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Development of an IPM Program for Management of the Potato Psyllid to Reduce Incidence of Zebra Chip Disorder in Potatoes

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ABSTRACT

The seasonal phenology of the potato psyllid, *Bactericera cockerelli* (Sulc.) was monitored in insecticide-treated commercial potatoes, untreated experimental plots and in stands of native host plants, using a combination of yellow sticky traps and leaf samples in the Lower Rio Grande Valley of Texas. Adult potato psyllids entered the growing areas in late fall shortly before emergence of potatoes to overwinter and reproduce on cultivated and native host plants including *Lycium* spp. Potato psyllids showed no oviposition preference for Atlantic or FL1867 potato cultivars, however nymphal densities were higher on the Atlantic variety. Management of the pest, using a program which included imidacloprid at planting and foliar applications of spiromesifen and dinotefuran led to low egg and nymphal densities in both varieties as compared to the untreated control plots. Incidence of zebra chip was below economic levels in the fields where the potato psyllid was kept at low density for the entire season. High levels of *Xylella fastidiosa* and phytoplasma were found in three psyllid species tested. The role these pathogens in the disease is still unknown; therefore, their presence remains circumstantial until Koch's postulates are completed. The IPM method developed in this research for sampling and management of potato psyllid may be applicable to other growing areas in the Southwestern U.S., Mexico and Central America impacted by zebra chip.

Additional index words: potato insects, Lower Rio Grande Valley of Texas, integrated pest management.

In Texas, potato production was valued 91.6 million dollars in 2005 (Rosson et al. 2006). The majority of potatoes are processed for crisps, but fresh market potatoes are also important to the industry. All the major potato production areas in Texas and parts of Mexico have recently experienced a new disorder of potato known as 'zebra chip' (ZC) (Cadena-Hinojosa & Guzman-Plazola 2003, Secor et al. 2006, Munyaneza et al. 2007a,b). The ZC disorder not only leads to lower yields, but also to the rejection of chips processed from infected potatoes. The disorder was first noted in the United States in the Lower Rio Grande Valley of Texas in 2000 (Secor et al. 2006).

ZC plant symptoms resemble those caused by potato purple top and psyllid yellows diseases (Munyaneza et al. 2007a,b). Potato purple top disease is caused by phytoplasmas that are usually transmitted by leafhoppers, planthoppers, and psyllids. To date, vectors and causal agents of ZC are not well known. However, the insect that has most often been associated with ZC is the potato psyllid, *Bactericera cockerelli* (Sulc) (Rubio-Covarrubias et al. 2006, Goolsby et al. 2007, Munyaneza et al. 2007a, b). The potato psyllid is indigenous to the southwestern U.S. and northern Mexico and is known to migrate long distances to exploit its solanceaous host plants, which include the native wolfberry, *Lycium* spp. and nightshades, *Solanum* spp. (Romney 1939, Wallis 1955, Rowe 1993, Drees & Jackman 1999, Liu & Trumble 2004).

It is not known if the disorder is caused by a pathogen or toxins injected into the plant by the potato psyllid, or some combination. Considerable effort has been made to identify causal pathogens. To date, none have been positively linked with ZC (Bextine, unpublished data). Lee et al. (2006) and Secor et al. (2006) reported that ZC was associated with phytoplasmas, including "*Candidatus* Phytoplasma americanum", a previously unknown phytoplasma in the stolbur phytoplasma group. However, recent studies by Munyaneza et al. (2007a, b) indicated that these plant pathogens seem not to be involved in ZC.

Based on the evidence that insects, in particular the potato psyllid, were directly linked to ZC, an intensive sampling and integrated management plan was developed to control these insects. Populations of psyllids and leafhoppers were compared in commercial fields and in untreated control plots. The goals of the study were to: 1) determine if management of potato psyllid using strategies developed by Liu and Trumble (2004) for management of this pest in tomatoes would lead to lower levels of ZC; 2) investigate the role of native plants in the seasonal phenology of *B. cockerelli*; and 3) sample populations of potato psyllid to test for the presence of ZC putative pathogens.

MATERIALS AND METHODS

Study Sites. Nine commercial potato fields with a history of ZC occurrence were selected for the study. The fields were located 10 miles north of McAllen, TX in Hidalgo Co., bounded on the east by Wallace Rd. and Hwy 490 to the north. A general description of the commercial potato fields and growing area can be found in Goolsby et al. 2007. For comparison, untreated control plots were planted at the USDA research farm in Weslaco, TX, approximately 30 miles to the southeast of commercial potato fields. All fields were planted with 'seed' potato varieties Atlantic and Frito Lay (FL) 1867 from Colorado sources. The commercial fields were planted between Dec. 12, 2006 and Feb. 18, 2007, with the research plots planted by hand on Jan. 18, 2007. The commercial potatoes were planted with an in-furrow application of imidacloprid, Admire Pro® (Bayer Crop Science, Kansas City, MO) insecticide followed by several in-season applications of spiromesifen, Venom® (Valent Biosciences, Walnut Creek, CA) and Dinotefuran, Oberon® (Bayer Crop Science) used in a rotation and generally applied at weekly intervals until the two week pre-harvest interval. Above ground emergence of plants from tubers occurred 30-45 days after planting, with harvest from April 9-28, 2007. At harvest, yields per hectare were estimated by counting the number of truckloads per field divided by the number of hectares per field. A subsample of potatoes from each field was chipped and fried on-site, according to Frito Lay protocols, to estimate the occurrence of ZC.

