with comparatively low numbers of adult potato psyllids captured by the small yellow and neon-orange traps. In most cases, more potato psyllids were found on the neon-green traps. Although the differences between standard yellow and neon-green were not significant in the Dalhart trial (Fig. 1A), significantly more psyllids were collected on the neon-green traps in the Olton trial, and in the pooled data (Figs. 1B and 1C, respectively). These results suggest that improved psyllid detection and monitoring may be possible by considering sticky traps with alternate colored backgrounds, reflectance, hues, etc. These results may offer insights into how potato psyllids orient to visual cues so that better monitoring traps can be developed.

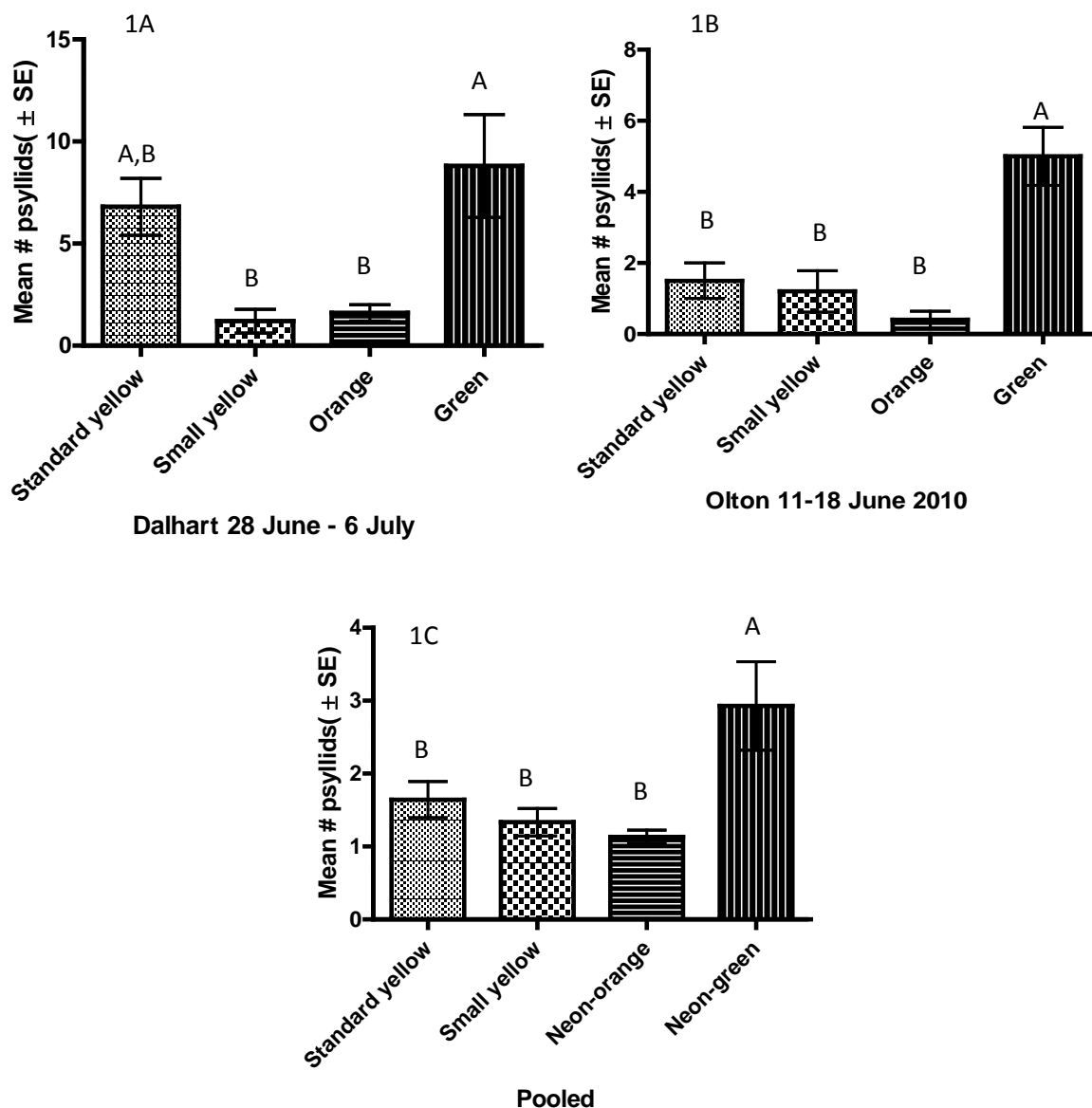


Figure 1. Results of sticky trap tests at two locations in the Texas Panhandle (untransformed data shown). Bars with the same letters are not significantly different at $\alpha=0.05$. 1A Dalhart, TX June 28-July 6; 1B Olton, TX June 11-18 2010; 1C Dalhart and Olton data pooled.

Effect of *Liberibacter solonacearum* on wild solanaceous hosts. Different results were obtained from each alternate host evaluated. We were unable to infect two wolfberry plants with *Liberibacter* (Ct values >36). The plants were vigorous and growing, even 3-4 months after the trials ended. Buffalobur nightshade was killed by *Liberibacter* infection. Within 2-3 weeks of infection, the infected plant began shedding its primary leaves and producing small secondary leaves, similar to the secondary proliferation seen in commercial potato plants that have been infected by *Liberibacter*. Silverleaf nightshade appeared to be unaffected by *Liberibacter* infection. Ct values between 22 and 35 were found in this plant, depending on plant part. Leaves had a higher titer than the stem parts, which is contrary to

Lso titer distribution in potato plants. More testing of alternate hosts should be performed to verify these results, particularly with Wolfberry and Silverleaf nightshade. Both of these plants are very common in Texas and may be important reservoir hosts of *Liberibacter*.

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FREQUENCY OF “*CANDIDATUS LIBERIBACTER SOLANACEARUM*” IN POTATO PSYLLID AND SOLANACEAOUS WEEDS

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Summary

“*Candidatus Liberibacter solanacearum*” (Lso) and “*Ca. L. psyllauros*” (Lps) are known to infect a number of solanaceous plant hosts in addition to potato and tomato. Previous studies conducted using weed species from Texas determined that silverleaf nightshade, black nightshade and wolfberry were hosts for Lso. In the current study, solanaceous weed species were collected in the High Plains of Texas and from Western Nebraska and Eastern Wyoming. These solanaceous weeds included silverleaf nightshade, buffalo bur, black nightshade, and groundcherry. Frequencies of Lso infection in all weed species were very low suggesting these solanaceous hosts are not epidemiologically significant in the development of the zebra complex (ZC) disease. Psyllid eggs, nymphs and adults collected in Texas, Nebraska and North Dakota were also assayed for Lso infection using three PCR primer sets. Lso was not detected in any of the psyllid eggs assayed, however, the ZC bacterium was detected in adults and nymphs at most locations at frequencies approaching 20%. These results are surprising since only 1-2% of psyllid adults trapped and assayed for Lso are positive. PCR primer ZCf/OI2c detected Lso at higher frequencies in psyllid adults and nymphs compared to other primers used. Further studies to improve the detection of Lso in psyllids are warranted. There are two hypotheses to explain these data. Either psyllid adults collected in the trapping network have Lso titers too low to efficiently detect or there is pathogenic variation in the Lso population with avirulent types present in high frequency among psyllid nymphs.

Introduction

In addition to potato and tomato (Hansen et al., 2008; Liefting et al., 2008a), ‘*Candidatus Liberibacter sp.*’ (“*Candidatus Liberibacter solanacearum*” (Lso) and “*Ca. L. psyllauros*” (Lps)) are known to have a number of solanaceous hosts including Tamarillo (*Solanum betaceum*) and Cape gooseberry (*Physalis peruviana*) (Liefting et al., 2008b). In the USA, ‘*Candidatus Liberibacter sp.*’ has been detected in silverleaf nightshade (*Solanum elaeagnifolium*), wolfberry (*Lycium barbarum*) and black nightshade (*S. ptychanthum*) in the High Plains of Texas (Wen et al., 2009). Other solanaceous weed species in Texas, such as buffalo bur (*Solanum rostratum*) have not been evaluated as a host for ‘*Ca. Liberibacter sp.*’ Additionally, the incidence of ‘*Ca. Liberibacter sp.*’ in other locations outside of Texas have not been examined.

To our knowledge, the potato psyllid (*Bactericera cockerelli*) has not been reported previously in North Dakota. In 2010 the potato psyllid was confirmed in three locations in North Dakota which was not surprising given the high populations of this pest found in sticky traps in Western Nebraska (see J. Goolsby weekly trapping network results elsewhere in these proceedings).

The objectives of these studies were to 1) determine the presence of '*Ca. Liberibacter* sp.' in solanaceous weed species in TX and NE and 2) determine the frequency of this bacterium in psyllid eggs, nymphs, and adults in TX, NE and ND.

Materials and Methods

Plant material and DNA extraction. Samples of solanaceous weed species including silverleaf nightshade (*S. elaeagnifolium* Cav.) and groundcherry (*Physalis* sp., probably *P. virginiana*) were collected from Western NE and Eastern WY by Robert M. Harveson and Jeff Bradshaw and shipped to our laboratory. Additional samples in North Central NE were collected by NC Gudmestad and A. Naslund, CSS Farms. Samples of silverleaf nightshade and buffalo bur (*S. rostratum*) were collected in Dalhart, TX by NC Gudmestad and B. Zens, CSS Farms, and shipped to our laboratory. DNA was extracted from taproot roots as described previous (Li et al, 2009).

Psyllid material and DNA extraction. Plant samples (mainly potato plants unless stated otherwise) infested with psyllid in ND, NE and TX were collected by collaborators and cooperators identified above and shipped to our laboratory. All psyllid adults, eggs and nymphs were immediately placed into 70% ethanol after capture and stored in this solution until processed. DNA extraction from single eggs, nymphs, and adults was carried out using method described previously (Li et al, 2009).

Detection of Lso. Detection of Lso in weed samples was performed using PCR assays described by Wen et al (2009) and Liefting et al (2009). Detection of Lso in psyllid samples was conducted using conventional PCR assays as described above as well as the PCR assay described by Hansen et al (2008).

Results and Discussion

The frequency of Lso detection in all solanaceous plants collected, regardless of location or plant species was low (Table 1). In a previous preliminary study the incidence of Lso in silverleaf nightshade was reported range from 23-36% depending on the cPCR primers used (Wen et al., 2009). However, in data reported here Lso was detected in only a single silverleaf nightshade plant collected near Dalhart, TX and only when the ZCf/OI2c primer was used. Lso was never detected in any silverleaf nightshade plants collected in NE or WY. Similarly, the frequency of Lso detected in groundcherry was also low and was only detected using the ZCf/OI2c primer. Lso was detected in approximately 22% of the plants examined when the ZCf/OI2c primer was used but none of the plants were positive when using the OA2/OI2c primer (Table 1).

The incidence of '*Ca. Liberibacter* sp.' was varied in the psyllid adults and nymphs assayed (Table 2). The frequency of Lso detected in psyllid adults and nymphs also varied by the location where they were collected and the PCR primers used (Table 2). For example, PCR primers ZCf/OI2c detected Lso in approximately 19% of the psyllid nymphs collected while OA2/OI2c and 1611F/480R primers detected Lso in 11 and 1% of the samples, respectively. Interestingly, the highest detection of Lso was in psyllid nymphs collected in ND. Although relatively few psyllid adults were evaluated from most locations during these studies it is interesting to note that those collected from tomatoes in Western NE had a high frequency of Lso infection (Table 2). Approximately 20% and 19% of all psyllid adults

Table 1. Frequency of Lso tested in solanaceous weeds (Lso positive/total sample)

Species	Location	Date of collection	ZCf/OI2c (Wen et al., 2009)	OA2/OI2c (Liefting et al., 2009)
Buffalo bur	Dalhart, TX	7/29/2010	2/7	0/7
	Dalhart, TX	9/9/2010	2/11	0/11
Groundcherry	Cody, NE	7/13/2010	0/4	0/4
	Cody, NE	9/9/2010	3/48	0/48
	Banner County, NE	9/15/2010	0/4	0/4
	Box Butte County, NE	9/16/2010	0/11	0/11
Silverleaf Nightshade	Dalhart, TX	7/15/2010		
		healthy	0/6	0/6
		intermediate symptom	0/3	0/3
		severe symptom	0/6	0/6
	Dalhart, TX	8/22/2010	0/4	0/4
	Dalhart, TX	9/9/2010	1/21	0/21
	Scottsbluff, NE	9/16/2010	0/5	0/5
	Kimball, NE	9/16/2010	0/7	0/7
	Cheyenne, WY	9/16/2010	0/6	0/6

assayed were Lso positive when using the ZCf/OI2c and the OA2/OI2c primers, respectively. Only 2% of the psyllid adults were Lso positive when using the 1611F/480R PCR primers. Lso was not detected in any psyllid eggs examined regardless of the primer set used (Table 2).

Impacts. The very low frequency of Lso infection in silverleaf nightshade is surprising given the frequency of infection during our initial surveys conducted two years ago. The low frequency of Lso infection among solanaceous weed species suggests they are unlikely to be epidemiologically important in the development of ZC. However, more intensive sampling of weed species is warranted given the variability of the results to date.

The data on Lso infection of psyllids and nymphs are also surprising. Lso infection rates reported here are substantially higher than those reported in the weekly surveys (see Goolsby et al. and J. Crosslin reports in these proceedings). There are two hypotheses that explain these results. First, the Lso titer in migratory psyllid adults may be so low that detection efficiency is much lower than that found in feeding nymphs. A second hypothesis, and perhaps more likely, is that there is pathogenic variability in the Lso population with avirulent types being the predominate pathotype present in psyllid nymphs. If true, then psyllid trapping data could be enhanced by molecular tests developed to distinguish these pathotypes.

There is substantial variability in the frequency of Lso detection among the PCR primers used in these studies. Overall Lso detection was higher using the ZCf/OI2c (Wen et al., 2009) primers compared to the standard OA2/OI2c primers (Liefting, et al., 2009) used by other labs. The 1611F/480R primers (Hansen, et al., 2008) had the lowest frequency of Lso detection. These results suggest that PCR primers should continue to be developed and

evaluated for their efficiency of Lso detection, as has been previously suggested (Wen, et al., 2009).

