

## Impact of Different Potato Psyllid Populations on Zebra Chip Disease Incidence, Severity, and Potato Yield

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### ABSTRACT

Zebra chip (ZC) is an emerging and damaging potato disease that is causing millions of dollars in losses to the potato industry in the southwestern United States, Mexico, and Central America. ZC plant symptoms resemble those caused by potato purple top and psyllid yellows diseases. Tubers produced by ZC-infected potato plants exhibit internal necrosis that affects the entire tuber. Fried chips processed from ZC-infected tubers have a characteristic striped pattern of necrosis and are unmarketable. This potato disease has recently been associated with the potato psyllid (*Bactericera cockerelli* Sulc). A field experiment was conducted in southern Texas, under controlled cage conditions, to document the impact of different geographic populations of potato psyllid on ZC incidence, severity, and potato yield. Nine different colonies of psyllids were used in the study. Results showed that potato plants exposed to potato psyllids developed typical ZC symptoms in raw tubers and fried chips. ZC incidence in potato plants ranged from 0 to 100%. Not all insects used in the study were infective and only six psyllid colonies induced ZC symptoms in potatoes. No ZC symptoms were observed in psyllid-free control plots. Results indicated that ZC symptom severity in fried chips was correlated with ZC severity in raw tubers and with tuber weight. The impact of potato psyllid on potato yield and processing quality was highly significant. The number of commercially acceptable tubers per plant was significantly reduced and up to 93% potato yield loss occurred when potato plants were exposed to psyllids. Tubers from ZC-infected plants produced unmarketable potato chips.

*Additional Index Words:* *Bactericera cockerelli*, potato psyllid, zebra chip, disease incidence and severity, potato yield.

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Zebra chip (ZC), an important and emerging potato disease, is causing millions of dollars in losses to potato producers and processors in the southwestern United States, Mexico, and Central America (Goolsby et al. 2007a,b; Munyaneza et al. 2007a,b). This disease is characterized by symptoms that develop in fried chips from infected potato tubers that consist of a striped pattern of necrosis in tuber cross-section (Munyaneza et al. 2007a,b). ZC plant symptoms resemble those caused by potato purple top and psyllid yellows diseases and include purpling, chlorosis, aerial tubers, and leaf scorch (Munyaneza et al. 2007a,b). To date, the exact causal agents of ZC are unknown. However, recent studies (Goolsby et al. 2007a,b; Munyaneza et al. 2007a,b) have shown that the potato

psyllid, *Bactericera cockerelli*, is strongly associated with ZC. Growers in the Lower Rio Grande Valley of Texas, one of the regions seriously affected by the disease, have recently managed to keep ZC incidence in potatoes under manageable levels by applying insecticides targeted against the potato psyllid (Goolsby et al. 2007a). However, little is known on the impact of this insect pest on the incidence and severity of ZC. In addition, information on the impact of ZC on potato yield and factors that influence ZC symptom expression and severity in potatoes is lacking. Furthermore, there have been reports that some geographic populations of the potato psyllid may fail to induce ZC symptoms in potatoes (J.E. Munyaneza and G. Bester, unpublished data). The main objective

of the present study was to document, under controlled field conditions, the impact of different potato psyllid populations on ZC incidence and severity. Also, the impact of ZC and potato psyllid on potato yield was investigated.