To study the phenology of the potato psyllid on non-crop hosts, sites were established with *Lycium berlandieri* M. Dunal (Solanaceae), Berlandier wolfberry and *Lycium carolinianum* T. Walter, Carolina wolfberry, which are known native hosts of this insect. *Lycium berlandieri* occurs in the drier, upland locations of Hidalgo Co. *Lycium carolinianum* grows only along the Gulf Coast in saline, clay soils. *Lycium berlandieri* stands were sampled at three sites in the native brushland of Hidalgo Co. within the vicinity of the commercial potatoes, near Faysville, McCook, and Palmview, TX. *Lycium carolinianum* was sampled in the in Cameron Co. east of San Benito, TX.

Insect Sampling. Yellow sticky cards were used (Goolsby et al. 2007) to capture adult psyllids and leafhoppers in the fields. Each of the commercial fields and the USDA farm with the control plots had a single transect with five traps placed at intervals of 61 meters. In-field sampling began on Jan 18, 2006 with traps changed weekly through May 5, 2007. Non-crop sites were sampled bi-weekly between October 2007 and November 2008. All traps were returned to the laboratory for identification and counts of the leafhoppers and psyllids. Potato psyllids were counted separately. Counts of other psyllid species were pooled. The most common leafhoppers were categorized by species. All other leafhoppers were counted as unknown brown or green leafhoppers. From each sample date, a subsample of potato psyllids was removed from the traps and screened for potential plant pathogens.

Vacuum sampling was used to detect the presence of adult potato psyllids in the plant canopy of the potatoes. Three of the nine commercial fields were selected for vacuum sampling. These fields represented early, mid and late-season plantings. Each week, ten locations on the perimeter of each field were vacuumed for one minute. The vacuum socks containing the insects were returned to the laboratory, held in the refrigerator at 5° C for one hour and then counted.

Leaf samples were used to assess the density of potato psyllid eggs and nymphs. Samples were collected weekly from the same three commercial fields selected for vacuum sampling, plus the two untreated control plots located at the USDA research farm in Weslaco, TX. The control plots were planted with the two most common commercial varieties in the Lower Rio Grande Valley, Atlantic and FL1867. From the commercial fields, one hundred fully mature,

compound leaves (6-12 leaflets) were selected and clipped from plants 5 m inside the perimeter of the field. Because the untreated control plots were smaller (and to avoid the effect of destructive sampling), 25 leaves were selected from each variety. Leaves were returned to the laboratory for counts of eggs and nymphs using a dissecting microscope set at 30X. Counts of eggs and nymphs were analyzed using ANOVA, with cultivar, sample date, insecticide treatment and their interactions as fixed effects (SAS, 2004)

Pathogen Screening. Collected insects were tested for the presence of *X. fastidiosa* and multiple groups of phytoplasma. Residual adhesives were removed from the insects by dipping them into orange oil and washing with de-ionized water. Samples collected from the same location and times were then sorted into groups of five individuals for DNA extraction. Extractions were carried out using DNeasy® Blood & Tissue Kit following the total insect DNA purification protocol (Bextine et al. 2004).

PCR for *Xylella fastidiosa* (*Xf*) detection was carried out by performing an initial denaturing step of 3 min. at 95°C, followed with 30 cycles of 1 min. at 95°C, 1 min at 55°C and I min. at 72°C, with a final extension at 72°C for 10 min. A final volume of 20µl reaction mixture consisted of 10 µl of iQ™ Supermix containing 10mM KCL, 4mM Tris-HCl, pH 8.4, 0.16mM dNTPs, 5 U/ml iTaq DNA polymerase, 0.6mM MgCl2 and stabilizers (BIO-RAD, Hercules, CA), 2 µl of DNA extracted from samples, 0.2 µM of the forward primer MopB139 BBF, and reverse primer MopB777 BBR. Each PCR product was separated on a 1% agarose gel at 10 V/cm for 120 min in TAE buffer. Gel were stained with ethidium bromide (0.5 µg/ml). A sample was considered positive for the presence of *Xf* if a 638-bp band was visualized using UV light. Primer MopB139 BBF has a sequence of 5'-GGTTCCACCGGGTTTAACTT-3' and MopB777 BBR has a sequence of 5'-GCACTTGTCGTCACAGTCGT-3'. The same protocol was adopted and modified base on *Xf* detection protocol published by Bextine et al. (2005).