Table 2. Frequency of Lso in psyllid samples collected in North Dakota, Nebraska and Texas

Origin and stage of psyllid	ZCf/OI2c (Wen et al, 2009)	OA2/OI2c (Liefting et al, 2009)	1611F/480R (Hansen et al, 2008)
Pearsall TX			
egg	na	na	na
nymph	3/9	2/ 9	0/9
adult	na	na	na
Dalhart, TX			
egg	0/23	0/23	0/23
nymph	3/27	1/27	1/27
adult	na	na	na
Linton, ND			
egg	na	na	na
nymph	3/6	0/6	0/6
adult	3/6	2/6	0/6
Lisbon, ND			
egg	na	na	na
nymph	4/7	2/7	0/7
adult	na	na	na
Inkster, ND			
egg	0/2	0/2	0/2
nymph	21/92	15/92	0/92
adult	na	na	na
Minden, NE			
egg	na	na	na
nymph	0/1	0/1	0/1
adult	na	na	na
Scottsbluff, NE			
egg	na	na	na
nymph	1/3	1/3	0/3
adult	1/2	1/2	0/2
Scottsbluff, NE (tomato)			
egg	na	na	na
nymph	5/47	1/47	1/47
adult	31/163	30/163	4/163

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DETECTION OF *CANDIDATUS LIBERIBACTER SOLANACEARUM* IN TRAPPED INSECTS AND NON-CROP PLANTS IN NEW ZEALAND

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Summary

The impact of the Tomato Potato Psyllid (*Bactericera cockerelli*) and its associated pathogen, *Ca. L. solanacearum* has threatened the future production of potatoes, tomatoes, capsicums and tamarillos in New Zealand. Seasonal variation in the incidence of *Ca. L. solanacearum* in TPP and non-crop plants may provide essential information to aid in pest management decisions. In the current study, molecular screening of TPP DNA from sticky traps has shown that *Ca. L. solanacearum* is present in all NZ monitoring locations over most monitoring periods. Molecular screening of non-crop plant DNA from twelve survey sites throughout NZ, reveals that *Ca. L. solanacearum* has a much broader host range than previously believed. Further studies are required to determine the effect of *Ca. L. solanacearum* in TPP and non-crop host plants on acquisition, transmission and ensuing disease expression.

Introduction

In New Zealand, since the discovery of the Tomato Potato Psyllid (*Bactericera cockerelli*) (Hemiptera, Trioziidae) (TPP) in 2006 and *Candidatus Liberibacter solanacearum* in 2008, there has been a critical need to acquire knowledge about these organisms. Potatoes NZ has recently estimated that the complex has cost the industry an estimated \$50-60million in both the 2008-09 and 2009-10 growing seasons. In addition increased ongoing crop management costs are estimated from between \$700/ha to \$1200/ha.

TPP/ *Ca. L. solanacearum* can cause a range of similar foliar symptoms in potato crops, some of which seem to be associated with the presence of *Ca. L. solanacearum* and some with TPP alone. Studies by Munyaneza et al. (2008) suggest that not all TPP populations induce ‘zebra chip’ symptoms. Other US studies have suggested that there may be seasonal variation in the occurrence of *Ca. L. solanacearum* in TPP populations (Crosslin et al. 2010). In NZ, numbers of TPP caught on sticky traps in 2008-09 and 2009-10 were highly variable between sites and regions (Berry & Jorgensen, unpubl. data) In addition, the prevalence of visual ‘zebra chip’ symptoms varied between sites and regions during these seasons.

A variation of the percentage of TPP carrying *Ca. L. solanacearum* during the growing season may provide essential information to aid in pest control decisions. Furthermore, data on the incidence and distribution of *Ca. L. solanacearum* and its insect vector (*B. cockerelli*) can provide essential biological information to aid in pest management.

The objectives of the current study were to 1; determine the incidence of *Ca. L. solanacearum* in TPP's caught on sticky traps and 2; determine the incidence of *Ca. L. solanacearum* in non-crop plants in the 2009-2010 growing season.

Materials and Method

TPP numbers were monitored weekly using yellow sticky traps in thirteen monitoring sites in North Island (NI) potato and field tomato and South Island (SI) potato sites throughout NZ from mid October 2009 – April 2010. Detailed results of two monitoring locations are included in this report. TPPs from single traps were sorted into groups of up to 5 individuals for DNA extraction. TPP DNA samples contained either bulked (maximum of 5 individuals) or individual TPP.

During 2009 – 2010, plant specimens from within crops and adjacent boundaries (weeds, bush and tree species) were collected in twelve field surveys (5 SI, 7 NI). Plant specimens were identified and assessed for invertebrates. Two leaf and stem samples were removed from each plant specimen for DNA extraction.

DNA was extracted from TPP and plant material using a modified CTAB protocol (IAW Scott, pers comm.). Insect and plant DNAs were tested for the presence of potential PCR inhibitors using appropriate primers. Appropriate positive and negative controls were used.

A semi-nested PCR protocol was used to test insect and plant DNA samples for the presence of *Ca. L. solanacearum*. The specificity of this test had previously been confirmed by DNA sequence analyses of PCR fragments amplified (IAW Scott, pers.comm.). Appropriate controls were used during PCR screening. *Ca. L. solanacearum* positive plant DNA had been extracted from tomatoes and capsicums (MAF BNZ) as well as previously tested potato material (PFR).

PCR amplification products were separated on agarose gels alongside DNA size markers and visualised using ethidium bromide. Digital images of each gel were taken, scored for presence/absence and checked to ensure that bands were of the correct size.

Results and Discussion

Numbers of TPP on sticky traps were highly variable between sites and regions during the monitoring period of early November 2009 to end of April 2010 (Berry 2010). Numbers of TPP rose significantly in early January and declined by early April in most regions. Numbers of TPP found on traps were higher at NI monitoring sites than at SI monitoring sites (Berry 2010).

Molecular screening of TPP DNA samples has shown that *Ca. L. solanacearum* was present in TPP populations from the first appearance of TPP on sticky traps in NI and SI monitoring regions. Molecular screening results of TPP DNA from traps for the presence of *Ca. L. solanacearum* in one NI (Manawatu) and one SI (mid Canterbury) potato monitoring site are described in detail below.

Manawatu

Of the 73 TPP DNA samples screened, 68 (93.15%) were positive for *Ca. L. solanacearum*. *Ca. L. solanacearum* positive TPP DNA samples were first detected from sticky traps in potatoes in the 7th week of trap monitoring (1st TPP caught, week ending 23/12/2009, Figure 3). On 14 of 16 trap out dates (where TPP were caught) at least some TPP DNA samples tested were *Ca. L. solanacearum* positive.

On 12 of those 14 trap out dates all samples tested positive for a *Ca. L. solanacearum*.

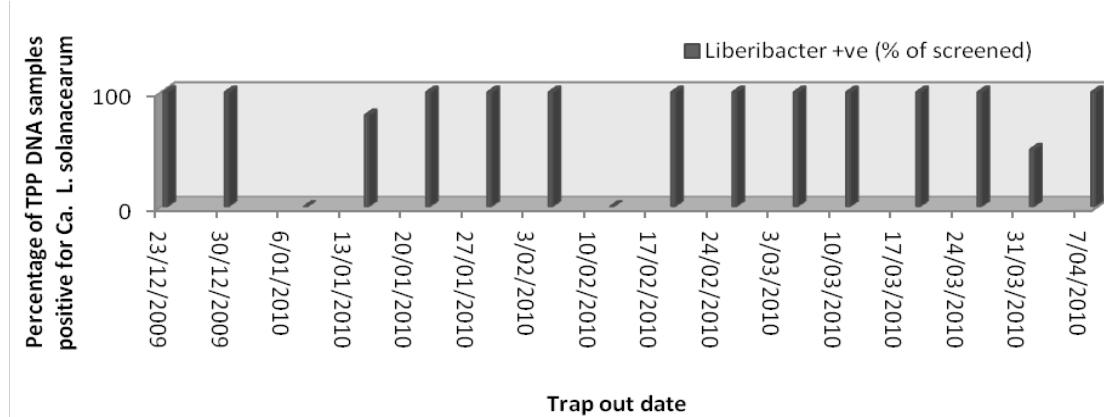


Figure 1. Percentage of TPP DNA samples (bulked and/or individual TPP) positive for *Ca. L. solanacearum* during the monitoring period of mid December 2009 – early April 2010 in potatoes, Manawatu.

Mid Canterbury

Of the 31 TPP DNA samples screened, 30 (96.77%) were positive for *Ca. L. solanacearum*. *Ca. L. solanacearum* positive TPP DNA samples were first detected from sticky traps in potatoes in the 7th week of trap monitoring (1st TPP found, week ending 11/1/2010, Figure 2). *Ca. L. solanacearum* positive samples were detected on all 12 trap out dates (where TPP were found) and on 11 of those trap out dates all samples tested positive.

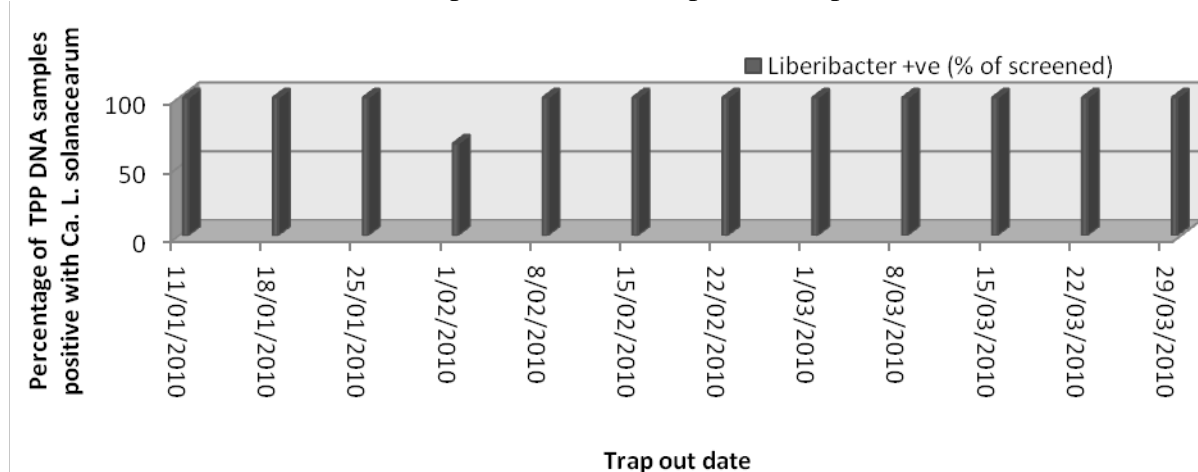


Figure 2. Percentage of TPP DNA samples (bulked and/or individual TPP) positive for *Ca. L. solanacearum* during the monitoring period of early January 2010 – early April 2010 in potatoes, mid Canterbury.

The percentage of TPP DNA samples (bulked and/or individual TPP) positive for *Ca. L. solanacearum* over all monitoring dates for each of thirteen sites are summarised in Table 1. Results of the current study show that *Ca. L. solanacearum* was detected at most monitoring dates in all monitoring sites. An estimation of the proportion of infected versus uninfected TPP individuals during the monitoring period or between regions could help to understand differences in disease expression at different sites and regions in NZ.

Table 1. Percentage of TPP DNA samples positive for *Ca. L. solanacearum* over the monitoring period at each of six monitoring sites.

Site	Crop	Percentage <i>Ca. L. solanacearum</i>
North Island, NZ		
Hawke's Bay	Tomatoes	73.8
Hawke's Bay	Potatoes	67.2
Waikato, NI	Potatoes	73.8
Manawatu	Potatoes	93.2
Pukekohe	Potatoes	74.6
South Island, NZ		
North Canterbury	Potatoes	53.6
North Canterbury	Potatoes	70.0
Mid Canterbury	Potatoes	72.9
Mid Canterbury	Potatoes	96.8
Mid Canterbury	Potatoes	46.2
South Canterbury	Potatoes	81.8
South Canterbury	Potatoes	50.0

As a newly discovered pathogen (Hansen et al. 2008, Liefting et al. 2008), studies on the host range of *Ca. L. solanacearum* have focussed primarily on solanaceous plants (Liefting et al. 2008, Wen et al. 2009). In contrast, the TPP has been associated with a broader range of hosts. For example Wallis (1955) lists TPP adults found on plants from 20 families, though TPP was found to breed on only 3 of these families. The current research programme in NZ focuses on the common host range of both the TPP and *Ca. L. solanacearum*.

A total of 108 plant species collected from 12 survey sites were assessed for invertebrates and screened for the presence of *Ca. L. solanacearum*. Of these, DNA samples from 34 plant species belonging to 17 families yielded PCR bands of the same size observed for *Ca. L. solanacearum*. Seven of the 34 plant species were determined by semi-nested PCR to be positive at more than one of the survey sites (Table 2). These putative *Liberibacter* PCR bands are to be confirmed by DNA sequence analysis.

Table 2. Plant species determined by semi-nested PCR to be *Liberibacter* positive at multiple sites, 2009-2010.

Family	Species
Malvaceae	<i>Malva parviflora</i> (small flowered mallow)
Polygonaceae	<i>Polygonum aviculare</i> (wireweed)
Polygonaceae	<i>Polygonum persicaria</i> (willow weed)
Fabaceae	<i>Pisum sativum</i> (peas)
Cupressaceae	<i>Cypress</i> sp.
Solanaceae	<i>Solanum nigrum</i> (black nightshade)
Solanaceae	<i>Solanum tuberosum</i> (volunteer potatoes)

The above preliminary range of plants that may act as reservoirs of *Ca. L. solanacearum* may have important implications for the management of *Ca. L. solanacearum* and TPP. Such information may provide an insight into the role of immigrant TPP in the spread of *Ca. L. solanacearum* in potato fields.

Molecular screening of TPP DNA has shown that *Ca. L. solanacearum* is present in all NZ monitoring locations over most monitoring periods. Preliminary screening of non crop plant DNA reveals that *Ca. L. solanacearum* has a broader host range than previously believed. Further research will be carried out to determine the effect of *Ca. L. solanacearum* on TPP acquisition, transmission and subsequent disease expression.

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CONSEQUENCES OF “*CANDIDATUS LIBERIBACTER SOLANACEARUM*” ON PSYLLID POPULATIONS

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Summary

The potato/tomato psyllid transmits “*Candidatus Liberibacter solanacearum*” (CLs), the pathogen causing zebra chip. Deciphering the relationship between vectors and pathogens is essential to improve the management of vectors and diseases. Here we investigated the effect of CLs on psyllid population growth and psyllid longevity. The goal of this work is to evaluate the effects of CLs on vector populations to improve our understanding of population dynamics and disease epidemiology.