## MATERIALS AND METHODS

The field experiment was conducted at the USDA-ARS Research Farm in Weslaco, TX. Certified clean potato seed (Atlantic variety) was obtained from Black Gold Farms, Pearsall, TX. The potatoes were hand-planted in plots that consisted of 1.8 m of a single row each on 15 January 2008 (Fig. 1). Six potatoes were planted in each plot. The plots were arranged in a randomized complete block design. Each treatment was replicated four times. Selected plots were each covered individually with a cage that consisted of a 2 m tent-like narrow model cage designed to cover a single row of potato plants. Each cage was made of fiberglass tree stakes (GEOTEK, Inc., Stewartville, MN) for frame, insect screen fabric (USGR, Inc., Seattle, WA) for cover, and tent stakes (Figs. 1 and 2). There were 11 treatments consisting of uncaged plants, caged plants without psyllids (controls), and caged plants with psyllids from one of nine colonies. The psyllids used in the study were obtained from the following sources: 1) three psyllid colonies from USDA-ARS Wapato (WA) referred to as Munyaneza Lab colony, Horton Lab colony, and Washington-field collected colony; Munyaneza colony was established with psyllids originally collected from a potato field severely affected by ZC in Dalhart, TX, in late fall 2005; psyllids in Horton colony originated from insects collected from south Texas and Dalhart, TX; 2) a colony referred to as "Mixture", originally made of psyllids from Munyaneza Lab, Horton Lab, and Washington-field collected colonies, 3) North Texas colony obtained from the laboratory of Dr. Charlie Rush, Texas A&M AgriLife Research in Bushland, TX; the psyllids were originally collected from Dalhart, TX in 2007, 4) two colonies from the laboratory of Dr. Tong-Xian Liu, Texas A&M AgriLife Research in Weslaco, TX; the colonies are referred to as Liu-Potato and Liu-Tomato and were originally collected in south Texas, 5) Texas field collected colony whose psyllids were directly collected from potatoes, and 6) South California colony that was obtained from the laboratory of Dr. John Trumble at University of California, Riverside, CA. In the treatment involving Texas potato field collected psyllids, the insects were collected from a block of potatoes that had been planted next to the experimental plots and were immediately transferred to potatoes in each of the assigned caged plots. The

remaining colonies consisted of insects that had been reared in the laboratory for several generations on potato, except the South California and Liu-Tomato colonies which had been reared on tomato, in addition to the Munyaneza Lab colony that had been reared on egg plant. Approximately 350 psyllid adults were introduced into each assigned caged plot, except for the Texas field collected psyllids treatment in which nymphs were used because not enough psyllid adults could be collected. The first insect release (200 psyllids per cage) was made on 4 March 2008, when the plants were in the pre-bloom stage (Fig. 1) and naturally occurring psyllids had been observed on the uncaged plants. Caged plots were inspected for psyllid establishment on 14 March 2008 and a second insect release (150 psyllids per cage) was made to augment the psyllids in these plots.

Caged plots were covered immediately after potato planting. The insect screen fabric covered the entire cage and was buried in the ground at the bottoms of the cage to exclude unwanted insects. Irrigation of the potato plants was accomplished by a drip tape that was buried in the hill just after planting and fertilizer was delivered through this irrigation as needed. A pre-plant herbicide (Eptam® 7-E, Gowan, Yuma, AZ) was used to control weeds in the cages and no insecticides were applied to the potatoes throughout the study. Potato psyllids were monitored weekly at the research site by deploying five yellow sticky traps for adults and counting psyllid eggs and nymphs on 100 leaves collected from an untreated potato block next to the experimental plots. Potatoes were harvested on 19 May 2008. Potato tubers from each individual plant in the experiment were collected and shipped to the USDA-ARS Laboratory in Wapato, WA, for processing. The tubers were weighed individually to estimate potato yield and checked for ZC symptoms by making a cross-section cutting near the stem end. The tubers were then sliced into chips and fried according to Munyaneza et al. (2007a) to check for ZC symptoms and to estimate disease incidence. In addition, tubers were selected from seven treatments with high ZC incidence and shipped to Frito-Lay Inc. in Rhinelander, Wisconsin, for chip processing to assess ZC symptom severity in tubers from an infected plant. The chips and raw tuber material were scored using the Frito-Lay scale of 0 (no visible ZC symptoms) to 5 (highly visible ZC symptoms); acceptable color score in fried chips is 1 or 0. Furthermore, due to the resemblance of ZC symptoms and those caused by the potato purple top disease, 70 tubers exhibiting typical ZC symptoms were randomly collected from seven treatments (10 tubers each) and tested for phytoplasmas using a nested polymerase chain reaction (PCR) assay with universal primer pairs



**Fig. 1.** Experimental design layout and potato plots; plots were arranged in a complete randomized block design with for 4 replications for each treatment (A). Potato plant growth stage at the time of potato psyllid release in the cages (B).



