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Seasonal Abundance of Sharpshooters, Leafhoppers, and Psyllids Associated with Potatoes Affected by Zebra Chip Disorder

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ABSTRACT

A survey of psyllids, leafhoppers and sharpshooters in commercial potatoes near McAllen in the Lower Rio Grande Valley of Texas was conducted using yellow sticky traps. Species from these insect groups that were collected in the survey were evaluated for the presence of *Xylella fastidiosa*, a putative contributing factor of zebra chip (ZC) disorder in potatoes. Potato psyllid, *Bactericera cockerelli*, was the most abundant species collected in the study, entering the fields in mid-January and reaching a peak in mid-March. More than 87 % of the psyllids tested positive for *X. fastidiosa*. Leafhoppers were less common, with populations peaking in mid-February. Several species tested positive including; *Empoasca* spp., *Scaphytopius* sp., *Ceratagallia* sp., *Penestragania* sp., *Erythroneura* sp. *Graminella* sp. and *Dikraneura* sp. It is believed that none of these species, including *B. cockerelli*, are capable of transmitting *X. fastidiosa*, but they should be further investigated in transmission studies and for other roles in ZC expression. Although nearly all sharpshooters, which are known to transmit *X. fastidiosa*, tested positive, they were rare, and therefore do not appear to play a role in ZC. Thirteen common species of plants in the vicinity of the potato fields were evaluated as potential reservoirs of *X. fastidiosa*, with only *Funastrum clausum*, climbing milkweed vine, testing positive.

RESUMEN

Un examen de psyllids, de chicharitas y de tiradores de primera en patatas comerciales cerca de McAllen, TX en el valle más bajo de Río Grande fue conducido usando trampas pegajosas amarillas. Las especies de estos grupos que fueron recogidos en el examen fueron evaluadas para la presencia del *Xylella fastidiosa*, un factor que contribuía supuesto del desorden de la viruta de la cebra (ZC) en patatas. Los psyllids de la patata, *Bactericera cockerelli* eran las especies más abundantes recogidas en el estudio, incorporando los campos en mediados de-Enero y alcanzando un pico en marcha mediados de. Más de 87 % de los psyllids probaron el positivo para el *X. fastidiosa* del Chicharitas era menos común, con las poblaciones enarbolando a mediados de Febrero. Positivo probado varias especies incluyendo; sp. de *Empoasca* spp., de *Scaphytopius*, sp. de *Ceratagallia*, sp. de *Penestragania*, sp. de *Graminella* de los sp. de *Erythroneura* y SP de *Dikraneura*. Se cree que ningunas de estas especies, incluyendo *cockerelli* del *B.*, son capaces de transmitir *X. fastidiosa*, pero él debe ser investigada más a fondo en estudios de la transmisión y para otros papeles en la expresión de ZC. Aunque casi todos los tiradores de primera, que se conocen para transmitir fastidiosa del *X.fastidiosa*, probaron el positivo, eran raros, y por lo tanto no aparecen desempeñar un papel en ZC. Trece especies comunes de plantas en la vecindad de los campos de la patata fueron evaluadas mientras que los depósitos potenciales del *fastidiosa* del *X.*, con solamente *Funastrum clausum*, subiendo milkweed el positivo de prueba de la vid.

Additional index words: insect vectors, *Xylella fastidiosa*, Lower Rio Grande Valley of Texas, pest management

In Texas, more than 8,496 hectares of potatoes were planted in 2004, with a value of $54 million (USDA 2005). Approximately 1,200 hectares are grown in the Lower Rio Grande Valley (LRGV) during the winter and harvested in early spring for the chipping industry. A disorder called “zebra chip”, which may be caused by a pathogen is impacting potato production and quality. The disorder is called zebra chip (ZC) because of the striped pattern of necrosis in the tubers which becomes more prominent when tubers from affected plants are processed into fried chips. This disorder has been causing serious quality losses in the southwest United States since 2000 (Secor et al. 2006; Munyaneza et al. 2007). This same disorder is also present in Central and South America, particularly in Guatemala and Mexico (Cadena-Hinojosa et al. 2003; Munyaneza et al. 2007; Secor et al. 2006). In the LRGV, affected potato plants initially exhibit scorch symptoms similar to Pierce’s Disease in grapes. During late season, a portion of the infected plants develop a purple top wilt syndrome similar to those observed in the potatoes of the Pacific Northwest in the United States (Munyaneza et al. 2006). However, known phytoplasmas and other plant pathogens such as bacteria, fungi or viruses have not been demonstrated by Koch’s postulates to be the causal agent of ZC. Therefore, neither the causal pathogen(s) and/or vector(s) of the ZC disorder have been positively identified.