Introduction

Associations between insects and intracellular bacteria (endosymbionts) are well known (Baumann, 2005; Buchner, 1965; Dale & Moran, 2006). Often, these endosymbionts have important effects in their hosts. They can influence fundamental biological processes such as synthesis of essential nutrients and recycling of nitrogenous wastes (Douglas, 2003), reproduction (Hoffmann & Turelli, 1997) and other fitness traits (Brownlie *et al.*, 2009; Huigens *et al.*, 2004; Kremer *et al.*, 2009). Obligate endosymbionts of phloem-feeding insects, such as psyllids, are believed to compensate for the nutritional deficiency of essential amino acids (Baumann, 2005; Baumann *et al.*, 1995; Douglas, 1998). The role of secondary endosymbionts is often unknown, but some of them are known to confer protection against natural enemies, provide protection against heat stress, and broaden the range of suitable food plants (Oliver *et al.*, 2010). Vector-borne plant pathogens also manipulate the behavior of their insect vector to promote their propagation (Mayer *et al.*, 2008).

The potato psyllid, *Bactericera cockerelli* (Sulc), is an important pest of solanaceous plants (Liu & Trumble, 2004) and vectors the bacterium causing zebra chip, “*Candidatus Liberibacter solanacearum*” (CLs) also known as *Candidatus Liberibacter psyllaureus* (Hansen *et al.*, 2008; Liefting *et al.*, 2008; Munyaneza *et al.*, 2007). Very little is known about the relationship between these two organisms. It is plausible CLs may affect life-history and ecologically-important traits of the potato psyllid. Therefore, we studied the effect of CLs on psyllid population growth and psyllid longevity. Innovative techniques for monitoring, trapping or controlling this insect vector might result from our findings.

Materials and Methods

Insect colonies: CLs-uninfected and CLs-infected *B. cockerelli* colonies were maintained in separate insect cages (BioQuip, Rancho Dominguez, CA) on tomato plants. The colonies were maintained at 23°C and 24 h light. Levels of CLs are tested regularly using multiplex PCR as described in (Nachappa *et al.* 2010, submitted). No individuals from CLs-uninfected colony have detectable levels of CLs, whereas 70 to 80% of insects from CLs-infected colony test positive (Nachappa *et al.* 2010, submitted).

Isofemale lines: After eclosion, young adults were allowed to mate for 7 days. Ten females from each colony were used to create isofemale lines. Each female was isolated on a 4-5 week old tomato plants. After a 7-day oviposition period, females were removed and plants were inspected daily until adult eclosion. Number of eggs, nymphs and adults were assessed daily.

Total number of eggs was determined as the number of eggs present on the plant plus all nymphs the day the female was removed.

Larval count was assessed as first, second, third, fourth and fifth instar based on body size and development of wing pads.

Hatching percentage was determined as maximum number of newly hatched first instar nymphs divided by maximum number of eggs produced during the 7-day oviposition period.

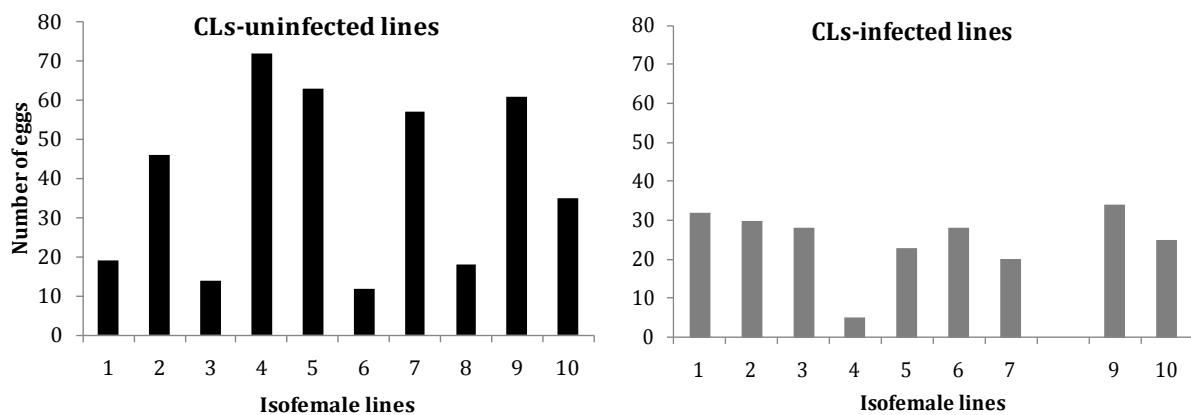
Longevity studies: L5 nymphs from each colony were placed in 15-cm petri dishes with tomato leaves. After adult eclosion, their survival was recorded daily.

Statistical analyses: Due to non-normal distribution of data, comparisons between all parameters (egg counts, total larval counts and hatching percentage) were made using non-parametric Kruskal-Wallis test (MINITAB v.14, Minitab, Inc., State College, PA).

Results and Discussion

Population growth: Ten isofemale lines (female potato psyllid reared on a plant for 7 days) were established from CLs-uninfected and CLs-infected colonies. No significant difference in the oviposition rate of CLs-uninfected and CLs-infected females after a one-week oviposition period was found (Kruskal-Wallis test: $P=0.47$; Fig 1). Although, the CLs-uninfected lines appeared to have a higher oviposition rate than the CLs-infected lines, there was greater variability in oviposition among CLs-uninfected lines (Fig. 1).

Figure 1. Mean oviposition rate of CLs-uninfected (left panel) and CLs-infected (right panel) lines under controlled laboratory conditions. (CLs-infected line 8 failed to lay any egg)



There was a significant reduction in the total larval counts (all five larval stages) of CLs-infected lines compared to CLs-uninfected lines at first adult eclosion (Kruskal-Wallis test: $P=0.01$; Fig. 2). This reduction in larval counts may be explained by the significant reduction

in hatching percentage of CLs-infected lines compared to the CLs-uninfected lines (Kruskal-Wallis test: $P=0.02$; Table 1).

Figure 2. Mean larval counts of CLs-uninfected (left panel) and CLs-infected (right panel) lines under controlled laboratory conditions.

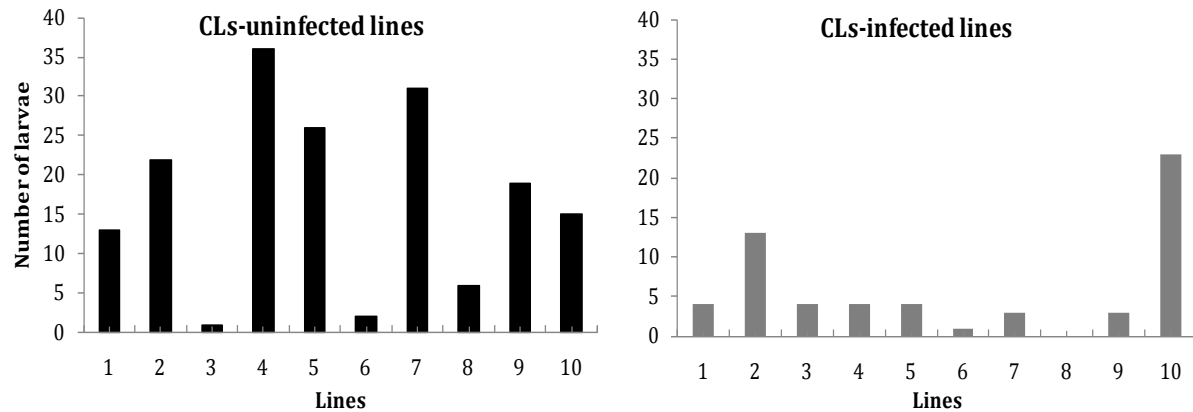


Table 1. Hatching percentage of CLs-uninfected and CLs-infected lines under controlled laboratory conditions.

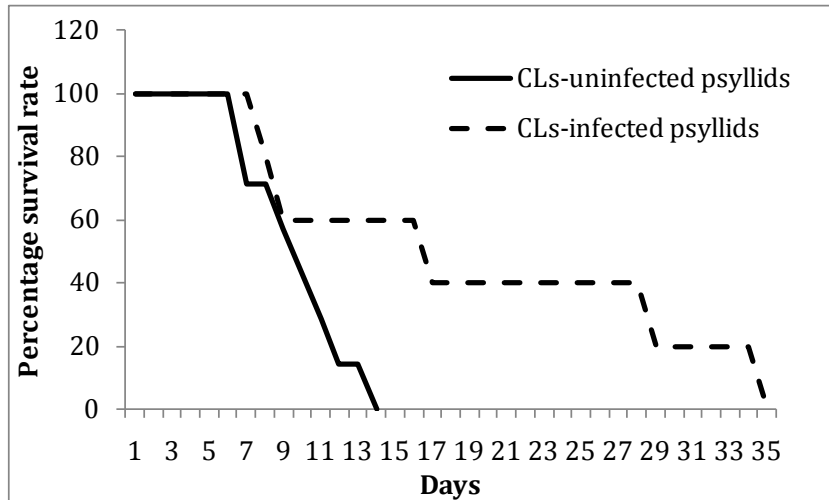
Isofemale lines	CLs-uninfected	CLs-infected
1	100.00	37.50
2	66.13	80.56
3	76.92	52.50
4	100.00	26.09
5	59.18	35.71
6	76.92	28.57
7	52.94	19.23
8	72.22	-
9	57.14	85.71
10	58.82	44.86

These results suggest that the bacterium, CLs, negatively affects population growth rate of the vector.

Longevity: We examined longevity of CLs-uninfected and CLs-infected adult psyllids in petri-dish bioassays under laboratory conditions. We observed that longevity of CLs-infected psyllids was greater than CLs-uninfected psyllids (Mean \pm SD: 16.33 ± 4.68 and 7.57 ± 4.86

days respectively, Fig. 3). Our sample size was small (n=10 per population), more replicates are needed to confirm this finding and preferably conducted on whole plants.

Figure 3: Longevity study of CLs-infected and CLs-uninfected adult psyllids



Adult longevity is an important aspect of disease incidence/vector competence. Longer longevity might lead to greater disease transmission during the adult stage, while a shorter longevity might lead to less disease transmission as it was reported in the mosquito-*Plasmodium* interaction (Black IVth & Moore, 2005; Hackett, 1958).

Conclusion

Our results suggest that CLs-uninfected population have a greater population growth rate than CLs-infected population. However, CLs-infected adults have greater longevity than the CLs-uninfected adults. Understanding the effects of CLs on vector populations might help predict potato psyllid and/or Zebra Chip outbreaks. The impact of CLs on psyllid population fitness should be taken into account when testing pesticides or designing monitoring strategies.

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ZEBRA CHIP DISEASE DEVELOPMENT OVER TIME

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Summary

A study was conducted to determine the length of time required to produce zebra chip following exposure of potato plants to infective potato psyllids and to investigate the impacts of zebra chip infection on yield and levels of reducing sugars in tubers through time. Results indicate that tuber symptoms appear 3 weeks following exposure. Yield is stunted quickly and reducing sugar levels elevate upon symptom expression.

Impact Statement

Results of this experiment show that zebra chip tuber symptoms appear within 3 weeks following exposure to infective psyllids. This information can be useful in late season invasions of infective psyllids to assist growers in making harvest decisions to avoid the economic impacts of this devastating disease.

Introduction

Zebra chip (ZC) is an emerging disease in potatoes that has caused significant economic impacts in potato growing areas in the southwestern United States, Mexico, Central America and New Zealand. The unique symptom is a darkened discoloration of the perimedullary tissue of the tuber which may become more pronounced after frying which renders the tubers unmarketable. Whole plant yield loss and reduction of tuber size is also associated with the disease (Munyaneza 2009). The suspected causal agent for the disease, *Candidatus Liberibacter solanacearum* syn. *psyllaureus* (Lso), was found to be associated with the potato psyllid, *Bactericera cockerelli* (Sulc) (Hansen 2008) and with diseased potato tubers (Liefting 2008). The potato psyllid has also been shown to be associated with ZC in controlled field studies (Munyaneza 2007, 2009). Currently, little is known about the time-course development of the disease, in particular, the time needed after exposure for the characteristic symptom to develop in the tuber. This study was conducted to define the time-course of symptom development in the tuber and to determine how the disease affects yield, tuber size and reducing sugar levels through time. This knowledge, in combination with effective monitoring, could assist growers in making harvesting decisions following an outbreak of infective potato psyllids in their fields and also expands our basic understanding of how this devastating disease affects the potato plant.

Materials and Methods

A controlled field enclosure study was conducted at the USDA-ARS in Wapato, WA during 2009-2010. Potato psyllids were collected from untreated potatoes growing at the USDA-ARS Research Farm in Moxee, WA during the fall of 2007. The psyllids had been routinely tested positive for Lso by PCR. Six whole potato seed pieces (cultivar: Atlantic) were planted within each insect-proof enclosure. To expose plants to psyllids, 20 psyllids were released

onto each plant at bloom stage and were eliminated with insecticides after 4 to 7 days. For every enclosure receiving psyllids, a second enclosure was left psyllid-free to serve as a control. Destructive harvests were conducted at periodic intervals (4 exposed enclosures and 4 control enclosures). Individual tuber weights and total plant yield were recorded for each plant. Incidence was measured by cutting tubers from each plant near the apical end for the diagnostic symptom. If any plant had symptomatic tubers the entire plant was considered diseased. A pooled sample of ten tubers was selected from each enclosure and assayed using a YSI 2700 Biochemical Analyzer (YSI, Inc.) to measure levels of reducing sugars (glucose and sucrose). In 2009, enclosures were harvested at 3, 5, 7, 9 and 11 weeks following psyllid exposure. In 2010, based on 2009 results, the harvest schedule was changed to 1, 2, 3, 4, 6 and 8 weeks following psyllid exposure.

Results and Discussion

Incidence and yield results from both field seasons are summarized in Table 1. In 2009, plants exposed to psyllids three weeks before harvest had 100% incidence; all subsequent harvests also showed 100% incidence. In 2010, the first two harvests were asymptomatic (1 and 2 weeks after exposure) but the harvest three weeks following psyllid exposure had 100% disease incidence;

again, all subsequent harvests showed 100% incidence.

In 2009, the total yield of plants exposed to psyllids was significantly different from unexposed plants 5 weeks after exposure. There was no significant increase in yield found through time. In 2010, the total yield of the exposed plants was significantly different from unexposed controls beginning 6 weeks following exposure. Yields plateaued at the third through the final harvests (3 to 8 weeks following exposure). Similar trends were found with tuber size.

The results from the sugar analysis are found in Figures a-d. In 2009, all exposed plants had higher levels of glucose and sucrose relative to unexposed plants. In 2010, a similar trend in both reducing sugar levels was found beginning in the plants harvested three weeks after exposure and remained high relative to the controls throughout the rest of the experiment.