**Fig. 2.** Caged potato plants without psyllids (A) and caged plants with psyllids exhibiting potato psyllid damage and zebra chip (ZC) symptoms (B), five weeks after insect release.

P1/P7 and fU5/rU3 according to Crosslin et al. (2006). Testing was performed at USDA-ARS Laboratory in Prosser, WA.

Percentage of plants with ZC symptoms in both raw tubers and fried chips were calculated for each treatment and comparisons were made between the different psyllid populations. Comparisons between populations were also made for the total number of tubers and number of commercially acceptable tubers (at least 50 g in weight or 5 cm in diameter, according to Frito-Lay standards) per plant. In addition, total and commercial yields per plant were estimated and compared between populations. Moreover, individual tuber weight was compared among seven treatments that produced ZC symptoms after psyllid exposure. Analysis of variance was performed by using SAS general linear models procedures (PROC GLM; SAS Institute 2003). Percentage data were transformed using  $\arcsin \sqrt{x}$  prior to performing analysis of variance. Spearman rank correlation test was used to assess correlation between ZC symptom severity scores between raw tuber material and fried chips, in addition to determining whether tuber weight affects ZC symptom severity (SAS Institute 2003). Furthermore, the Kruskal-Wallis non-parametric test was performed, after averaging the severity scores for tubers from each individual potato plant, to assess whether ZC symptom severity in raw tubers and fried chips differed among different treatments (SAS Institute 2003). The level of significance was set at  $P=0.05$  and the Ryan-Einot-Gabriel-Welsch multiple range test (REGWQ) was used to separate means.

## RESULTS

Potatoes emerged approximately four weeks after planting. By mid-February, naturally occurring potato psyllids were present at the study site and eggs and nymphs were observed on uncovered potato plants in late February (Fig. 3). Three weeks after insect release, psyllid-induced damage symptoms were visible on the plants in both uncovered and caged plots with psyllids. Five to six weeks after psyllid exposure, potatoes were exhibiting severe psyllid-induced plant damage (Fig. 2) and typical ZC symptoms in raw tubers (Fig. 4). Plant symptoms included rolling upward of the top leaves developing into a basal cupping of the leaflets, accompanied with yellowish discoloration; proliferation of axillary buds, shortened internodes, swollen nodes, and leaf scorching (Fig. 2). Tuber symptoms included collapsed stolons, brown discoloration of the vascular ring, necrotic flecking of internal tissues, and streaking of the medullary ray tissues (Fig. 4).

No single potato plant in caged plots without

psyllids (controls) showed ZC symptoms in raw tubers or fried chips (Table 1). In contrast, 100% of plants from plots exposed to psyllids from Munyaneza Lab, Horton Lab, Washington-field, Mixture, and South California colonies exhibited ZC symptoms in both raw tuber material and fried chips (Table 1). Potato plants exhibiting ZC symptoms averaged 66.7 and 87.5% for uncovered plots and those exposed to Texas-field collected psyllids, respectively (Table 1). Interestingly, none of the plants from plots exposed to psyllids from Liu-Potato, Liu-Tomato, and North Texas colonies showed ZC symptoms (Table 1). Statistical analysis showed that there were significant differences in ZC incidence between treatments (ANOVA;  $P < 0.0001$ ; Table 1).

Although statistical analysis showed significant difference in the total number of tubers per plant between treatment (ANOVA;  $P = 0.0004$ ), there was no difference between the number of tubers per plant produced from the control plots and the remaining treatments (Table 1). However, the plants from the control plots produced a higher number of commercially acceptable tubers (greater than 50 g or 5 cm in diameter) compared to those plants exposed to psyllids (ANOVA;  $P < 0.0001$ ; Table 1).