Scorch symptoms associated with ZC in potatoes appear similar to those in grapes called Pierce’s Disease which is caused by the phytopathogenic xylem-dwelling bacterium, *Xylella fastidiosa*. This bacterium was detected through species-specific PCR in low levels in potato plants, but presence of *X. fastidiosa* was not consistently associated with ZC symptoms (Bextine et al., unpublished data). Further, two strains of *X. fastidiosa* were previously isolated from ZC affected potatoes, although its role as a pathogen was not known (L. Morano, pers. comm.). Glassy-winged sharpshooter, *Homalodisca vitripennis* Say (= *Homalodisca coagulata*), which invaded California from the southeastern U.S. in the 1990s, is the primary vector of *X. fastidiosa* in grapes (Purcell 1997, Hoddle 2004). Other sharpshooters and leafhoppers are also known to transmit the disease to grapes but are less efficient vectors (Almeida et al. 2005). *Homalodisca vitripennis* is native to the LRGV and is commonly found in the native vegetation in the vicinity of the potato production areas. Although *H. vitripennis* had not been reported as a pest of potatoes, it was suspected that it could be transmitting *X. fastidiosa* to potatoes and therefore associated with ZC.

Incidence of ZC has been associated with infestations of the potato psyllid, *Bactericera cockerelli* (Sulc) (Munyaneza et al. 2007). However, *B. cockerelli* is not known to be a vector of xylem pathogens, therefore, it does not appear to have a suitable biology to act as a vector of *X. fastidiosa* (Liu et al. 2005). Potato psyllids are reported to overwinter in south Texas as adults on native wolfberry, *Lycium* spp. (Solanaceae) (Romney 1939, Wallis 1955, Drees and Jackman 1999). It is not known if *Lycium* spp. are asymptomatic reservoirs for *X. fastidiosa*.

Based on our understanding of *X. fastidiosa* transmission in grapes we conducted a study to evaluate the potential of *H. vitripennis* or other sharpshooters, leafhoppers or psyllids as potential vectors of this pathogen in commercial potatoes. The goals of our study were to: 1) conduct surveys for sharpshooters, leafhoppers, and psyllids in potato fields and analyze for presence of *X. fastidiosa* in these populations; 2) determine if these insects were present in sufficient numbers to account for ZC expression in potatoes; and 3) assess the uncultivated plants near potato fields and determine if they could be potential reservoirs of *X. fastidiosa*.

MATERIALS AND METHODS

Study Sites. Commercial potato areas with a history of ZC occurrence were selected for the study. The fields were located 10 miles north of McAllen in Hidalgo County, Texas and were bounded on the east by Wallace Road and Hwy 490 to the north. Nine fields were planted with ‘seed’ potato varieties Atlantic, FL (Frito Lay) 1867 and FL 2053, sourced from Colorado, Nebraska and Wisconsin in succession between December 12, 2005 and February 18, 2006. Potatoes were planted with an in-furrow application of Thimet® insecticide followed by several in-season applications foliar insecticides including Leverage® (imidacloprid), Phaser® (endosulfan) and Monitor® (methamidophos), all Bayer CropScience products. Above ground emergence of plants from tubers occurred 30-45 days after planting with harvest between April 9-28, 2006. At harvest, yields were estimated by counting the number of truckloads per field. A subsample of potatoes from each field were chipped and fried according to Frito Lay protocols to estimate the occurrence of ZC.

The potatoes were grown under pivot irrigation on a fine sandy loam soil, which drains locally to low spots or basins called ‘lagunas’ (Fig. 1). The lagunas are seasonally inundated and used for irrigation water storage. They are surrounded by native vegetation that is characteristic of riparian habitats including black willow, *Salix nigra* Marsh and sandbar willow, *Salix exigua* Nutt.