PCR for general phytoplasma detection was performed on a 20 µl reaction mixture with 10 µl of iQ™ Supermix containing 10mM KCL, 4mM Tris-HCl, pH 8.4, 0.16mM dNTPs, 5 U/ml iTaq DNA polymerase, 0.6mM MgCl2 and stabilizers (BIO-RAD, Hercules, CA), 2 µl of DNA extracted from samples, 1 µl each of 20 µM primer solution of P1 and Tint. PCR reaction was performed with the following steps: 3 min at 95°C, then 30 cycles of 95°C for 1 min, 56°C for 1 min, 72°C for 2min, followed by final extension at 72°C for 5 min. Each PCR product was separated on a 1% agarose gel at 10 V/cm for 120 min in TAE buffer. Gel was stained with ethidium bromide (0.5 µg/ml). A sample was considered positive for the presence of *Xf* if a 1600-bp band was visualized using UV light. Sequences of primers P1, Tint and the original PCR protocol followed Smart et al. 1996.

Primer pair P1/WXint, P1/BLTVAint, and P1/AYint were used to identify X-disease group (16SrIII), Aster Yellows group (16SrI), and Stolbur group (16SrXII), respectively. Direct PCR to amplify the respective phytoplasma groups were performed under the following conditions: 3 min at 95°C; then 30 cycles of 95°C for 1 min, 48°C for 1 min (P1/BLTVAint and P1/WXint) or 56°C for 1min (P1/AYint), 72°C for 2min; followed by final extension at 72°C for 5 min. A total of 20 µl final reaction mixture is composed of 10 µl of iQ™ Supermix containing 10mM KCL, 4mM Tris-HCl, pH 8.4, 0.16mM dNTPs, 5 U/ml iTaq DNA polymerase, 0.6mM MgCl2 and stabilizers (BIO-RAD, Hercules, CA), 2 µl of DNA extracted from samples, 0.5 µM of each appropriate primer sets. 1% agarose gel containing ethidium bromide was used to perform electrophoresis with the above products and visualized under UV light. Primer sequences and PCR conditions were adopted from Smart et al. 1996.

Nested PCR consisted of primer set P1/P7//fU5/BLTVAint specific for phytoplasma clover proliferation group (16SrVI) were performed with 3 min at 95°C; 25 cycles of 1 min at 94°C, 1 min at 48° C, 2 min at 72°C; and extension for 5 min at 72°C for the direct PCR. 2 µl of DNA extracted from samples was used in direct PCR. Nested PCR was performed with 1 µl of direct PCR product and fU5/BLTVAint primers. Both the direct and nested PCR were carried out in 25 µl reaction mixture containing 12.5 µl of iQ™ Supermix containing 10mM KCL, 4mM Tris-HCl, pH 8.4, 0.16mM dNTPs, 5 U/ml iTaq DNA polymerase, 0.6mM MgCl2 and stabilizers (BIO-RAD, Hercules, CA). Nested PCR products were viewed in 1% agarose gel after electrophoresis under UV light with ethidium bromide staining. Primers and nested PCR procedures were modified from Khan et al. 2004 and Crosslin et al. 2006. The data were analyzed using a chi-square analysis.

RESULTS

The first capture of potato psyllids was well in advance of the potato season on November 7, 2006, which coincided with the first cold front of the winter season in the Lower Rio Grande Valley. Population levels remained low until after emergence of the potato plants in late January 2007. Numbers of adult potato psyllids on the sticky traps peaked in all fields, including the control plots, during mid-March (Fig 1.).