Total potato yield per plant was significantly higher for psyllid-free control plots than those exposed to psyllids (ANOVA;  $P < 0.0001$ ; Table 1). The yield loss ranged from 48 to 78% (Table 1). Similarly, the commercially acceptable yield was significantly higher for potato plants from plots without psyllids (ANOVA;  $P < 0.0001$ ; Table 1) and the commercial yield loss ranged from 55.2 to 93% (Table 1). The impact of psyllids on potato yield was significant even for treatments with psyllids that did not induce ZC symptoms (Table 1). Early tuber sprouting before harvest was observed in plots exposed to these non ZC-inducing psyllid colonies and plants with early sprouting tubers averaged 12.5, 33.3, and 72.7% of the plants in plots exposed to psyllids from Liu-Potato, Liu-Tomato, and North Texas colonies, respectively.

Correlation coefficients and P-values from the Spearman rank correlation test indicated that there were positive correlations between the rank scores of ZC symptom severity in raw tubers and fried chips for each treatment (Table 2), suggesting that ZC symptom severity in fried chips depends on ZC severity in raw tubers. However, a range of symptom severity between tubers from the same plant was observed in some of the treatments and included tubers with no visible symptoms but showed ZC symptoms in fried chips. Also, results from the Spearman rank correlation test indicated that there was evidence in several treatments that ZC symptom severity in fried chips increased with increasing tuber weight (Table 2).