Insect Sampling. Yellow sticky traps were chosen as a survey tool for their utility in sampling a broad array of insects including sharpshooters, leafhoppers, psyllids over a large area. Blua et al. (2001) reported that yellow sticky traps provided useful data on both population density and flight activity of sharpshooters in grape and citrus plantings. However, it should be noted that the data generated from yellow sticky traps may not reflect insect population densities on the potato plants. Our intention was to survey extensively in the commercial potato production area for sharpshooters or other insect species that were sufficiently abundant as to plausibly fit the profile of a vector of *X. fastidiosa* or other pathogens that may be associated with ZC.

Cross-directional transects were laid out from each of the six lagunas into the potato fields, native brush and unplanted farmland (Fig 1). Each transect had 11 traps placed at intervals of 200 ft. Yellow sticky cards (Trécé Inc., Adair, OK) were placed 1 m above ground on a wooden stake. To secure the traps during the frequent high winds experienced in the LRGV, they were attached to the stake by a twist-tie and looped through a staple inserted into the stake. Trap sampling began on Jan. 17, 2006, just as the plants began to emerge from the tubers, with traps changed bi-weekly through April 24, 2006. Traps were returned to the laboratory for identification and counts of the sharpshooters, leafhoppers, and psyllids. Counts of the insect groups from the traps for each of the transects and lagunas were pooled by date and presented as means plus standard errors. Representative morpho-species for these groups were curated and held for identification. *Empoasca* spp. were not identifiable to the species level and may represent a complex. Counts of *Empoasca* spp. were pooled with *Penestragania* sp. as 'green' leafhoppers. Several species of 'brown' leafhoppers were collected from the traps and the numbers were pooled. It was determined that the most common brown leafhoppers were *Scaphytopius* sp., *Erythroneura* sp. and *Ceratagallia* sp. From each sample date, a sub-sample of the common insects were removed from the traps and shipped to the University of Texas - Tyler for molecular diagnostics. Mouthparts of the insects were removed and screened for presence of *X. fastidiosa*. At the conclusion of the sampling period, seasonal population levels of the insects were categorized as abundant, common, uncommon or rare. Insects categorized as abundant or common were collected in most or all of the transects throughout the season. Insects that were common early season were collected in most or all of the transects, but primarily in January