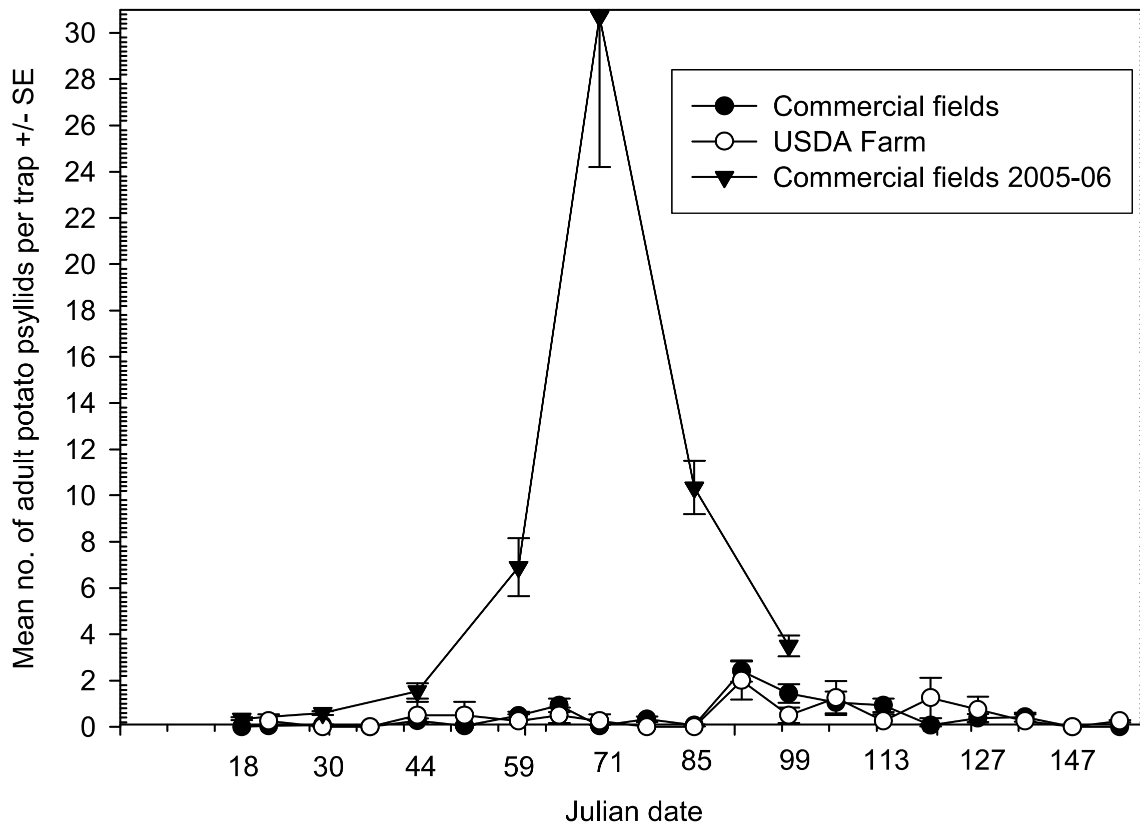

Fig 1. Populations of adult potato psyllids in potatoes on yellow sticky card traps near McAllen, TX during the 2006-07 potato growing season.

Potato psyllid numbers from December 2006 through February 2007 were similar to those encountered during the same period in 2005-06. However, peak populations in the 2005-06 season reached a mean of 60 adults per trap in mid-March (Goolsby et al. 2007). Adult psyllids in the vacuum samples showed a similar trend but were not detectable during the early season using this method. The complex of leafhopper species was similar to the 2005-06 season (Goolsby et al. 2007). Four other psyllid species were common during the season and were identified to be *Pachypsylla* sp. *Leuronota maculata* Crawford, *Heteropsylla* sp. and *Trioza arizonae* Aulmann. The first two psyllid species are known to occur on *Celtis* spp., hackberry and the second two on *Salix* spp. (willows). Both of these plant species are common in the potato growing area (Goolsby et al. 2007).

ANOVA testing the effects of cultivar, sample date, and insecticide on egg density found no significant interaction between cultivar and sample date ($F = 0.82$, $df = 3, 2085$, $P = 0.4850$), or between cultivar and insecticide ($F = 1.96$, $df = 1, 2080$, $P = 0.1612$), therefore data for each cultivar were pooled and analyzed using ANOVA for the treatment effect (insecticide) for each sample date (Fig 2). There were significantly more eggs on the untreated controls than in the treated commercial fields for all three sample dates, except 68-75, in which no eggs were found in the untreated controls.

ANOVA testing the effects of cultivar, sample date, and insecticide on nymphal density found significant interaction between cultivar and insecticide, insecticide and sample date, but not cultivar by sample date (Table 1). Therefore, cultivars were analyzed separately to test the effect of insecticide by sample date. Nymphal populations in the FL1867 potatoes were significantly higher in the untreated control plots on the second sample date 66-73 (Fig. 3). Following this spike in numbers of nymphs, the FL1867 potatoes declined dramatically showing classic ZC symptoms (stunting, purple terminals, aerial tubers, and scorched leaf margins). In contrast, nymphal numbers in the commercial treated

Table 1. Type 3 Tests of Fixed Effects for nymphal potato psyllid

Effect	df	F Value	*P*
Cultivar	1, 2141	22.47	< 0.0001
Sample Date	3, 2141	59.93	< 0.0001
Insecticide	1, 2141	161.25	< 0.0001
Cultivar*Sample Date	3, 2141	0.86	0.4597
Cultivar*Insecticide	1, 2141	29.31	< 0.0001
Insecticide*Sample Date	3, 2141	75.33	< 0.0001