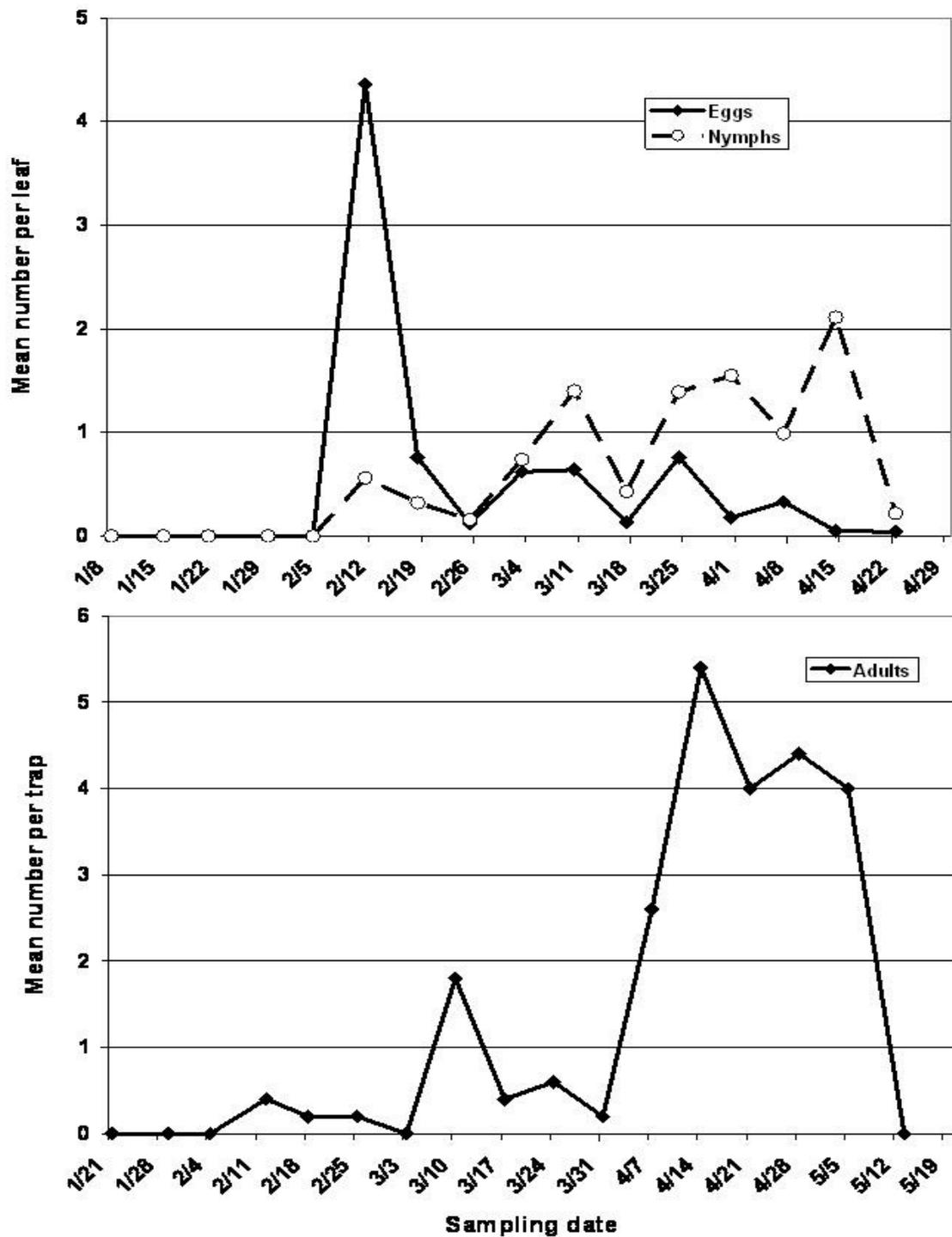
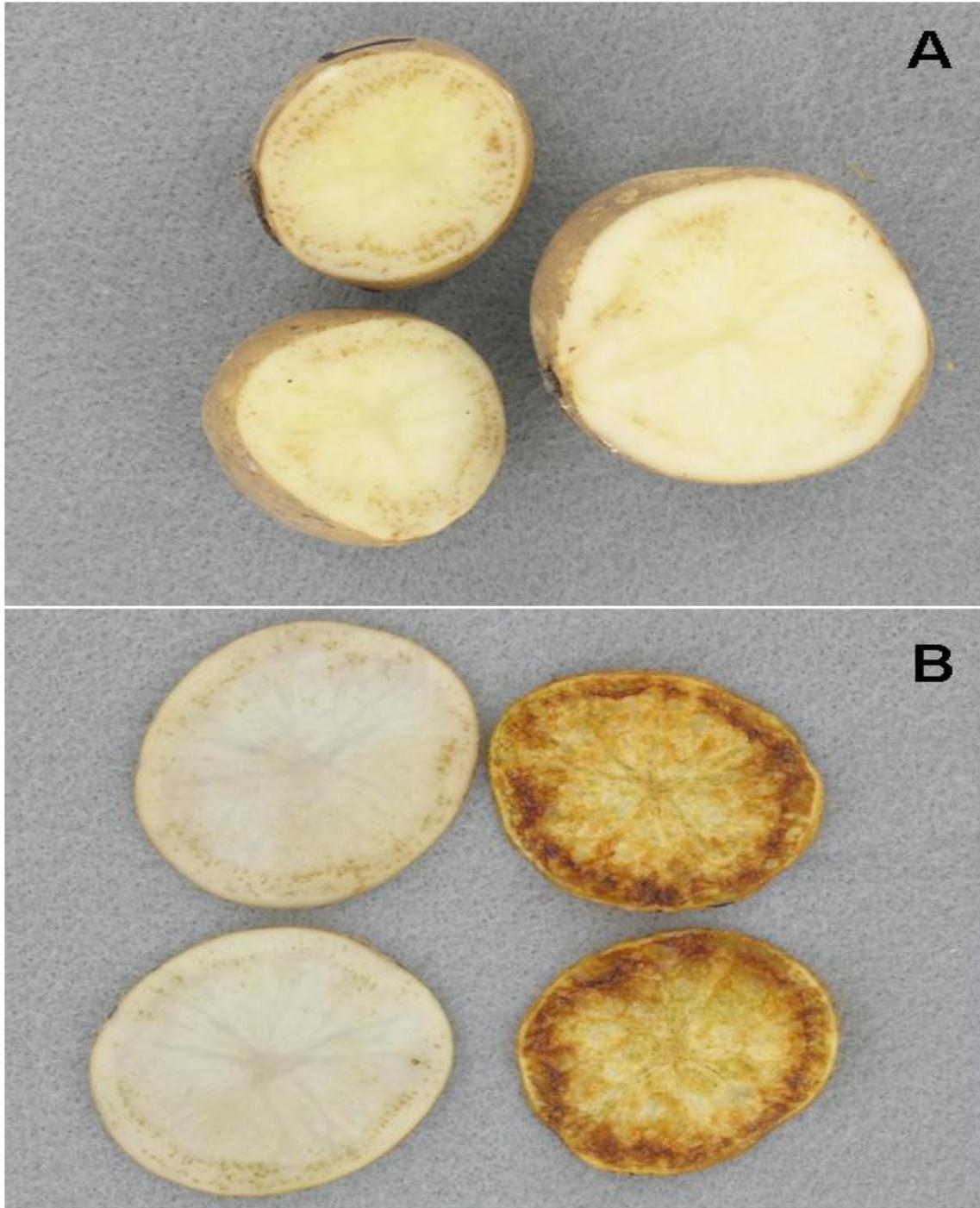