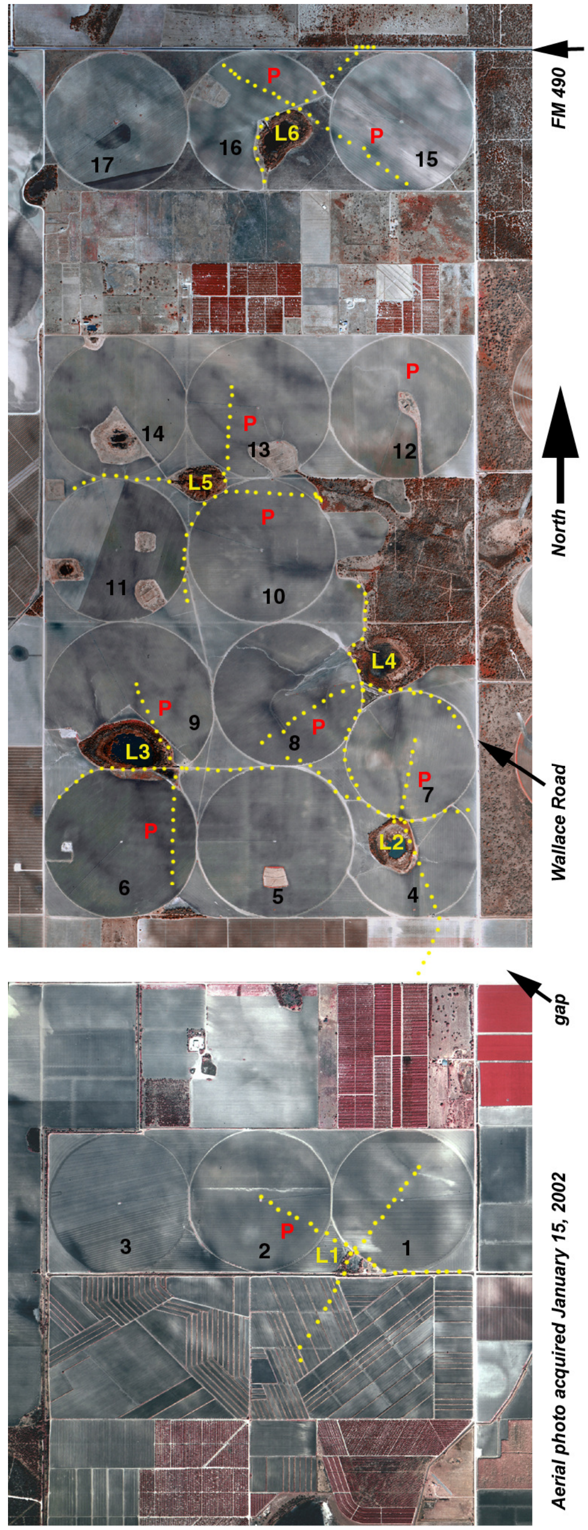

Fig. 1. Aerial view of potato fields near McAllen, TX, Hidalgo Co. Yellow dots represent trap locations. Lagunas are labelled L1-L6, fields 1-17, and potato fields 'P' (Photo by R. Fletcher, USDA-ARS).

and February. Uncommon or rare insects were collected only in some transects at various times during the study.

Plant Surveys. In addition to the insect surveys, samples of the common herbaceous and woody plants growing around the lagunas were collected on January 17, 2006. Plant samples were analyzed to determine if they were positive for the presence of *X. fastidiosa*. *Lycium* spp. were collected on May 21, 2006. *Lycium berlandieri* samples were collected from the native brush 1 km east of the study site. *Lycium carolinianum* was collected from native stands growing in the salty, clay soils east of San Benito in Cameron County, Texas.

Pathogen Screening. Adult insects were removed from sticky traps and analyzed for the presence of *X. fastidiosa*. Upon arrival, insects were immediately stored at 4°C. For all insects other than psyllids, heads were removed from the insect's bodies using a sterile razor blade and DNA was extracted following the protocol developed by Bextine et al. (2005). Insects were beheaded with a sterile razor blade by cutting from the suture anterior to the forelegs to the suture posterior to the pronotum. Insect heads were pinned in a microfuge tube with an insect pin (size 3) (Bioquip Inc., Rancho Dominguez, CA) placed through the back of the insect head and forced through the frons, so that the tip of the pin protruded slightly. Individually, 500 µl of phosphate-buffered saline (PBS) was added to the tube so that the head was completely submerged. All tubes were loaded into a tube rack and placed in a vacuum desiccator and placed under pressure (20 bars) three times. The buffer mixture was then pipetted and used for DNA extraction with the DNeasy Tissue kit. For psyllids (which were too small to be used in the vacuum extracted protocol), five individuals were macerated and extracted via the DNeasy Tissue kit protocol.

Samples of plant tissues including new growth, mature leaves and stems were collected and sent to B. Bextine for analysis. Plant DNA was extracted using ExtractN amp (Sigma-Aldrich, St. Louis, MO) following manufacturers protocol. PCR was run using primers BDnF2 and BBnR1 as previously mentioned.

For detection of *X. fastidiosa*, QRT-PCR was performed in a Rotor Gene 3000 (Corbett Research, Mortlake, Australia) by using iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA) (100 mMKCl, 40mMTris-HCl, pH 8.4, 0.4mMeach dNTP, 50 U/ml iTaq DNA polymerase, 6 mM MgCl2, SYBR Green I, and 20 nM ßuorescein) and 4 µl of DNA template in 20- µl reactions using PCR primers: BBin F2, 5' GTT TGA TTG ATG AAC GTG GTG AG 3' and BBin R1 5' CAT TGT TTC TTG GTA GGC ATC AG 3'. After an initial denaturing step of 3 min at 95°C, the reactions were cycled 40 times under the following parameters: 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. At the end of the PCR, the temperature was increased from 72 to 99°C at a rate of 1°C/15 s, and the fluorescence was measured every 1^{o}C increase to construct the melting curve. Because SYBR Green fluoresces in the presence of double-stranded DNA (Bates et al. 2001), nontarget amplication increases when primer dimers form. The target amplicon is created with the XfF2/XfR2 primer at 86.1°C. Therefore, only samples with a melting temperature of 86.1°C were considered positive. This was confirmed by comparing melting temperature-positive samples with agarose gel analysis of the same samples amplified by conventional PCR methods. In all QRT-PCR reactions, a nontemplate control (NTC) was run as a negative control and a known positive sample was run as a positive control.