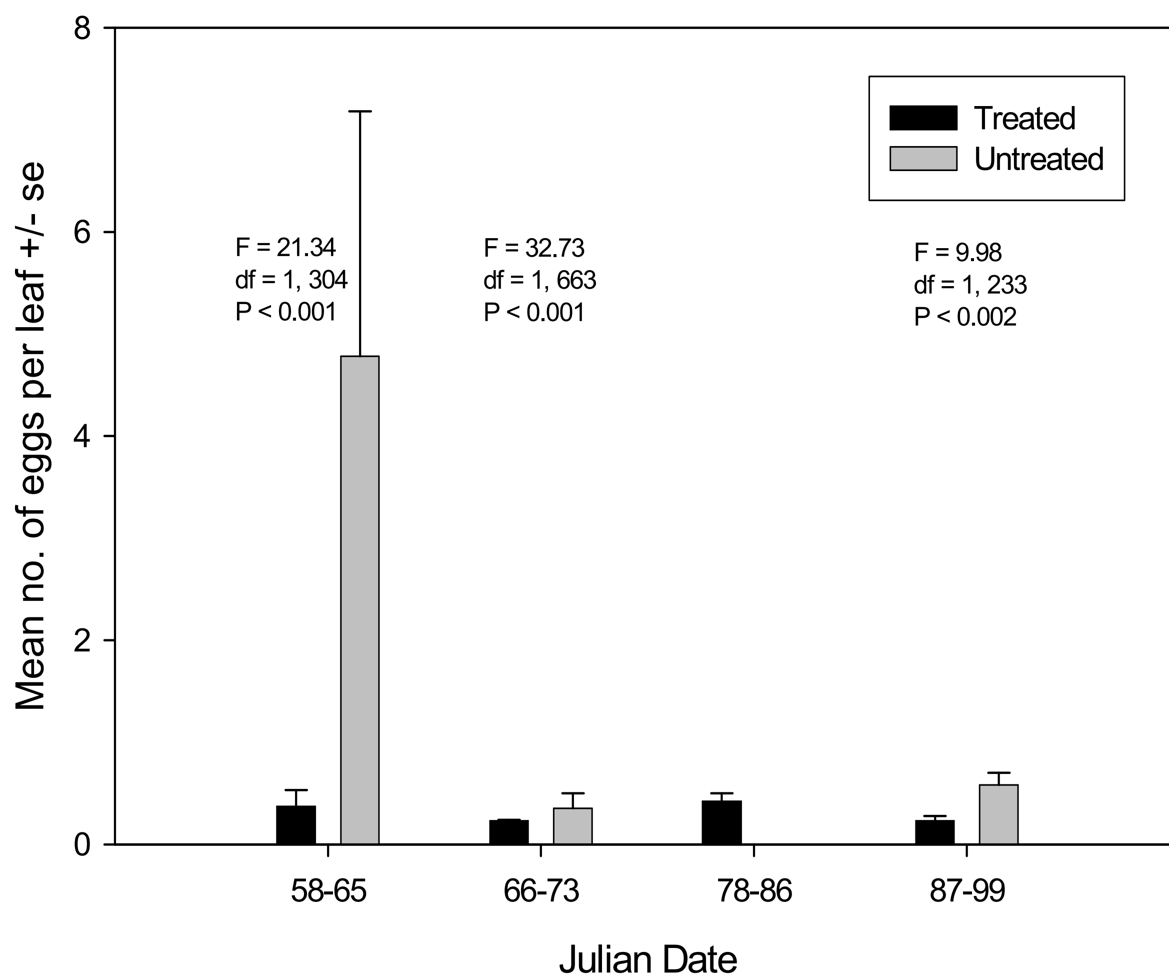

Fig 2. Mean density of potato psyllid eggs in treated commercial potato fields vs. untreated control plots by sample date. No eggs were recorded.