Table 1. Disease progression ZC disease incidence and yield

2009 Field season

Time after exposure, weeks	Disease incidence, %		Total plant yield, g		Tuber size, g	
	Exposed	Control	Exposed	Control	Exposed	Control
3	100	0	493.62 ^{aA}	583.98 ^{aA}	89.97 ^{aA}	79.49 ^{aA}
5	100	0	472.88 ^{aA}	963.27 ^{bB}	73.66 ^{aA}	108.81 ^{aB}
7	100	0	520.39 ^{aA}	1265.69 ^{cB}	74.25 ^{aA}	159.94 ^{bB}
9	100	0	666.21 ^{aA}	1790.15 ^{dB}	79.38 ^{aA}	205.56 ^{cB}
11	100	0	565.15 ^{aA}	1926.86 ^{dB}	78.18 ^{aA}	194.96 ^{cB}

2010 Field season

Time after exposure, weeks	Disease incidence, %		Total plant yield, g		Tuber size, g	
	Exposed	Control	Exposed	Control	Exposed	Control
1	0	0	613.71 ^{aA}	563.93 ^{aA}	38.25 ^{aA}	43.16 ^{aA}
2	0	0	998.95 ^{aA}	962.53 ^{abA}	63.56 ^{abA}	66.82 ^{abA}
3	100	0	1138.99 ^{bcA}	1134.87 ^{bcA}	78.39 ^{bA}	67.70 ^{abcA}
4	100	0	1287.85 ^{bcA}	1511.00 ^{cA}	83.38 ^{bA}	84.15 ^{bcA}
6	100	0	1405.12 ^{cB}	2031.98 ^{dB}	77.28 ^{bB}	130.92 ^{dB}
8	100	0	1402.14 ^{cB}	2771.98 ^{eB}	77.30 ^{bB}	137.56 ^{dB}

**Means within each column followed by same lowercase letter are not statistically different (Tukey's test). Control and exposed means having different uppercase letters are not statistically different.*

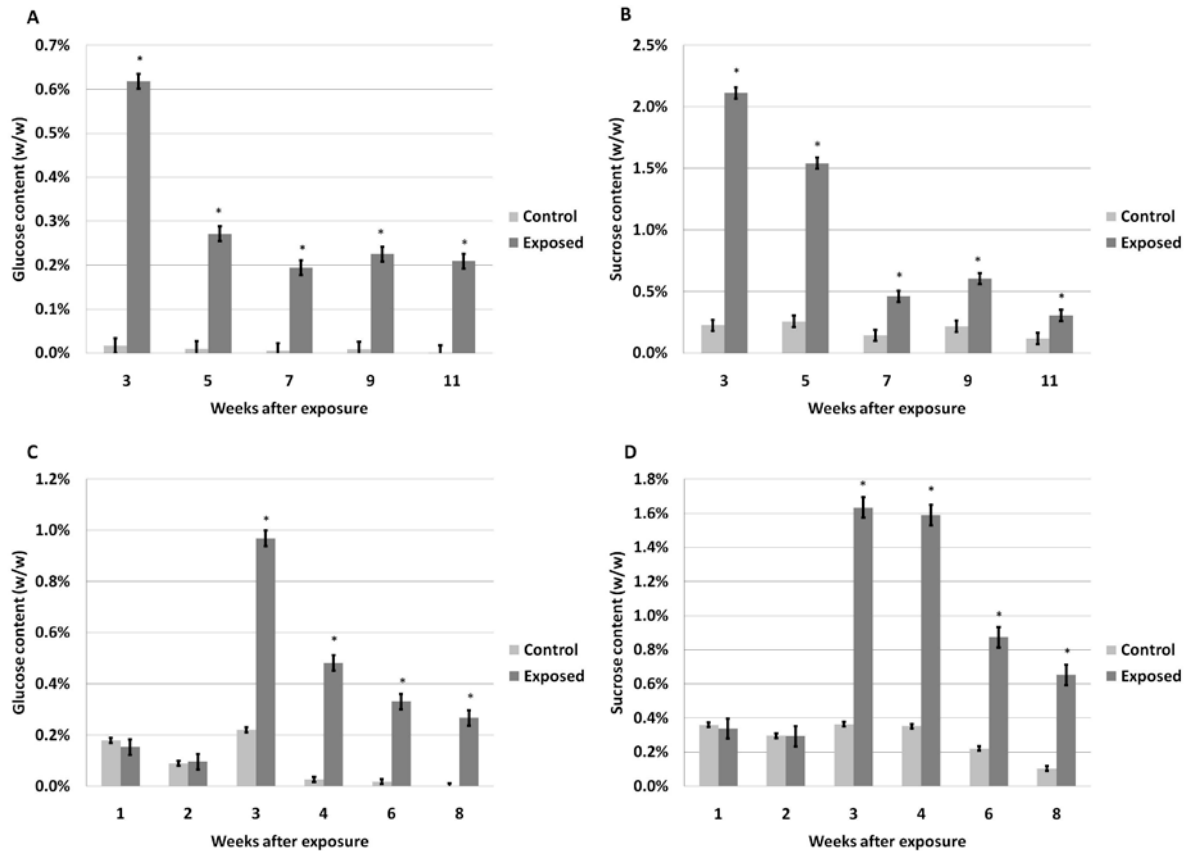


Fig. 1 The concentration of reducing sugars, glucose (a and c) and sucrose (b and d) found in tubers of plants either exposed to Lso-infective potato psyllids or not exposed over two field seasons, 2009 (a and b) and 2010 (c and d). *Asterisks denote significant treatment differences (Tukey's test).

Based on the results from this study, symptoms develop rapidly in plants following exposure to liberibacter-infective psyllids. Glucose contributes to darkening in fried potato products and thus the simultaneous rise in glucose levels and the arrival of symptoms may be linked. Both glucose and sucrose are known components of defense pathways in plants (Gibson 2003) and the elevated levels in psyllid-exposed plants may be related to the plant's response to pathogen invasion. Finally, the stunted yield development in exposed plants suggests a rapid shutdown of the bulking processes normally observed in tuber development. Stolon degeneration is another symptom related to ZC and if it occurs as the symptoms develop it may indicate that the yield flattening effect following infection was related to interruption of photosynthetic products needed by the tubers to bulk.

This study has shown that growers have a very short window of opportunity following the invasion of liberibacter-infective psyllids before ZC symptom expression appears. We caution that this study examined only a single variety and one plant stage (bloom) for all exposures. It would be valuable to examine the timing of symptom development among different potato plant stages and between different cultivars.

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EFFECTS OF INSECTICIDES ON BEHAVIOR OF ADULT *BACTERICERA COCKERELLI* (HEMIPTERA: TRIOZIDAE) AND TRANSMISSION OF “*CANDIDATUS LIBERIBACTER PSYLLAUROUS*”

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Summary

The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae), is a serious pest of potatoes that can cause yield loss by direct feeding on crop plants and by vectoring a bacterial pathogen, *Candidatus Liberibacter psyllaeus/solanacearum* (LPS). Current pest management practices rely on the use of insecticides to control potato psyllids to lower disease incidences and increase yields. While many studies have focused on the mortality that insecticides can cause on potato psyllid populations, little is known regarding the behavioral responses of the potato psyllid to insecticides or if insecticides can decrease pathogen transmission. Thus, the objectives of this study were 1) to determine the effects of insecticides on adult potato psyllid behaviors through direct observations and with electrical penetration graphs (EPGs); 2) determine the residual effects of insecticides on potato psyllid behaviors over time; and 3) determine effects of these insecticides on LPS transmission. Insecticides tested included imidacloprid, kaolin particle film, horticultural spray oil, abamectin, and pymetrozine. All insecticides significantly reduced feeding durations and increased the amount of time adult psyllids spent off the leaflets suggesting that these chemicals may be deterrents to feeding as well as repellents. Non-feeding behaviors such as tasting, resting, and cleaning showed variable relationships with the different insecticide treatments over time. EPG recordings of psyllid feeding revealed that imidacloprid significantly lowered salivation, ingestion of phloem and ingestion of xylem sap. The insecticides imidacloprid and abamectin significantly lowered transmission of LPS compared to untreated controls. We discuss the implications of our results for the use of imidacloprid in an integrated pest management program for potato psyllid and disease control.

Introduction

The potato psyllid is a major pest of solanaceous crops in Central and North America (Cranshaw, 1994, Jackson et al. 2009). Insecticidal control of the potato psyllid has been the subject of extensive research and is the current pest management practice used for potatoes in the U.S.A. to control the potato psyllid and lower zebra chip (ZC) incidence. While these studies focused on the mortality caused by insecticides on the potato psyllid, little is known regarding the behavioral responses of the potato psyllid to insecticides, or if insecticides can prevent or lower transmission of pathogens. This information would be useful for the selection of insecticides for potato psyllid control (Liu and Trumble 2004). Behavioral responses of insects to insecticides often provide important contributions to chemical control efforts because insecticides can interfere with the normal behavior patterns and might

therefore contribute to management of populations (Pluthero and Singh, 1984, Haynes, 1988). Previous research by Liu and Trumble, (2004) documented the behavioral responses of the potato psyllid to insecticides and tomato plant lines. Their results indicated that there can be interactions between potato psyllid behavioral responses to insecticides and tomato lines (Liu and Trumble 2004). Behavioral assays on potato plants are now needed to assess the impact of insecticides, given the significance of the potato psyllid both as a pest of potatoes as well as the vector of the ZC associated pathogen. Thus, the objectives of this study were to: 1) determine the effects of insecticides on adult potato psyllid behaviors, 2) determine the residual effects of insecticides on potato psyllid behaviors over time on potato plants, and 3) determine if these insecticides can decrease transmission of LPS. Analysis of behavioral responses can be used to evaluate insecticides that have the potential to impact psyllid population development and decrease the incidence of ZC. The long-term goal of this research is to maximize the effectiveness and use of insecticide selection, and to increase options for future management of potato psyllid both as a pest and a vector.

Materials and Methods

Bactericera cockerelli were originally obtained from field collections in Texas. Host plants were tomatoes (*Solanum lycopersicum* L. cv. 'Yellow Pear'). A host plant other than potato was chosen as the rearing host to avoid insects developing a preference for their natal host plant (Tavormina, 1982). Adult females were used in all behavioral tests. Potato (*Solanum tuberosum* L. cv. 'Atlantic') plants were used in all chemical trials. Plants were treated with insecticides once the plant produced 8-12 leaves, and selecting the uppermost fully expanded leaves standardized behavioral assays.

We evaluated five insecticides: one soil-applied systemic material and four that were applied to foliage. Chemicals and rates included in this study were imidacloprid (Admire Pro[®], Bayer Corporation, Kansas City, MO; 0.54 ml Admire/liter, applied to the soil), kaolin clay particle film (Surround WP[®], Engelhard Corporation, Iselin, NJ; 50 g/liter, applied with a pressurized sprayer), horticultural spray oil (Pure Spray Oil[®], Petro-Canada, Mississauga, Ontario, CA; 10 ml/liter, applied with a pressurized sprayer), abamectin (Agri-Mek[®] 0.15 EC, Syngenta Corporation, Greensboro, NC; 1.25 ml/liter, applied with a pressurized sprayer), and pymetrozine (Fulfill[®], Syngenta Corporation, Greensboro, NC; 0.42 g/liter, applied with a pressurized sprayer). Controls were treated with distilled water. Plants treated with imidacloprid were tested weekly for 6 wks post-application. With foliar-applied insecticides, leaves were used 24 h after treatment and were further examined 1 wk and 2 wks post-application. Imidacloprid residues in potato leaf tissue were measured by ELISA (QuantiPlate[®] kit for imidacloprid available from EnviroLogix, 500 Riverside Industrial Parkway, Portland, ME). At 3 and 6 wks after treatments, leaves were sampled (N = 12) from the plants.

Behavioral assays. All assays were based on the protocols of Liu and Trumble (2004). A newly emerged adult was placed into the arena and allowed to adjust for 5 min before initiating behavioral recording. An observation period lasted 15 min. Preliminary studies (Liu and Trumble, unpublished data) indicated that the 15-min observation period was sufficient for the psyllids to exhibit most of the behaviors. The observations were recorded using the

Noldus Observer program (Noldus, Wageningen, The Netherlands). Specific behaviors recorded included cleaning (using legs to cleanse or wipe antennae, appendages or abdomen), feeding, jumping (leaping from one point to another on the leaflet), off-leaflet (exiting or abandoning the leaf surface), tasting (tapping the mouthparts on the leaf surface sporadically), resting (no activity on the leaflet and mouthparts not in contact with the leaflet), and walking (walking on the leaf surface). The behavioral observations were replicated 20 times with different psyllids for each of the insecticides and for each of the time periods.

Electrical penetration graph (EPG). Potato plants were treated with imidacloprid as described above, and were both examined one week post-application. Adult female potato psyllids had a 10- μ m-diameter 1-cm long gold wires that were glued to their pronotums with electrically conductive water-based glue. The wire was then attached to the EPG input and the tethered insect was placed on the abaxial surface of a leaflet of a potted potato plant. To complete the electrical circuit, another copper electrode was inserted into the pot soil. EPG recordings were recorded using a DC-monitor, GIGA-8. Each psyllid was monitored for 5 h and the data of 20 individuals were analyzed for each treatment.

Transmission assays. Ten psyllids (subsequently determined to be infected, see below) were caged on a 7 x 7.5 cm cage on the terminal leaflet of one of the fully expanded potato leaves for a 24-h inoculation access period for each of the foliar-applied insecticides treatments 24 h post-application, and for the plants treated with imidacloprid 1 wk and 4 wks post-application. After 24 h, the psyllids were removed from the leaflet and placed in 100% ethanol and stored at -20°C until real-time PCR analysis. The plants were held for 2 wk after potato psyllid exposure to allow disease development. The potato leaf was then removed from the plant and placed in a Ziploc® bag and stored at -80°C until real-time PCR analysis.

Statistical analysis. For the imidacloprid treatments, durations of behaviors were analyzed using analysis of variance (ANOVA) in a general linear models procedure of SAS version 9.2 (PROC GLM; SAS Institute, 2008). When effects were significant ($P < 0.05$), multiple comparisons tests using the LSMEANS/PDIFF option were accomplished to discriminate differences among treatment means. A nonparametric Kruskal-Wallis Test (PROC NPAR1WAY; SAS Institute, 2008) was used to test the differences between the mean amounts of imidacloprid in the potato leaf disc samples. For the foliar-applied insecticides, durations of behaviors were analyzed separately for each time period using ANOVA in a general linear models procedure of SAS version 9.2 (PROC GLM; SAS Institute, 2008). When treatment effect was significant ($P < 0.05$) a least significant-difference (LSD) test was used to discriminate significant differences among treatment means. The number of waveform events per insect, and waveform duration per insect were analyzed with t-tests. Treatment differences in the number of potato plants infected with *Ca. L. psyllaureus* were compared using a Fisher's exact test (SAS Institute 2008).