RESULTS

Potato psyllids, *B. cockerelli,* were the most abundant insects collected in the study (Table 1). Psyllids began to enter the fields in mid-January and reached a peak in mid-March (Fig 2). The mean density of psyllids and leafhoppers by date in Fig. 2 represents the pooled data from ten separate potato fields. A high percentage of the psyllids tested positive for *X. fastidiosa*. The potato leafhopper, *Empoasca* spp. and *Penestragania* sp. were common throughout the season (Fig 2). A high percentage of individuals from both species tested were positive for *X. fastidiosa*. *Scaphytopius* sp., *Ceratagallia* sp. and *Erythroneura* sp were common throughout the season, but a lower percentage were positive for *X. fastidiosa*. Of the few sharpshooters that were collected nearly all were positive for *X. fastidiosa*.

Of the 13 plant species tested for *X. fastidiosa*, only one, *Funastrum clausum,* was positive for the bacterium (Table 2). Climbing milkweed vine is common around the lagunas and is found climbing on fences in the vicinity of the potato production. Cut foliage placed near infested potatoes attracted psyllid adults. Cut foliage from other plant species did not induce the same response from psyllid adults.

The incidence of ZC in the potato fields ranged from 12.92 to 20.42 percent (Table 3). The later planted fields had a lower percentage of ZC. Adjacent commercial chipping potato fields that were managed with similar agronomic and pest management practices exhibited similar levels of ZC (C. Burns, personal

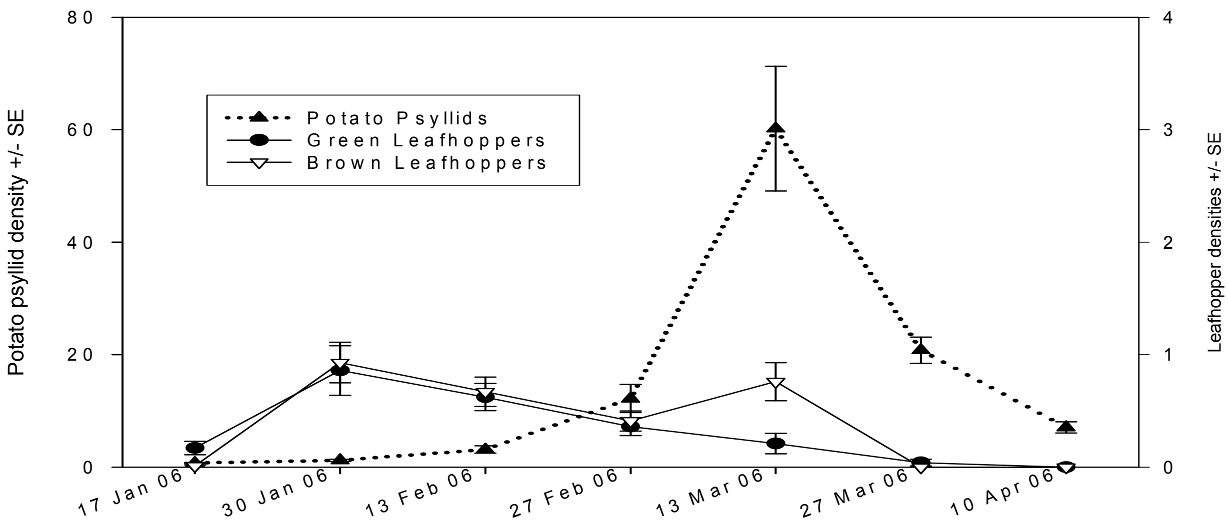

Fig. 2. Mean density of potato psyllids and leafhoppers on yellow sticky traps placed in potato fields in McAllen, TX with high incidence of zebra chip disorder. Brown leafhoppers are mixed populations of *Scaphytopius* sp., *Ceratagallia* sp. and *Erythroneura* sp. Green leafhoppers are populations of *Empoasca* spp. and *Penestragania* sp.