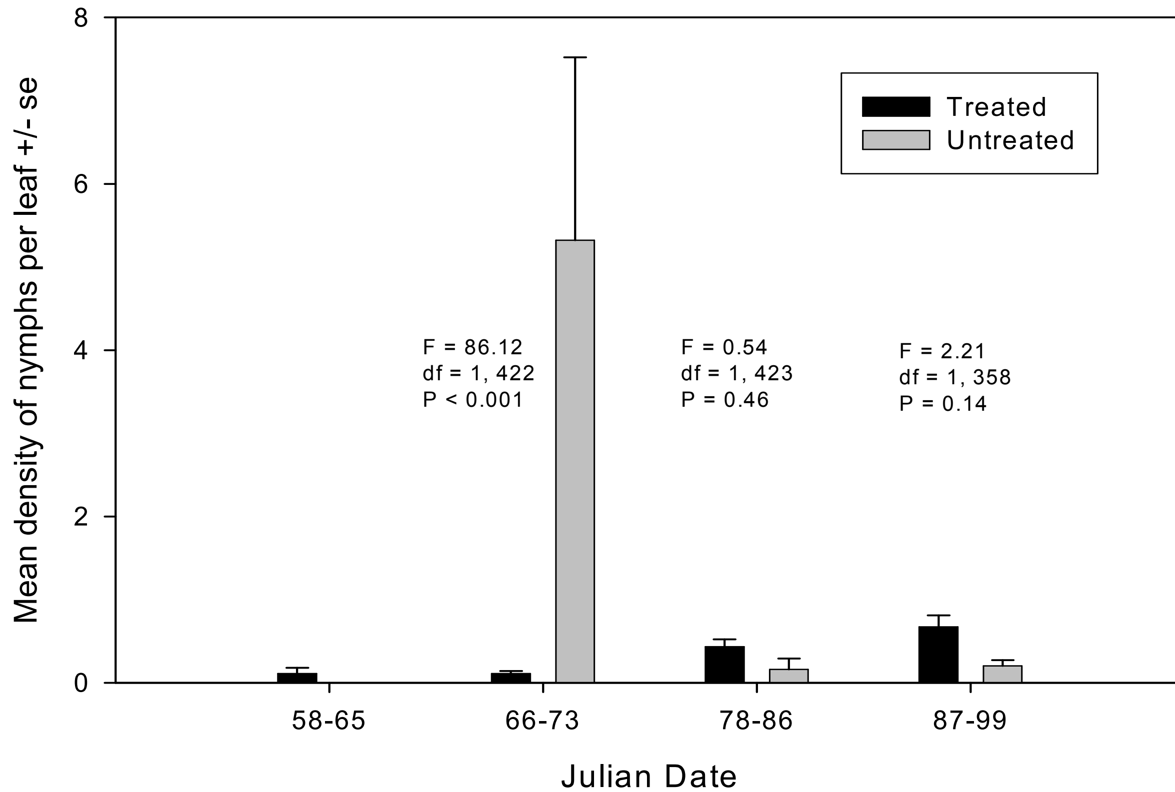

Fig 3. Mean density of potato psyllid nymphs in FL 1867 potatoes in treated commercial fields vs. untreated control plots by sample date. No nymphs were recorded.

FL1867 potatoes showed a slight trend upward towards the end of the season but remained below 1 nymph per leaf. Nymphal populations in the Atlantic potatoes followed a different trend (Fig. 4). In the untreated Atlantic potatoes, nymphal populations were significantly higher for all of the sample dates, except 78-86. Although the plants in the untreated controls exhibited ZC symptoms, they kept growing and had more foliage than the 1867 potatoes. Populations of nymphs in the treated commercial fields remained low throughout the season peaking below one nymph per leaf on the 78-86 sample date.

Potato yields were slightly higher in the 2006-07 as compared to 2005-06 season (Table 2) (Goolsby et al. 2007). Zebra chip (ZC) levels were also substantially lower, and nearly all the potatoes were accepted by the processors. There did not appear to be an effect of planting date on yield or ZC incidence. Other potato growers in the LRGV, who followed equivalent pest management practices experienced similar quality and yield (C. Burns, pers. comm.). In contrast, both potato varieties studied in the untreated research plots had extremely high ZC levels and were unmarketable. Observed ZC incidence averaged 50 and 72% for FL1867 and Atlantic, respectively, when tubers were processed into fried chips (Munyaneza et al. 2007b).

Potato psyllid populations on *L. berlandieri* and *L. carolinianum* were detectable at very low numbers from November to April. Hand searches of *L. berlandieri* revealed eggs and nymphs of *B. cockerelli* showing the suitability of this plant as a host. Additional searches of the native brush in south Texas revealed that *L. berlandieri* was common to the south of the potato growing areas along the Rio Grande and to the north and west in the uncleared native brush. Likewise, *Lycium carolinianum* was found to be very common to the east along the coastal salt flats and barrier islands. Potato psyllid nymphs were collected on one occasion from *Solanum americanum* Mill., American nightshade (R. Coleman, unpublished data). From April to November 2007 no potato psyllids were collected in the traps at the *Lycium* locations.

Screening of the potato psyllids and two other common psyllids did show the detectable presence of two pathogens in their bodies. Of the 143 samples of psyllid DNA samples screened, 74 (51.75%) were positive for phytoplasma, and 60 (41.96%) were

positive for *X. fastidiosa.* When data were pooled across dates and species, the number insects testing positive for phytoplasma or *X. fastidiosa* were not significantly different ($\chi^2 = 2.752$, $P = 0.0971$). For *B. cockerelii*, only 30.34% tested positive for phytoplasma, whereas 96.55% of the non potato psyllids tested positive ($\chi^2 = 24.219$, $P < 0.001$). Interestingly, 71.43% of all psyllids collected from *Lycium* tested positive for phytoplasma, whereas only 59.70% of the psyllids collected from potato tested positive ($\chi^2 = 0.938$, $P = 0.332$).