Results and Discussion

Our results indicate that the use of these insecticides can reduce feeding times, increase abandonment of potato leaflets, and applications of imidacloprid and abamectin can decrease

disease transmission of LPS as compared to controls. Psyllids spent significantly less time feeding on potato plants treated with imidacloprid compared to the controls ($F = 131.60$, $df = 1$, 225 , $P = <0.0001$) and there were significant differences in feeding durations of psyllids over the six wk experimental period ($F = 2.40$, $df = 5$, 225 , $P = 0.0383$). This effect was most profound 1 wk after treatment in which the duration of feeding averaged 5.39 s, a 99% decrease compared to the control. This was significantly less than the time spent feeding for psyllids exposed to plants four (198.92 ± 75.92 s), five (258.92 ± 85.78 s), and six (201.29 ± 76.31 s) wks post-application. Psyllids spent more time cleaning (except for wk 1) when exposed to plants treated with imidacloprid compared to controls ($F = 4.69$, $df = 1$, 225 , $P = 0.0314$). On average psyllids spent 32.07 ± 10.83 s cleaning on plants treated with imidacloprid versus 7.39 ± 3.41 s on control plants. Psyllids consistently spent significantly more time resting ($F = 54.47$, $df = 1$, 225 , $P = <0.0001$) and more time off the potato leaflet ($F = 7.81$, $df = 1$, 225 , $P = 0.0056$) on potato plants treated with imidacloprid compared to controls. On average psyllids spent 443.94 ± 34.96 s resting on plants treated with imidacloprid versus 123.28 ± 23.67 s resting on control plants. Psyllids spent on average 253.39 ± 33.38 s off the potato leaflet on plants treated with imidacloprid whereas psyllids spent 135.99 ± 25.40 s off the potato leaflet on control plants. Systemic imidacloprid application can significantly reduce the durations of feeding times, and increase the amount of time spent off the potato leaflet for up to 6 wks post-treatment. These results suggest that imidacloprid can act as both a feeding deterrent and as a repellent (i.e., adults orient themselves away from treated surfaces). This compound appears to have residual activity for at least 6 wks, which may lower the need for additional applications.

The percentages of imidacloprid present within the leaves sampled at 3 and 6 weeks were 78% and 52%, respectively. There were significant differences in the mean amounts of imidacloprid in the potato leaf disc samples ($X^2 = 32.33$, $df = 2$, $P = <0.0001$). The average amount of imidacloprid in the controls (0.00 ± 0.00 $\mu\text{g/g}$, $N = 12$) was significantly less than the amounts in the treated plants sampled three (129.65 ± 6.44 $\mu\text{g/g}$, $N = 12$) and six (78.26 ± 4.60 $\mu\text{g/g}$, $N = 12$) wks after application, which indicate that these levels were high enough to impact behaviors.

Furthermore, EPG recordings of psyllid feeding revealed five EPG waveforms which include: (NP) non-probing, (C) intercellular stylet penetration, (D) initial contact with phloem tissue, (E1) salivation into phloem sieve elements, (E2) phloem sap ingestion, and (G) ingestion of xylem sap. Psyllids spent significantly more time non-probing ($p = <0.0001$) on plants treated with imidacloprid compared to controls. In addition, psyllids spent significantly less time salivating into sieve elements ($p = 0.0283$), ingesting phloem sap ($p = 0.0002$), and ingesting xylem sap ($p = 0.0417$) on plants treated with imidacloprid compared to controls. The effectiveness of imidacloprid on potato psyllid behaviors remains to be tested in the field, but the recommendations for imidacloprid application at the time of planting (UC IPM Online 2008, Goolsby et al. 2007) suggests the strategy has proven useful.

For the foliar-applied insecticides, the durations of feeding and abandonment of leaflets were significantly different by treatment. Durations of feeding were significantly less for psyllids exposed to insecticides 24 h ($F = 2.60$, $df = 4$, 91 , $P = 0.0414$), 1 wk ($F = 5.08$, df

= 4, 91, $P = 0.0010$), and 2 wks ($F = 5.27$, $df = 4, 91$, $P = 0.0007$) after application as compared to controls. Durations of time feeding averaged 243.33 ± 72.71 s for psyllids feeding on control plants which was significantly more than the time spent feeding for psyllids treated with horticultural spray oil (83.45 ± 52.36 s) and abamectin (41.49 ± 23.97 s) 24 h post-application. Feeding time durations were all significantly less for all insecticides tested 1 wk and 2 wks after application when compared to the controls (Table 4). Time spent off the potato leaflet was significantly greater for psyllids exposed to insecticides 24 h ($F = 2.47$, $df = 4, 91$, $P = 0.0499$) and 2 wk ($F = 2.93$, $df = 4, 91$, $P = 0.0250$) post-application when compared to controls.

All of the psyllids tested were infected with *Ca. L. psyllaurous*. There were significant decreases in the number of potato plants that were infected with *Ca. L. psyllaurous* based on treatment compared to controls (imidacloprid, 1 wk post-treatment: $X^2 = 4.46$, $df = 1$, $P = 0.0412$; imidacloprid, 4 wk post-treatment: $X^2 = 4.89$, $df = 1$, $P = 0.0341$; abamectin, 24 h post-treatment: $X^2 = 4.29$, $df = 1$, $P = 0.0433$). None of the other foliar-applied insecticides were significantly different from the control. The insecticide treatments of imidacloprid at 1 wk and 4 wks post-application, and abamectin at 24 h post-application decreased infection by 59%, 64%, and 64%, respectively. Thus, for imidacloprid and abamectin, the behavioral modifications resulting from antifeedant effects, repellency, toxicity, or a combination of these activities on psyllid adults are sufficient to lower disease transmission. Furthermore, like imidacloprid, the effectiveness of abamectin for reducing feeding, increasing abandonment of leaflets, and lowering disease transmission remains to be tested in the field.

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FEEDING BIOLOGY OF THE POTATO PSYLLID, *BACTERICERCA COCKERELLI*

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Summary

Our Knowledge of piercing-sucking arthropod feeding behavior has changed dramatically since the introduction of the earliest form of the modern day electrical penetrating graph (EPG) monitor in 1964. This system makes the insect part of an electrical circuit and produces patterned fluctuations in voltage known as waveforms. These can be used to describe certain behaviors, such as salivation and ingestion, through a process of correlation using techniques such as histology, honey dew analysis, and artificial diet studies. During the present study, EPG was used to analyze the stylet penetration behaviors of the potato psyllid, *Bactericera cockerelli*, on Atlantic, a well documented zebra chip susceptible potato cultivar. *Bactericera cockerelli* waveforms were for the first time identified and then defined using light microscopy of salivary sheath termini in plant tissue after probes were artificially terminated; the different waveforms are discussed herein.

Identifying and defining waveforms of *B. cockerelli* is the essential first step towards implementing EPG as a tool for increasing the understanding of feeding behavior or pathogen transmission by this insect vector of zebra chip potato disease. Also, EPG could be a very useful tool for screening potato varieties for resistance to zebra chip. In addition, this technique could help evaluate effectiveness and mode of action of insecticides used to control this insect pest.

Introduction

Our knowledge of piercing-sucking arthropod feeding behavior has changed dramatically since the introduction of the earliest form of the modern day electrical penetrating graph (EPG) monitor in 1964 (McLean & Kinsey, 1964; Backus & Bennett, 2009). This system makes the insect part of an electrical circuit, allowing for the measurement of resistance changes along with biopotentials that occur during probing (McLean & Kinsey, 1964; Tjallingii, 1978, 1985, 1988; Walker & Janssen, 2000; Backus et al., 2000). These measurements of patterned fluctuations in voltage known as waveforms can be used to describe certain behaviors i.e. salivation and ingestion through a process of correlation using techniques such as histology, honey dew analysis, and artificial diet studies (McLean & Kinsey 1964, 1965; Tjallingii, 1978; Kawabe & Mclean, 1980; Auclair et al. 1982; Rapusas & Heinrichs 1990; Backus 1994). This is essential for understanding the process of plant pathogen transmission by insect vectors, including acquisition and inoculation of the pathogen from/to host plants. While probing behavior activities and transmission processes have been studied in great detail with aphids and some of the phytopathogenic viruses they transmit, little is known of other important hemipteran insect vectors such as leafhoppers and psyllids (Prado & Tjallingii 1994; Bonani et al., 2009). One such example is the potato psyllid, *Bactericera cockerelli*, a widely dispersed vector of “*Candidatus Liberibacter solanacearum*” (also known as *Ca. Liberibacter psyllae*) (Hansen et al. 2008; Liefting et

al. 2009), a phloem limited pathogen that is suspected to cause an economically important defect in potato known as “zebra chip” (Munyaneza et al., 2007).

Zebra chip caused millions of dollars in losses to potato growers in the 2004, 2005 and 2006 growing seasons in the United States and Mexico (Munyaneza et al. 2007). The disease has also been documented in Central America and New Zealand and is associated with the potato psyllid (Crosslin et al. 2010). Despite this insect being such an important vector, very little is known about the potato psyllid probing activities including phloem ingestion in which *Liberibacter* is both acquired and inoculated in susceptible potato plants. This knowledge is crucial for developing pest management strategies that would impede upon the insects feeding behavior and lessen its efficacy as a vector. Here in, we report on how EPG was used to study and establish for the first time the stylet penetration behaviors of the potato psyllid feeding on potato (cv. ‘Atlantic’). Waveforms produced by adult *B. cockerelli* on Atlantic were also defined using light microscopy of salivary sheath termini in plant tissue after probes were artificially terminated to correlate the identified waveforms.

Materials and Methods

The study was conducted at laboratories of both USDA-ARS in Parlier, CA and Wapato, WA. Adult *B. cockerelli* were obtained from a colony that had been established at the USDA-ARS Laboratory in Wapato, WA. Psyllids were maintained under greenhouse conditions with a photoperiod of 16:8 (L: D) and temperature of 24-29°C. The live insects were immobilized by grasping their wings with insect forceps and tethered using 0.001in. diameter gold wire and silver glue. Wired insects were then starved for 0.5 hr before being placed on the test plant. Feeding was observed and recorded for several psyllids using an AC/DC EPG monitor for a period of 24 hr each. Selected psyllids had their probing activities artificially terminated when particular waveforms were observed to correlate the activities with feeding behavior. Tissues containing the salivary sheath were histologically processed according to protocols described in Backus et al. (2007). Salivary sheath termini were then examined using light microscopy.

Results and Discussion

B. cockerelli adults produced a number of distinctive waveforms that were described for the first time in association with stylet sheath termini position in the plant tissue. Of these waveforms four main probing activities were described, including stylet pathway activities representing the secretion of the salivary sheath while probing in the vicinity of epidermal and parenchyma cells (C), ingestion from xylem tissue (G), salivation prior to phloem ingestion (E1), and phloem ingestion (E2) (Fig. 1). It is thought that during the duration of waveform E2 the pathogen is most likely acquired from an infected host plant (Prado and Tjallingii, 1994; Bonani et al., 2009), whereas the pathogen is most likely inoculated into the host plant via egestion occurring during salivation of the vector during the duration of waveform E1.

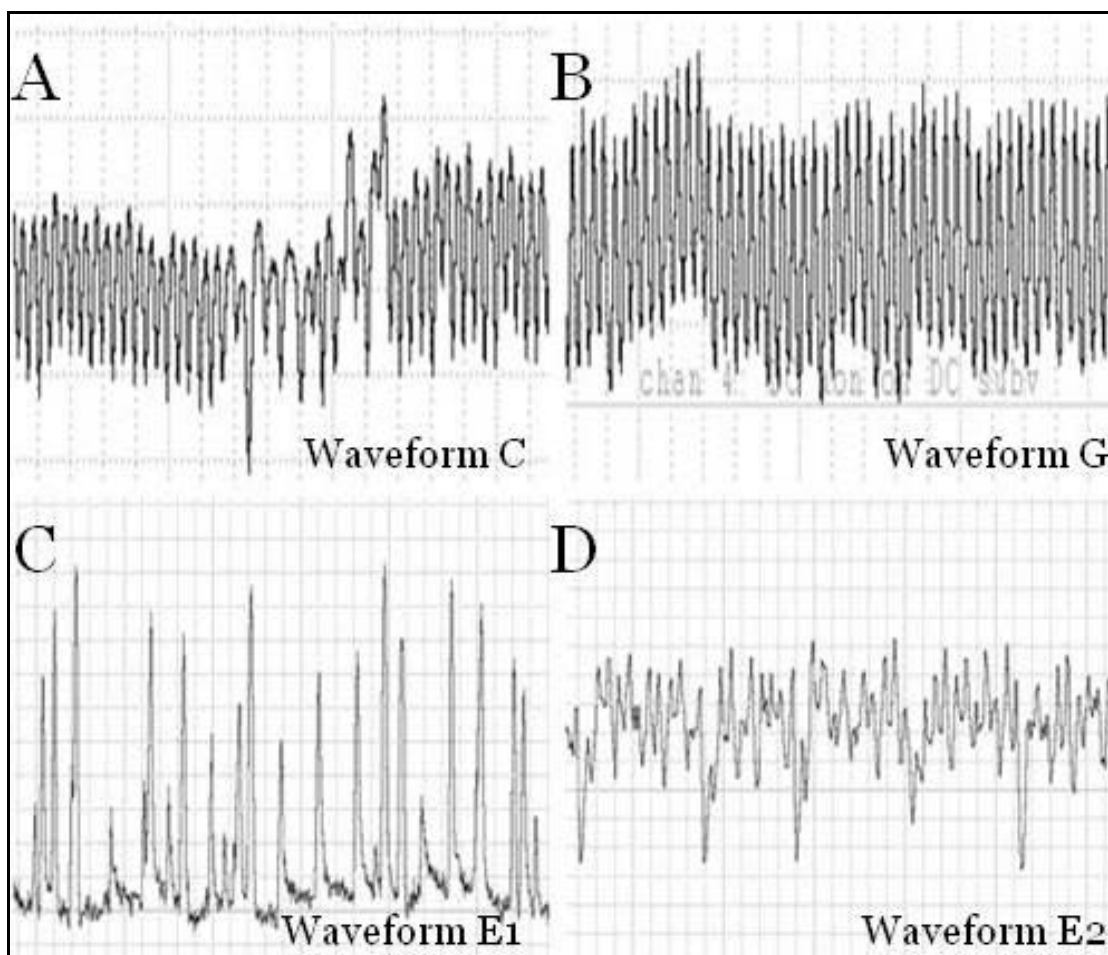


Figure 1. Electrical Penetration Graph (EPG) waveforms produced by *Bactericera cockerelli* while feeding on potato (cv. ‘Atlantic’): stylet pathway activities (A), xylem ingestion (B), salivation (E1), and phloem ingestion (E2).