Table 1. List of insect species collected in traps with reference to their potential to vector plant diseases.

Scientific name	Common name	Family	Occurrence in fields	No.adults positive for *Xylella fastidiosa*
Bactericera cockerelli	potato psyllid	Psyllidae	abundant	250/285
Empoasca spp.	potato leafhopper	Cicadellidae	common	22/39
Scaphytopius sp.	leafhopper	Cicadellidae	common	4/29
Ceratagallia sp.	clover leafhopper	Cicadellidae	common	3/3
Penestragania sp.	leafhopper	Cicadellidae	common	32/32
Erythroneura sp.	grape leafhopper	Cicadellidae	common	15/20
Graminella sp.	leafhopper	Cicadellidae	common early season	13/19
Colladonus sp.	leafhopper	Cicadellidae	uncommon	0/4
Dikraneura sp.	leafhopper	Cicadellidae	uncommon	2/2
Balclutha sp.	leafhopper	Cicadellidae	uncommon	
Idiocerus sp.	leafhopper	Cicadellidae	uncommon	
Exitianus exitiosus	leafhopper	Cicadellidae	uncommon	
Homalodisca insolita	sharpshooter	Cicadellidae	rare	13/30
Homalodisca vitripennis	glassy-winged sharpshooter	Cicadellidae	rare	2/2
Xerophloea trivitata	spittlebug	Cercopidae	rare	

Table 2. Native plants adjacent to potato fields assayed for presence of *Xylella fastidiosa.*

Plant Name/Family	Common Name	Xf detected	Notes
Salix nigra H. Marshall Salicaceae	black willow	no	Common in lagunas and drainage ditches
Salix exigua Nutt. Salicaceae	sandbar willow	no	Common in lagunas and drainage ditches
Ipomoea trichocarpa Ell. Convolvulaceae	sharppod morning glory	no	Very common on edge of lagunas and drainage ditches
Funastrum clausum (Jacq.) Schlechter Asclepiadaceae	climbing milkweed vine	yes	Attractive to psyllid adults when cut plants placed in potato field
Funastrum cynanchoides (Vail) R. Holm Asclepiadaceae	bearded swallow wort	no	Common in dry, upland areas
Chenopodium berlandieri Moq. Chenopodiaceae	pitseed goosefoot	no	Very common on field margins
Leucosyris spinosa (Benth.) Greene Asteraceae	spiny aster	no	Very common on edge of lagunas
Helianthus annuus L. Asteraceae	common sunflower	no	Common in disturbed areas
Baccharis neglecta N. Britton Asteraceae	dry-land willow	no	Common in disturbed areas adjacent to lagunas
Solanum eleagnifolium Cav. Solanaceae	silverleaf nightshade	no	Very common on field margins
Solanum americanum Mill. Solanaceae	American nightshade	no	Common in drainage ditches
Lycium berlandieri M. Dunal Solanaceae	wolfberry	no	Known overwintering host for potato psyllid, common in native brush near potatoes in northern Hidalgo Co.
Lycium carolinianum T. Walter Solanaceae	Carolina wolfberry	no	Known overwintering host for potato psyllid. Common in eastern Cameron Co. near coast

Table 3. Field production parameters and zebra chip incidence at harvest.

Laguna / Transect / Field #	Planting date	Hectares (ha)	Mean yield (kg/ha)	% ZC incidence
L1/NW/2	7 Dec. 2005	48	33,911	20.42
L2/N/7	13 Feb. 2006	25	55,235	12.92
L3/S/6	13 Dec. 2005	53	30,478	19.81
L3/N/9	20 Dec. 2005	45	30,478	19.81
L4/W/8	24 Jan. 2006	40	24,250	12.92
L5/N/13	28 Dec. 2005	46	27,950	18.08
L5/E/10	26 Dec. 2005	49	26,713	17.81
L6/NW/16	5 Jan. 2006	30	31,802	19.67
L6/SE/15	3 Jan. 2006	49	34,469	18.36

communication). Fresh market potatoes planted near the study site, which received only 1 application of imidacloprid, (Gaucho® Bayer CropScience), exhibited ZC level as high as 80% (Goolsby and Munyaneza, unpublished data).