DISCUSSION

Potato psyllid, *B. cockerelli* appears to move in mass across the landscape during early winter, most likely moving south with the cold fronts. *Lycium* spp. have been reported as alternate hosts (Romney 1939, Wallis 1955, Drees and Jackman 1999), and was confirmed in this study. However, populations are not detectable from late spring to late fall indicating that potato psyllids are not a year-round resident in South Texas. Populations of adults were fairly similar at the start of both growing seasons (2005-06 and 2006-07) despite cooler, wetter weather in second growing season. This may indicate that migrating population levels are influenced by biotic and abiotic factors in other parts of their annual range. Mid to late season population levels of adult potato psyllids varied dramatically between years and was most likely the result of pest management practices. Yellow sticky cards are an effective tool to detect potato psyllids in both crops and stands of native host plants, especially when they occur at low density. Yellow sticky cards

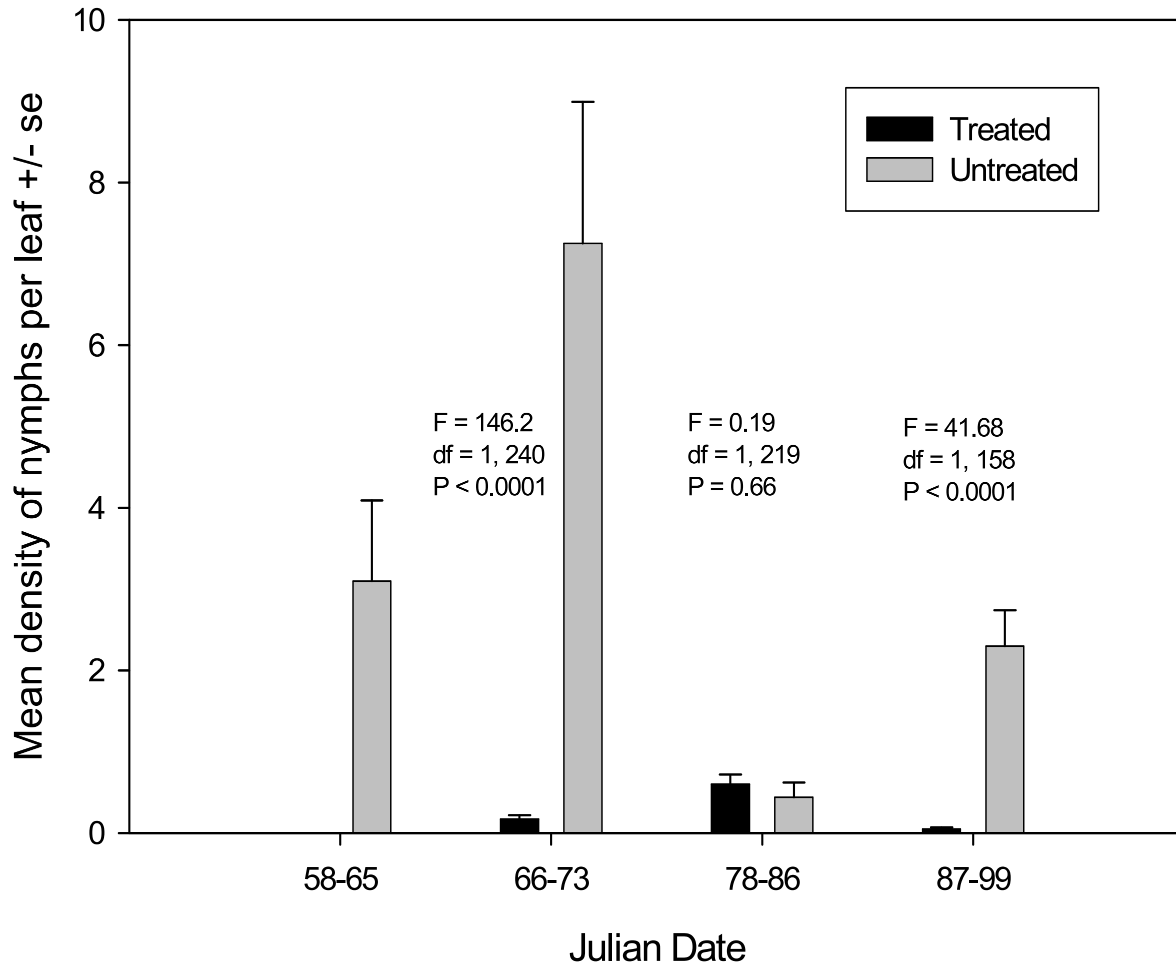

Fig 4. Mean density of potato psyllid nymphs in Atlantic potatoes in treated commercial fields vs. untreated control plots by sample date. No nymphs were recorded.

Table 2. Field production parameters and zebra chip incidence.