Identifying and defining waveforms of *B. cockerelli* is the essential first step towards implementing EPG as a tool for increasing understanding of liberibacter transmission by the potato psyllid and for screening plants for host plant resistance to either direct feeding or liberibacter transmission by this insect vector of zebra chip. With the establishment of waveforms for *B. cokerelli* it will also be possible to assess alternative host species which may act as reservoirs for liberibacter. In addition, these waveforms may be used in pesticide efficacy trials to develop regimens that maximize effectiveness of these potato psyllid management tools. Moreover, EPG waveforms of *B. cockerelli* could lead to faster development of host plant resistant varieties, strengthen integrated pest management strategies by incorporating alternative plant hosts, and maximize the efficiency of pesticides. In short, knowledge developed with the EPG technique will contribute to better understanding of mechanisms by which the potato psyllid transmit liberibacter to potato and other important solanaceous crops and help affected potato produce reduce damage caused by zebra chip disease.

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INVESTIGATING THE TRANSCRIPTOME OF THE POTATO PSYLLID (*BACTERICERA COCKERELLI*): TOWARD AN RNAI BASED MANAGEMENT STRATEGY

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Summary

The potato psyllid is the vector of the α -Proteobacteria *Candidatus* Liberibacter psyllaourous, the causal agent of Zebra Chip in potatoes. The disease is a major limiting factor in both the production and processing of potatoes into chips and there is currently no cure. RNA interference (RNAi) has the potential to limit the spread and severity of Zebra Chip by reducing the insect load per field per season. Pyrosequencing was used to identify target genes and synthetic dsRNA constructs were designed to block the activity of heat shock protein 70 and heat shock cognate 70. These molecular chaperones are intimately involved in essentially all life processes by folding and stabilizing other proteins. In this study these constructs were tested on potato psyllid cell cultures and morphological evidence showed shriveling and dispersal into the media. We plan to target other genes and deliver RNAi to insects via the plant through a root soaking procedure.

Introduction

Zebra chip (ZC) is a disease of the potato (*Solanum tuberosum* L.) (Abad et al 2009) characterized by chlorosis, yellowing, curling and scorching of leaves, swollen nodes and aerial tubers. When below ground tubers are sliced and processed into potato chips, alternating light and dark bands along the medullary rays become prominent due to the increased amount of soluble sugars in infected plants (Gao et al 2009). The disease is caused by an intracellular infection by the recently implicated phloem-limited α -Proteobacteria *Candidatus* Liberibacter psyllaourous (Hansen et al 2008; Liefing et al 2008, 2009). The potato psyllid (*Bactericera cockerelli*), a phloem feeding insect pest of solenaceous plants, has been associated with the disease (Munyanza et al 2007) and is now a known vector of the putative causal agent.

Once referred to as papa manchada or stained potato, Zebra chip was originally identified in potato fields surrounding Saltillo, Mexico, in 1994 (Secor, unpublished). Since then the disease has caused millions of dollars in losses (CNAS 2006; Secor and Rivera-Varas 2004) and continued to be a major threat to both producers and processors (Hernandez-Garcia et al 2006; Salas-Marina et al 2006), having spread north into the United States and south into Guatemala. In 2004 the disease was so prevalent that in the northeastern states of Coahuila and Nuevo Leon as many as 80% of plants in affected fields showed symptoms. Many fields with particularly heavy infestations of *Candidatus* Liberibacter infected potato psyllids have had to be abandoned entirely (Flores et al 2004).

When genes are active, or are coding, they produce mRNA which instructs the cell to manufacture a protein derivative of the gene. RNA interference (RNAi) is a method of down regulating specific genes in a cell by the application of synthetic double stranded RNA (dsRNA) molecules designed to be complementary to a gene's mRNA (Fire et al 1998). When the dsRNA taken into the cell, a protein called Dicer cleaves it into small segments. These segments are incorporated into the RNA induced silencing complex (RISC) and the cell's mRNA, using the synthetic RNA as a template, is enzymatically degraded thereby blocking protein production in a dose dependent manner. In an alternative pathway, the cleaved segments of dsRNA are used as templates for the RNA dependent elongation enzyme RdRP; in this way multiple off-target genes can be down regulated by a single dsRNA construct. Since its discovery, RNA interference has proved promising in determining a gene's function, controlling cancer and slowing viral replication (Huvenne and Smaghe 2010). Many studies have indicated that RNAi has great potential in managing insect pest – especially those that are capable of causing damage or disease in plants (Borovsky, 2005; Gordon and Waterhouse, 2007; Price and Gatehouse, 2008). In this study we used pyrosequencing to compile cDNA libraries for the adult and 5th instar lifestages of the potato psyllid. With this information we identified several targets for an RNAi-based management strategy.

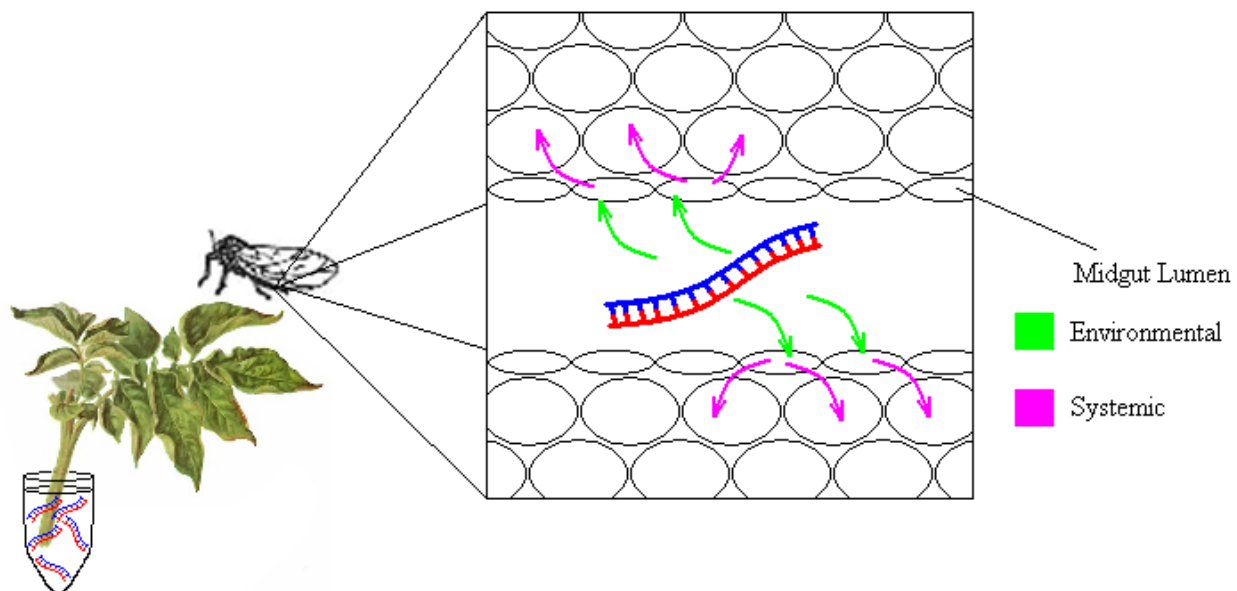


Figure 1. A novel dsRNA delivery mechanism for the RNAi in the Potato Psyllid. The roots of the plant are soaked with the constructs and drawn up into the plant to be delivered upon feeding.

Materials and Methods

Potato psyllids used to start a colony were provided by Drs. Tong-Xian Liu and Xiangbing Yang (Texas AgriLife Research) and maintained on potatoes (25°C; 40% humidity). Adult and 5th instar psyllids of mixed genders were collected and total RNA was extracted using the RNeasy mini Kit (Qiagen). Poly-A mRNA was isolated with the Oligotex mRNA mini kit (Qiagen), retrotranscribed using Stratagene's ZAP-cDNA synthesis kit and sent to the Research and Testing Laboratory of the Medical Biofilm Research Institute for pyrosequencing. Double-stranded cDNA was quantified and nebulized to 300-550bp

fragments and the pyrosequencing library was created according to manufacturer's instructions (Roche 454). Resulting sequences were assembled using DNASTar's NGEN assembler (Madison WI) and annotated using BLASTx - W.ND BLAST (Dowd et al. 2005) and cross referenced to functional annotations using DAVID (Huang et al. 2009).

Results and Discussion

Sequences homologous to previously identified cellular function and metabolic activity genes were recovered from the libraries. Sequences related to ribosomal functions, organelle construction, and muscular, neurological, and reproductive developmental processes were recovered. Stress response, ion transporters, nucleic acid binding, and primary metabolism sequences were also recovered. This information can be used to identify gender and life-stage-specific genes for RNAi-based management strategies, providing new direction for targeting single pest insects as opposed to the current broad spectrum insecticide regimes. Genes of interest include vitellogenin, an egg yolk precursor protein, ejaculatory bulb protein, a male reproductive protein, and actin II, one of the many genes responsible for proper wing function or formation.

Current targets are general house-keeping genes like heat shock proteins and heat shock cognates, molecular chaperones that influence essentially all cellular pathways by trafficking proteins to their proper locations in the cell and fold or refold proteins into their enzymatically active configuration. Presently, we are attempting to use a cocktail of 3 dsRNA constructs against the heat shock protein 70 (HSP 70) gene and 3 dsRNA constructs against the heat shock cognate 70 (HSC 70) gene to limit the viability of potato psyllid cells in culture. In response to the dsRNA cocktail, the cells began to shrivel, disconnect from the monolayer and disperse into the media, indications of overall poor health. We plan to investigate the gene knock down efficiency of the cocktail by quantifying the amount of mRNA specific to the target genes in non-treated, buffer treated and dsRNA treated cells. If this cocktail proves successful we plan to scale up the process and work with whole insects on potato plants to test the ability of dsRNA to limit psyllid populations via the digestive tract. In a separate study we have developed a novel delivery method for the potato psyllid based on saturation of the host plant's roots with dsRNA (Figure 1). The constructs are efficiently drawn up the roots, into the stems and delivered via the leaf veins to the insect.

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POTATO PSYLLID DENSITY AND FEEDING DURATION REQUIRED TO CAUSE ZEBRA CHIP

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Summary

Zebra chip is an emerging and economically important disease of potato in the United States, Mexico, Central America, and New Zealand. The disease has recently been associated with *Candidatus Liberibacter solanacearum* (syn. *Ca. L. psyllaureus*) and is transmitted by the potato psyllid, *Bactericera cockerelli*. A study was conducted to quantify the feeding duration and density of liberibacter-infective psyllids required to cause ZC and to determine if psyllid density can influence zebra chip disease incidence and severity. Results indicated single psyllids can successfully induce zebra chip disease in potato plants. In addition, results indicated that inoculation time for the potato psyllid appears to be very short, ranging from 1 to 6 hours, depending on whether multiple or single psyllids were used. Furthermore, it was found that psyllid density does not appear to affect disease severity in potato.

Impact Statement

Results of these series of experiments showed that a single liberibacter-infective potato psyllid is enough to cause zebra chip disease in potato, after a relatively short period of exposure to plants. Therefore, insecticides that can quickly kill potato psyllids or deter them from feeding are needed to effectively manage zebra chip disease in potatoes.

Introduction

Insect vector density, feeding time and infective status are critical elements to the eventual production of disease in insect-transmitted plant pathogen systems. The number of pathogen-infective insects and the access time required to effectively inoculate the host plant can vary by taxonomic group and species. Zebra chip (ZC) is an emerging disease of potato and the potato psyllid (*Bactericera cockerelli*) was identified as a primary vector (Munyaneza et al. 2007, 2008). The putative pathogen of ZC, *Candidatus Liberibacter psyllaureus/solanacearum* (Lso) (Hansen et al. 2008, Liefting et al. 2009) has been associated with individual psyllids and ZC-affected potato plants and tubers. The exposure and exclusion cage studies by Munyaneza et al. (2007, 2008) used high densities of psyllids over long periods of time which may not approximate the insect pressure and host exposure time experienced under natural field conditions. It is imperative that potato psyllid density and inoculation access period to effectively cause ZC be accurately determined. In addition to ZC incidence, vector density can also influence the severity of disease expression. The purpose of this study was to quantify the feeding duration and density of Lso-infective psyllids required to cause ZC and to determine if psyllid density can influence ZC disease severity.

Materials and Methods

The study was conducted under field and laboratory conditions at the USDA-ARS in Wapato, WA during 2009-2010. All potato plants (cultivar: Atlantic) used in the study were either grown from minitubers or certified seed. All psyllids used were from colonies originating from field collected insects from Moxee, WA during the fall of 2007 and had routinely tested positive for Lso by PCR. Disease incidence was evaluated by cutting the tubers at the stem end for presence of the typical ZC vascular discoloration. If any tubers from each plant had symptoms, the entire plant was considered diseased.

To determine the inoculation access period (IAP) of multiple potato psyllids, individual potato plants in pots were exposed to 20 potato psyllids confined to the plant by small hoop cages for the following durations: 24, 20, 16, 8, 4 and 1 hour(s). One plant was left psyllid-free to serve as a control and all treatments were replicated 5 times. To assess the single insect IAP, individual psyllids were exposed to potted potato plants for the following durations: 72, 24, 12 and 6 hours. One plant was left psyllid-free and all treatments were replicated 9 times. After each of the durations, the psyllids were recollected and the plants fumigated. The plants were maintained in the greenhouse and monitored for ZC foliar symptoms. After plant senescence, ZC incidence was measured.

To determine the density of psyllids required to produce ZC under laboratory conditions, individual potato plants were grown in pots in the greenhouse. At tuber initiation stage, psyllids were added to the plants and confined by small hoop cages over the pots at the following densities: 25, 16, 9, 4 and 1 psyllid(s) per plant. One plant was left psyllid-free as a control and all treatments were replicated 6 times and the entire study was repeated over two seasons. After 72 hours, the psyllids were recollected and the plants fumigated and were transplanted into field cages. After 60 days, the tubers were harvested from each plant and disease incidence was estimated.