DISCUSSION

Two *Homalodisca* spp. of sharpshooters tested positive for *X. fastidiosa* and are known xylem feeders, but were so rare that it is not plausible they could be vectors of the pathogen in the crop. While large numbers of *Bactericera cockerelli*, *Empoasca* spp., *Scaphytopius* sp., *Ceratagallia* sp., *Erythroneura* sp. and *Penestragania* sp. tested positive for the presence of *X. fastidiosa* and these insects occurred at densities which could explain the widespread incidence of zebra chip in all of the potato fields, none are known xylem feeders; therefore, their role as vectors is in question. It is possible that these phloem feeders occasionally probe into xylem tissue and come in contact with *X. fastidiosa*, yet have no role in pathogen transmission. The DNA extraction and QRT PCR protocols used in this study are the most sensitive to date (Bextine et al., 2005), allowing for detection within the extremely low 10-100 cell concentration per insect which could explain the high number positives.

A large proportion of insects tested positive for the presence of *X. fastidiosa*, despite not having a previously described relationship with *X. fastidiosa*. While the presence of *X. fastidiosa* in these insects indicates their ability to obtain the pathogen in their digestive system, it does not confirm their ability to transmit the pathogen to other plants. The occurrence *X. fastidiosa* in potatoes and non-agricultural crops is poorly understood and the interactions of these plants and the insects assayed in our study is not known. Therefore, it is possible that these insects could be obtaining *X. fastidiosa* from potatoes or other plants without the ability to transmit the bacterium. However, this study has presented preliminary evidence that the tritrophic interactions between insects, plants, and bacteria are occurring in the LRGV.

It does not appear that *X. fastidiosa* is common in the native plant community of the local growing area that we tested, in that only one plant. *F. clausum*, climbing milkweed vine, tested positive. If *X. fastidiosa* is a contributing factor of ZC, then the vector with the pathogen must migrate into the fields from outside the local area. It was surprising that the known overwintering hosts of potato psyllids, *Lycium* spp., did not test positive for *X. fastidiosa*. This could indicate that other overwintering hosts are visited by potato psyllids where they acquire the *X. fastidiosa*. Other milkweed species could be asymptomatic hosts and should be further investigated. However, if leafhoppers are vectors of *X. fastidiosa*, then many other host plant species could be reservoirs based on their individual feeding preferences. Several of the leafhoppers are known to have strong dispersal abilities and could be migrating into the potato fields from distant locations.

The relationship of weather and ZC incidence is unclear, but it appears that warm, dry winters, such as occurred in 2005-06 season, may increase incidence of this disorder. High winds from both the north and south did occur during the growing season. Northerly winds could bring potato psyllids and/or other insects associated with ZC from known stands of *Lycium berlandieri* from the native brush north of the Lower Rio Grande Valley. Southerly winds could bring potato psyllids from the subtropical and tropical areas of northern Mexico.

Additional research to determine the role of potential vector(s), host plant reservoirs and pest management strategies are needed to minimize the impact of zebra chip on commercial potatoes in Texas. Further pathological studies are needed to determine the causal agent of ZC. The role of *X. fastidiosa* in the ZC disease system remains unclear. Also, several of the species that tested positive for the presence of *X. fastidiosa* may have the bacterium in their mouthparts, but may be vector-incompetent. Phytoplasma or toxic saliva from potato psyllid nymphs could also be a factor in disease expression (Lee et al. 2006; Munyaneza et al. 2007). Vector transmission studies are needed for the insects that tested positive in the study. The molecular genetic diversity of *B. cockerelli* should be investigated for evidence of new haplotypes. Other growing areas, especially northwestern Mexico and southern California are experiencing similar changes in disease incidence in tomatoes which are attributed to a new haplotype of potato psyllid (Liu et al. 2005). The impact of the natural enemies on all the potential vectors, in particular the potato psyllid, is needed. Flowering refuge strips could be integrated into the potato fields to enhance parasitism of psyllid nymphs. Insecticide efficacy studies are needed to compare the efficacy of in-furrow insecticides followed by mid-season compounds that may hold promise for control of potato psyllids. The effect of high winds (north and south), rainfall and temperature should be investigated as triggers for migration of potato psyllids and other potential vectors. Finally, the potato seed producing areas should be surveyed to determine the potential role of insect vectors that contribute to the epidemiology of ZC disorder.

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