Field #	Planting Date	Size (ha)	Mean Yield (kg/ha)	% ZC incidence
Field 1 FL	11 Jan. 2007	46.0	34,972	1.5
Field 3 FL	7 Jan. 2007	46.0	34,951	1.9
Field 4 A	10 Jan. 2007	16.2	30,527	0.4
Field 7 FL	5 Feb. 2007	30.4	36,923	2.3
Field 8 FL	13 Feb. 2007	18.2	24,250	2.2
Field 11A	18 Dec. 2006	42.1	27,578	1.2
Field 14 FL	31 Dec. 2006	40.5	26,263	1.36
Field 17 FL	28 Dec. 2006	24.3	32,658	0.3
Field 18 A	20 Dec. 2006	32.4	42,093	0.4
Field 19 FL	21 Dec. 2006	40.5	32,160	0.5
Field 20 FL	27 Dec. 2006	36.4	43,817	0.5
Untreated A plots	12 Jan. 2007	n/a	n/a	>72.0
Untreated FL plots	12 Jan. 2007	n/a	n/a	>50.0

Potato Varieties: FL = Frito Lay 1867, A = Atlantic

could be used effectively in IPM programs for potatoes due to their ease of use, and applicability for transport to pest management specialists. Vacuum samplers were tested but were not found to be effective in sampling low density populations of potato psyllids. Leaf samples, although time consuming to collect and analyze, provide an even more detailed view of egg deposition and nymphal survival on the crop. The combination of yellow sticky cards and leaf samples appears to provide a complete view of the entire life cycle and the impact of the IPM program.

Potato psyllids do not appear to have an oviposition preference for the two potato varieties tested. Egg densities were high in the control plots as compared to the treated commercial fields, which may indicate that psyllids may be dying before oviposition. However, our sampling methods were biased towards detection of nymphs and may have underestimated egg densities, especially on larger, more robust, treated plants.

Nymphal density was significantly different between the varieties tested. The Atlantic variety is known to be one of the most susceptible varieties to potato psyllids (Munyaneza et al. 2007a,b), and our tests showed that nymphal populations were higher on this variety as compared to FL1867. Potato psyllid populations were higher on the untreated Atlantic than the FL1867 potatoes, but were low on both varieties in the treated commercial fields, which shows the strong potential of selected insecticides to control this pest and reduce the incidence of ZC. It is not known how low or how long during the season potato psyllid nymphs need to be controlled in order to keep ZC below economic levels. However, nymphal densities below 1 nymph per leaf are a bench mark for further refinement of the IPM program. We were not able to test different insecticides or their interactions, but the combination of imidacloprid, spiromesifen, and dinotefuran, used in a rotation, appears to be effective at controlling potato psyllids, even in susceptible varieties such as Atlantic. Although the impact of

predators was not measured, populations of key predators such as spiders, coccinellids, minute pirate bugs, and lacewings, were observed throughout the season. This may indicate that the insecticides had minimal impact on these beneficial arthropods which is critical to an effective IPM program. Further research is needed to test the efficacy of these insecticides and other potentially selective chemistries individually against *B. cockerelli*. This research is needed to prevent an over reliance on neonicotinoid insecticides. The timing of insecticide applications is also needed. It is not known if late season applications, or late season suppression of nymphs is needed to reduce ZC incidence. Yields did not appear to be dramatically higher on average between seasons but some fields showed increases which may have been attributed to slight differences in the timing or number of insecticide applications.

Sampling of the potato psyllid outside the agroecoystem revealed that the insect was common and widespread in the native plant community. Although extensive areas of the Lower Rio Grande Valley are intensively farmed or urbanized, native host plants are abundant. Outside of these areas in the vast plains of south Texas and northern Mexico, the native plants become even denser across the landscape. Based on our limited sampling, the bulk of the overwintering population appears to reside and reproduce outside of potato fields. This makes management of this pest outside of the crop impractical.

Screening for the presence of *X. fastidiosa* and several phytoplasma species in more than 700 insects was conducted (5 individuals per DNA sample) With 143 DNA samples tested during the 2007 growing season. *Bactericera cockerelli* was the most commonly collected species, and had relatively high incidence of pathogen contamination. Still, phytoplasma and *X. fastidiosa* were detected more frequently then expected. These high levels of pathogen detection could be misleading, indicating that both pathogens are present in the local area. *Xyella fastidiosa* has been investigated as a putative pathogen by several research groups and has been determined to be an unlikely causal agent of ZC (Bextine, unpublished). Phytoplasma presence in the adult psyllids populations also remains circumstantial considering that neither of these pathogens have been consistently detected in potato leaves or tubers exhibiting ZC symptoms (Munyaneza et al. 2007a).

In summary, intensive sampling and management of potato psyllid populations led to low levels of ZC in commercial potatoes in the Lower Rio Grande Valley. The methods developed in this research are applicable to other growing areas in the Southwestern U.S., Mexico and Central America. It is not known if the causal agent of ZC is a pathogen, toxic saliva, or a combination of these two factors, but extensive testing adult potato psyllids, other psyllids, and leafhoppers did not reveal any new suspect pathogens. Until the etiology of ZC is elucidated and more effective strategies can be developed to control ZC vectors and causal agents, management of the potato psyllid is critical to reducing the incidence of this destructive disorder of potatoes in affected areas.

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