To assess the role of insect density and ZC disease severity and to confirm the results of the laboratory study under field conditions, three densities of Lso-positive potato psyllids (9, 4 and 1 psyllid per plant) were released into field cages with 6 potato plants at tuber initiation stage. One cage was left psyllid-free as a control. All treatments were replicated four times and arranged in a Latin Square design and repeated over two field seasons. Subsets of the psyllids from the colony used were retained for PCR testing. After one week, the psyllids were eliminated with insecticides. Plants were monitored for symptoms and hand-harvested, 60 days following psyllid exposure. Disease incidence was measured by symptoms in cut raw tubers. Disease severity was assessed by individual mean plant yield, mean tuber weight, reducing sugar levels and ZC incidence percentage in tubers.

Results and Discussion

Multiple and single psyllid inoculation access periods

Results for inoculation feeding exposure times of 20 psyllids per plant are summarized in Table 1 whereas inoculation feeding exposure times of single psyllids are shown in Table 2. Potatoes exposed to multiple psyllids after only one hour were infected and showed a high rate of disease incidence after 4 hours. Single psyllid inoculations were successful after 6 hours but showed more variability across the exposure times. The exposure time differences observed between the multiple insect and single insect inoculations would be expected as more infective insects feeding on a single plant would likely be more effective at inoculating the host. Inoculation access periods are inclusive of a variety of factors and behaviors that an insect requires prior to actual feeding on the target tissue for successful inoculation including assessing overall plant acceptability, probing and chemosensory evaluation. Individual psyllids vary in the speed and efficacy of feeding and having the plant exposed to multiple psyllids would increase the chances that more psyllids would engage in feeding behavior resulting in inoculation in a shorter period of time.

Density of psyllids required to produce zebra chip

Table 3 summarizes how psyllid density impacts ZC incidence under controlled laboratory conditions. A density of one (1) psyllid was found to effectively inoculate potato plants and all higher

densities showed higher rates of inoculation and the trends were conserved between years. The results are consistent with our previous data that higher numbers of psyllids would

Table 1. Multiple psyllid (20 psyllids per plant) inoculation access periods

Exposure time, hours	# of plants	# of diseased plants	% diseased
0	5	0	0.0
1	5	1	20.0
4	5	5	100.0
8	5	5	100.0
16	5	5	100.0
20	5	5	100.0
24	5	5	100.0

Table 2. Single psyllid (1 psyllid per plant) inoculation access periods

Exposure time, hours	# of plants	# of diseased plants	% diseased
0	9	0	0.0
6	9	3	33.3
12	9	2	22.2
24	9	3	33.3
72	9	5	55.6

Table 3. Impact of psyllid density on zebra chip disease incidence under laboratory conditions

2009 Trial				2010 Trial			
# of psyllids	# of plants	# of diseased plants	% diseased	# of plants	# of diseased plants	% diseased	
0	6	0	0.0	6	0	0.0	
1	6	5	83.3	6	5	83.3	
4	6	6	100.0	6	6	100.0	
9	6	6	100.0	6	6	100.0	
16	6	6	100.0	6	6	100.0	
25	6	6	100.0	6	6	100.0	

be more effective agents of inoculation than single psyllids likely due to increased feeding behavior.

Psyllid density and zebra chip disease severity

Tables 4 and 5 summarize the ZC disease severity data collected over the 2009 and 2010 field seasons, respectively. Both the mean plant yield and the mean tuber size of treatments with Lso-infective psyllids were significantly different from the control, though no difference was found between the different densities of psyllids. A similar trend was observed in relation to the levels of reducing sugars, glucose and sucrose, with no significant difference found between the various density treatments but a difference between the psyllid exposed treatments and the control. Potatoes exposed to all densities of psyllid expressed high percentages of affected tubers between both field seasons.

Table 4. Impact of psyllid density on ZC disease severity under field conditions, 2009 field season

# of psyllids	Mean plant yield, g \pm SE	Mean tuber size, g \pm SE	Tuber glucose conc, w/w %	Tuber sucrose conc, w/w %	% of ZC symptomatic tubers
0	2696.32 \pm 239.18 ^b	204.23 \pm 19.99 ^b	0.0032 \pm 0.016 ^b	0.115 \pm 0.014 ^b	0.00
1	804.11 \pm 239.18 ^a	87.14 \pm 19.99 ^a	0.223 \pm 0.016 ^a	0.326 \pm 0.014 ^a	63.27
4	503.27 \pm 239.18 ^a	62.91 \pm 19.99 ^a	0.213 \pm 0.016 ^a	0.359 \pm 0.014 ^a	95.00
9	413.20 \pm 239.18 ^a	52.27 \pm 19.99 ^a	0.259 \pm 0.016 ^a	0.319 \pm 0.014 ^a	100.00

Table 5. Impact of psyllid density on ZC disease severity under field conditions, 2010 field season

# of psyllids	Mean plant yield, g \pm SE	Mean tuber size, g \pm SE	Tuber glucose conc, w/w	Tuber sucrose conc, w/w	% of ZC symptomatic tubers
0	2989.94 \pm 75.12 ^b	181.17 \pm 8.95 ^b	0.00082 \pm 0.011 ^b	0.110 \pm 0.014 ^c	0.00
1	1418.58 \pm 75.12 ^a	84.31 \pm 8.95 ^a	0.229 \pm 0.011 ^a	0.568 \pm 0.014 ^b	88.69
4	1263.95 \pm 75.12 ^a	77.34 \pm 8.95 ^a	0.264 \pm 0.011 ^a	0.504 \pm 0.014 ^{ab}	96.13
9	1099.53 \pm 75.12 ^a	70.80 \pm 8.95 ^a	0.265 \pm 0.011 ^a	0.476 \pm 0.014 ^a	99.58

**Means within each column followed by the same lowercase letter are not statistically different (Tukey's test).*

These results suggest that single psyllids are highly efficient vectors capable of inoculating plants with a bacterial titer sufficient to cause the disease. The results also suggest that the pathogen is the likely primary agent dictating the severity found in diseased plants as an increase in the number of Lso-infective psyllids gave no increase in severity measurements used in this study. The observed drop in tuber incidence under low psyllid pressure may be due to the feeding behavior of the psyllid and the number of plant stems. Potatoes planted from whole seed pieces, as used in this study, produce multiple stems. Each stem may need

to be inoculated individually thus the treatments with high densities of psyllids may allow for multiple sites of inoculation on multiple stems.

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GENETIC VARIATIONS OF “*CANDIDATUS LIBERIBACTER SOLANACEARUM*”

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Summary

Simple sequence repeat (SSR) primers based on the sequences of “*Candidatus Liberibacter solanacearum*” were validated in PCR experiments using 68 “*Candidatus Liberibacter solanacearum*” (Lso) strains isolated from potato plants collected from different geographical locations. Four primer pairs differentiated these Lso strains and divided them into two major groups, which are in agreement with the two groups based on 16S rRNA gene sequences revealed previously (Wen et al, 2009; Secor et al, 2009). Lso strains associated with zebra complex, psyllid-yellow, haywire and seedborne Zebra Complex were highly differentiated using the four primer pairs. Furthermore, the four primer pairs are efficient for epidemiology and population genetic studies.

Impact

SSR primers are well suited for differentiating strains of “*Candidatus Liberibacter solanacearum*” and can be used in further study of the ecology and epidemiology of the ZC bacterium.

Introduction

Previous studies have shown that “*Candidatus Liberibacter solanacearum*” (Lso) has associated with zebra complex (ZC), psyllid-yellow (PY), and haywire (HW) diseases of potato using specific primers developed based on 16S-ISR-23S rRNA gene sequence (Wen et al, 2009). Two types of Lso were differentiated by 8 SNPs based on 16S-ISR-23S rRNA gene sequence (Wen et al, 2009). Nonetheless, information regarding the population structure and genetic diversity of Lso is still unclear. A useful method of strain typing would facilitate source tracking, development of strategies for prevention and control, and the study of the Lso ecology and evolution.

Simple sequence repeats (SSRs) in DNA sequences are tandem iterations of a single nucleotide or a short oligonucleotide. SSRs are subject to slipped-strand mutations and a common source of phase variation in bacteria and antigenic variation in pathogens. SSR is commonly used for strain typing of pathogenic microorganisms.

In this study, SSR primers based on Lso genomic sequence developed by H. Lin (USDA-ARS, Parlier, CA) were used to study genetic variations among Lso strains isolated from potato plants displaying ZC, PY, HW, seedborne ZC (SBZC) and phytoplasma-like (unknown, UK) symptoms collected from different geographic locations.

Materials and Methods

Eighty-six freeze-dried tissue samples, Lso-positive tested previously (Wen et al, 2009), were selected based on host (potato, tomato, and silverleaf nightshade), geographical location (TX, NE, WY, CO, KS, MX and CA), symptom type (ZC, PY, HW, and SBZC), and year of

isolation (2007, 2008 and 2009). DNA was extracted from freeze-dried tissues as described previously (Wen et al, 2009) and quantified using Nanodrop (ThermoScientific). Quality of the 86 DNA samples were verified using a quantitative real time PCR based on *omp* gene (Lin, unpublished), and 68 of the DNA samples had PCR quality and were used in ZC SSR studies. PCR mixtures consisted of 20- μ L volumes containing 1.0 mM MgCl₂, 0.2 mM each deoxynucleoside triphosphate, and 0.5 U AmpliTaq Gold polymerase in 2 μ L of 10 x reaction buffer (Applied Biosystems, Foster City, CA), 0.5 μ M of each SSR primer with 1 μ L of genomic DNA (10 ng/ μ l). PCR reactions were conducted in a model ABI 9700 thermal cycler with the following thermal cycles: the initial denaturation step was 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 55°C for 45 s, and 72°C for 45 s, and a final extension at 72°C for 7 min. SSR PCR products were mixed with sample loading dye (10 mM NaOH, 95% formamide, 0.05% bromophenol blue, and 0.05% xylene cyanol) at a 1:2 ratio and denatured at 95 °C for 4 min, immediately put on ice. A 2- μ l aliquot of this mixture was resolved in a 5% polyacrylamide gel. The gel was run in 1 x TBE buffer (89 mM Tris, 89 mM boric acid, and 2.0 mM EDTA; pH 8.3) at a constant 100 W for 2.5 h. The gels were visualized by silver staining (Promega Biosciences Inc., San Luis Obispo, CA). Forty-four SSR primers designed by Lin (USDA-Parlier) were screened against 6 ZC DNA samples using PAGE gel with silver staining and candidate primer sets were found. The respective forward primers were fluorescent labeled. PCR products of the 68 ZC DNA samples using fluorescent labeled forward primer and regular reverse primer were genotyped using ABI 3130X1 and data were analyzed using GeneMapper (v.4.0). Data analyses were performed using PopGene Version 1.32 (Yeh et al, 1997), Multilocus version 1.3b (Agapow and Burt, 2001) and GeneAlex version 6.3 (Peakall and Smouse, 2006).

Results and Discussion

Polymorphism was detected by four Lso SSR primer pairs (Lso-SSR-1F/R, 4F/R, 8F/R and 9F/R) on 68 Lso strains, and two major groups of Lso were found (Table 1). Mexican Lso strains grouped together, whereas the genetic variation of Lso strains from the USA was not geographically related. Lso strains associated with ZC, HW, PY, UK and SBZC could be highly differentiated (Table 2 and Figure 1) (GSP = 0.8262 and Nm =0.1052). Lso strains associated with HW and SBZC are highly related with a 95.74% genetic identity, whereas Lso strains associated with PY and UK shared a 75.0% genetic similarity (Table 2). Among the Lso strains tested, two major groups formed: one group consisting of strains associated with ZC, HW and SBZC, and the other consisting of strains associated with PY and UK (Fig. 1). Lso strains are significantly diverse genotypically (AMOVA GD =0.7941 at p =0.001). Multilocus analysis showed that the primer pairs are efficient for differentiating Lso strains and for studying population genetics.

Table 1. Lso typing based on Lso SSR primer pairs (number of strains out of 68 strains tested)

Type	Lso-SSR-1F/R	Lso-SSR-4F/R	Lso-SSR-8F/R	Lso-SSR-9F/R
1	43	31	41	32
2	20	29	25	27
3	2	3	na	8
4	1	na	na	na
No amplification	2	5	3	1

Table 2. Genetic identity and genetic distance^x among Lso strains tested in this study

	ZC	HW	SBZC	PY	UK
ZC	*****	0.5977	0.5777	0.3415	0.2930
HW	0.5147	*****	0.9574	0.5833	0.3333
SBZC	0.5488	0.0435	*****	0.6963	0.4352
PY	1.0743	0.5390	0.3620	*****	0.7500
UK	1.2275	1.0986	0.8320	0.2877	*****

^x. Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

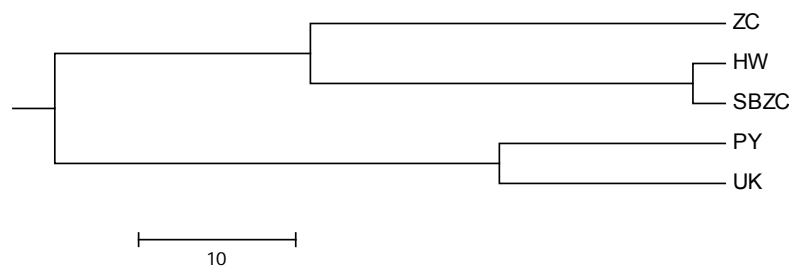


Figure 1. Genetic relatedness among Lso strains based on genetic distance (UPGMA)

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ASSESSMENT OF ACTIVE PSYLLID POPULATIONS IN CORRELATION WITH ZEBRA CHIP SEVERITY

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Summary

Zebra chip, a disease that causes internal brown coloration in the center of a tuber and a dark brown striped appearance in a fried tuber, first appeared in 1994 in southern Mexico and later spread to Texas around the year 2000. Plants show similar symptoms to purple top and psyllid yellowing. This disease along with zebra chip has been associated with the potato psyllid, *Bactericera cockerelli* (Munyaneza et al. 2007a; Liefting, L. W.). Ongoing research, including this project, is being used to understand this pathogen and determine if resistance and tolerance can be developed in the potato plants and prevent the spread of Zebra chip in other locations. Adult psyllids collected in this project will be used for conventional PCR and ISSR. If in fact these psyllids are migrating from one location to another, (Wallis 1955) preventative methods can be established to help reduce the incidence of zebra chip at each location.

Introduction

Causing millions of dollars to producers and potato processors, zebra chip (ZC) is emerging disease found in the southwestern United States, Mexico and Central America.(Munyaneza et al. 2007 a,b) The characteristics of ZC develop in the fried tuber. Brown strips of necrosis appear in the center and lead outward towards the edge of the chip. Aerial plant symptoms are similar to those of potato purple top disease, which include a slight purpling, aerial tubers, chlorosis, leaf rolling and leaf scorching. Ongoing research has proven the hardest hit areas are the lower Rio Grande Valley and Pearsall, Texas, which is where ZC first emerged. Little is known on how the active psyllid populations correlate with the incidence and severity of ZC, as well as the relationship between psyllids at each location (Goolsby, J.A et al. 2007). This project will primarily focus on the two topics.

Materials and Methods

This project compared the severity and incidence of zebra chip (*Candidatus Liberbacter*) at two different locations in Texas using the chipping cultivar Atlantic. These locations each had several different planting dates which corresponded with early, optimum, and late months. Locations included the USDA-ARS Research Facility in Weslaco, Texas with plantings in November, December, January and February and at Black Gold in Pearsall, Texas with plantings done in December, early January, late January, February, and March. Potato seed was obtained from Jack Wallace Farms in Edinburg, Texas and used for each location. Each location had plots established and maintained for each planting date. Seed pieces used were cut into three-to-four ounce pieces and treated with fir bark. At the Weslaco location, seeds were planted by the USDA-ARS personnel at the four different dates. Each plot had four rows and measured 150 feet in length. For the Pearsall location, seeds were cut in to three-to-four ounce pieces as well and planted in each of the established five plots, which were eight row plots also measuring 150 feet in length. Seeds were planted ten inches

apart in each row. For the location in Pearsall, wheat was planted in surrounding area of the plots including the ten foot spacing between each block. No insecticide was used in the project. Sampling was done weekly using the KISS® sampler (Beerwinkle et al., 1997) and manual leaf collecting. Weekly collection was done using the KISS® sampler and manual leaf collection. Using the KISS® sampler for this project ensures accurate numbers for DNA and PCR work for the reason that the net is in close proximity to the plant. Manual collection of leaves taken from the mid section of the plant was done to determine the number of immature psyllids, or nymphs, and eggs. Both mature and immature psyllids were identified in a laboratory setting, stored in a – 40 °C freezer in colored epindorff and labeled. Determination of severity and incidence of infected tubers was conducted at harvest. Each location was hand harvested and incidence was determined by cutting tubers at both ends and visually scoring using a simple “yes” or “no” on the presence or absence symptom. A yes was given if the tuber showed any signs or symptoms of ZC and the tuber was set aside for frying to determine severity. The tuber was sliced, fried for three minutes, and then examined for ZC symptoms. Adult and immature psyllids were identified, stored, labeled and frozen for DNA extraction and PCR analysis. The same DNA will also be used to determine relationships between psyllids at different locations. ISSR PCR will help to determine if, in fact, these psyllids are moving from one location to another. Adult psyllids will be used for DNA extraction, PCR, and ISSR work at a later date. PCR will be used to determine if the adult psyllid is positive for the bacterium *Candidatus Liberbacter*. ISSR will be used to determine if there is a relationship between psyllids collected in Weslaco and Pearsall.

Results and Discussion

The following reports are on the number of Adult psyllids collected at the three different locations using the KISS® Sampling method, the number of immature psyllids collected using manual leaf counts and the incidence of ZC at each location.

The following graph shows a slow incline of adult psyllid numbers, once the potato plant matured, followed by a slow decline due to the wet weather the Lower Rio Grande Valley experienced during this planting season. This graph also shows a high peak in April through May due to favorable temperatures the lower Rio Grande Valley experienced as well as mature plants. Once the plants senesced, the adult psyllid numbers slowly declined. This location, although experiencing very wet weather had a successful planting season as far as the plant itself is concerned. There were no reports of freeze damage or any pathogens other than ZC.

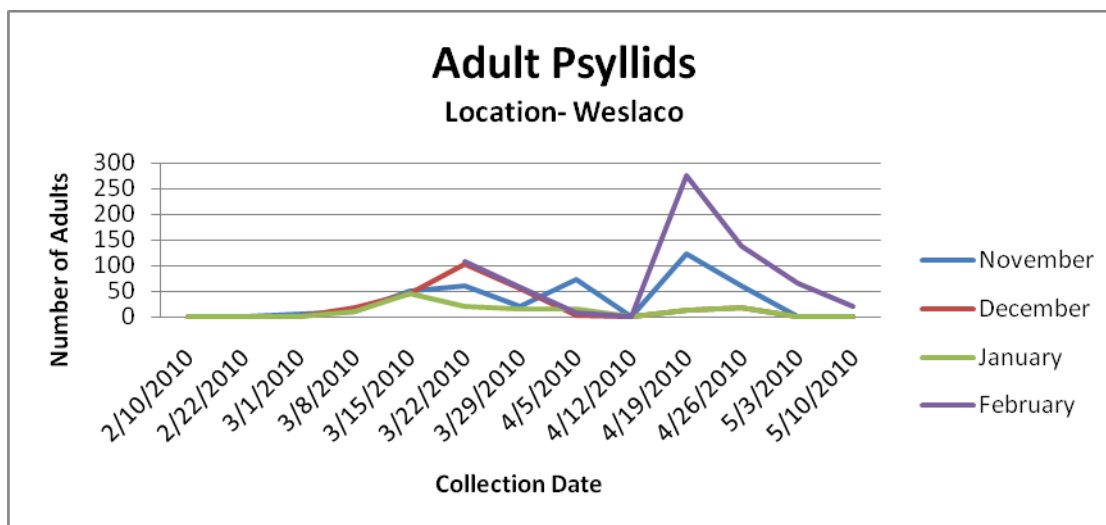


Figure 1. Adult psyllid numbers in a Rio Grande Valley potato crop following four planting dates at the same location.

For the Pearsall location, adult psyllids were not collected until mid-March due to weather issues such as freezing and excessive rain. In early January, this location experienced freezing weather, 20°F for eight hours, which caused damage to the December planting. During this time the first January planting had not germinated yet. The graph indicates a small incline in psyllid population the middle of March which was at the same time when the temperature was optimal (78°F) for psyllid activity. A sharp decline can be seen in April due to excessive rain in the area at the time of sampling. After experiencing a freeze, the December planting showed high psyllid populations soon after the last freeze once the plants began to mature once again. Harvest was conducted in late May.

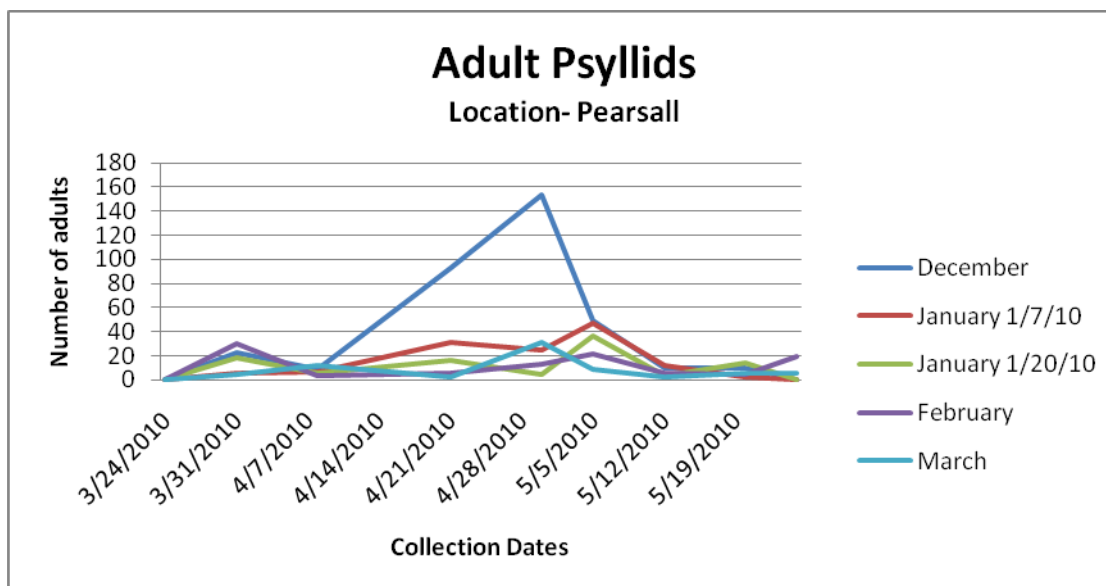


Figure 2. Adult psyllid numbers in a Pearsall potato crop following five planting dates at the same location.

Planting date	Zebra chip percentage
November 2009	40.64 %
December 2009	44.72 %
January 2010	44.62 %
February 2010	28.09 %

Table 1. Average zebra chip incidence per planting date in Weslaco.

Planting date	Zebra chip percentage
December 2009	1.81 %
January 2010 (1 st)	5.24 %
January 2010 (2 nd)	3.81 %
February 2010	3.00 %
March 2010	7.92 %

Table 2. Average zebra chip Incidence per planting date in Pearsall

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POTATO VARIETY SCREENING TRIAL FOR ZEBRA CHIP RESISTANCE UNDER CONTROLLED FIELD CAGE CONDITIONS

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Summary

Zebra chip, a newly emerging and economically important disease of potato is caused millions of dollars in losses to the potato industry in the Americas and New Zealand. The disease has recently been associated with *liberibacter* which is vectored by the potato psyllid. Breeding of zebra chip-resistant or tolerant varieties using traditional approaches or genetic engineering may offer the most efficient and useful way to manage this disease. Susceptibility of selected potato varieties and advanced breeding lines to zebra chip was evaluated under controlled field cage conditions in 2009 and 2010 at the USDA-ARS Research Farm at Moxee, WA. Results suggest that none of the currently available varieties tested are resistant or tolerant to zebra chip. An additional screening of 102 advanced breeding lines under similar controlled conditions indicated that a dozen of these lines may be tolerant to the disease and warrant further evaluation.

Results from this research project provided information on whether screened varieties and advanced breeding lines are differentially resistant to or tolerant to zebra chip. This information will help affected potato producers and processors to quickly minimize losses due to zebra chip by identifying and selecting cultivars that are less vulnerable to zebra chip.

Introduction

Zebra chip (ZC), a newly emerging and damaging disease of potato has caused millions of dollars in losses to the potato industry in the United States, Mexico, Central America, and New Zealand. The disease is associated with “*Candidatus Liberibacter solanacearum*” (syn. *Ca. L. psyllauros*) vectored by the potato psyllid, *Bactericera cockerelli* (Munyaneza et al. 2007a,b; 2008; Hansen et al. 2008; Liefting et al. 2008, 2009; Secor et al. 2009; Crosslin et al. 2010). Development and identification of potato varieties and/or advanced breeding lines with resistance or tolerance to zebra chip are crucial to development of effective and sustainable management strategies for this important potato disease. The main objective of this research was to conduct field experiments to screen potato varieties/lines for zebra chip resistance. The specific objective was to conduct *liberibacter*/psyllid transmission/exposure studies under controlled field cage conditions to quickly and accurately assess susceptibility of existing potato cultivars and advanced breeding lines to zebra chip.

Materials and Methods

In 2009 and 2010, susceptibility of nine fry and chipping potato cultivars to ZC was evaluated under controlled field cage conditions at the USDA-ARS Research Farm at Moxee in Washington. The varieties evaluated are Russet Burbank, Ranger Russet, Umatilla Russet, Russet Norkotah, Alturas, Shepody, Atlantic, FL1867, and FL 1879. Six potato seed pieces of each variety were planted in 6 small field tent-like cages (6 ft wide and 15 ft long each). For each variety, plants in 3 cages were exposed to 20 liberibacter-infective psyllids per plant at tuber initiation stage whereas plants in the remaining 3 control cages did not receive psyllids. The psyllids were eliminated from the plants with insecticides about a week after exposure. After the insect removal, plants were monitored for ZC symptoms. At the end of the experiment, tubers were harvested and checked for ZC symptoms to estimate disease incidence and severity. An additional screening trial was conducted in the summer of 2010 to quickly assess susceptibility of selected advanced potato lines to ZC. A total of 105 entries were evaluated. The plants were exposed to liberibacter-infective psyllids under field cage conditions as above, with a slight modification. Briefly, 6 potato seed pieces of each entry were planted in a small field cage as described above, along with 2 Atlantic seed pieces at each end of the cage. Atlantic was used as a control because this potato variety is well known to be very susceptible to ZC. Around tuber initiation stage, 20 liberibacter-infected potato psyllids per plant were released in each cage and eliminated with insecticides after about 2 weeks. Plants in the cages were monitored for above-ground ZC plant symptoms and the experiment was terminated when all the plants had died. At the end of the experiment, the plants in each cage were hand-harvested individually and the tubers from each plant checked for ZC symptoms.

Results and Discussion

All the fry and chipping varieties evaluated in both 2009 and 2010 were very susceptible to ZC, with almost 100% of plants showing severe ZC foliar and tuber symptoms (Table 1). Yield was significantly affected for all the varieties tested, with a range of 49.9 to 87. 2% yield loss (Table 1).

For the screening trial of advanced breeding lines in 2010, all the Atlantic control plants in the cages produced tubers with very severe ZC symptoms, indicating that *Liberibacter* inoculation by the insects during the trial was successful. Also, above ground plant symptoms were observed in all the cages and the plants generally died soon after ZC symptoms developed. Out of the 102 surviving entries (3 of the entries never emerged), 29 had ZC symptoms in raw tubers ranging from light to almost none (6 had almost no symptoms in raw tubers whereas 23 exhibited light ZC symptoms); the difference in symptom severity in these breeding lines compared to Atlantic was very significant, suggesting that these entries reacted differently to ZC (or maybe to insect feeding). All the remaining 76 entries had severe ZC symptoms in fresh tubers. Tuber samples of these 29 entries with no to light ZC symptoms were processed into chips and fried: 4 did not show typical ZC symptoms in fried chips, 2 produced chips with light ZC symptoms, and the rest had typical ZC symptoms. However, several of these entries with ZC symptoms in fried chips did not show any ZC symptoms in tubers when baked. Results of this study suggest that some of these potato lines may be promising and tolerant to ZC, *Liberibacter*, and/or

psyllid feeding, and warrant further investigation. Further potato variety screening under controlled conditions is planned for the 2011 growing season.

Table 1. Zebra chip incidence and yield loss in selected fry and chipping potato cultivars exposed to liberibacter-infective psyllids under controlled field cage conditions				
Cultivar	ZC Incidence in Plants (%)		Yield Loss (%)	
	2009	2010	2009	2010
Alturas	82.5	100	87.2	63.4
Ranger Russet	100	100	74.1	53.4
Russet Burbank	100	100	86.6	63.0
Russet Norkotah	100	100	63.4	62.7
Umatilla Russet	100	100	49.9	62.1
Shepody	100	100	84.4	63.3
Atlantic	100	100	73.8	58.4
FL1867	89	100	55.5	53.2
FL1879	100	100	61.7	62.0

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