Fig. 3. Densities of the potato psyllid population at the USDA-ARS Research Farm in Weslaco, TX, throughout the study in 2008. Mean numbers of potato psyllid eggs and nymphs per leaf in the field of potatoes adjacent to experimental plots and number of adults per yellow sticky trap at each sampling date.



**Fig. 4.** Raw potato tubers from plants exposed to potato psyllids exhibiting ZC symptoms (A), raw sliced (cross-section) and fried chips from harvested ZC infected potato tubers, resulting from plant exposure to potato psyllids (B).

**Table 1.** Impact of different potato psyllid populations on zebra chip (ZC) incidence and potato yield (Mean  $\pm$  SEM).<sup>a</sup>

Treatment (Psyllid Colony)	Plants with ZC symptoms in fried chips (%)	Total number of tubers/plant	Number of tubers > 50 g/plant	Total yield/plant (g)	Commercially acceptable yield/plant (g)
Control	0.0 $\pm$ 0.0b	4.5 $\pm$ 0.4ab	3.4 $\pm$ 0.3a	502.4 $\pm$ 32.2a	472.7 $\pm$ 32.4a
Mixture	100.0 $\pm$ 0.0a	5.2 $\pm$ 0.4ab	2.3 $\pm$ 0.2b	261.5 $\pm$ 22.0b	211.9 $\pm$ 26.1b
Texas Field	66.7 $\pm$ 20.4a	5.8 $\pm$ 0.4ab	2.1 $\pm$ 0.3b	223.7 $\pm$ 27.9bc	185.5 $\pm$ 32.2bc
Liu-Potato	0.0 $\pm$ 0.0b	5.9 $\pm$ 0.5a	1.8 $\pm$ 0.3bc	223.2 $\pm$ 21.4bc	154.6 $\pm$ 22.5bcd
Liu-Tomato	0.0 $\pm$ 0.0b	6.0 $\pm$ 0.6a	1.4 $\pm$ 0.2bcd	199.8 $\pm$ 20.8bcd	117.3 $\pm$ 21.4bcde
Munyaneza Lab	100.0 $\pm$ 0.0a	4.4 $\pm$ 0.3ab	1.6 $\pm$ 0.3bcd	199.6 $\pm$ 22.2bcd	135.4 $\pm$ 24.4bcd
South California	100.0 $\pm$ 0.0a	5.6 $\pm$ 0.5ab	1.4 $\pm$ 0.2bcd	183.7 $\pm$ 19.7bcd	117.8 $\pm$ 19.6bcde
Washington Field	100.0 $\pm$ 0.0a	4.4 $\pm$ 0.4ab	1.3 $\pm$ 0.2bcd	162.1 $\pm$ 17.0cd	104.2 $\pm$ 18.6cde
North Texas	0.0 $\pm$ 0.0b	4.5 $\pm$ 0.4ab	1.0 $\pm$ 0.2cd	134.7 $\pm$ 18.5cd	76.4 $\pm$ 20.5de
Horton Lab	100.0 $\pm$ 0.0a	3.8 $\pm$ 0.5b	0.9 $\pm$ 0.2cd	115.6 $\pm$ 14.2d	72.1 $\pm$ 16.9de
Uncovered	87.5 $\pm$ 12.5a	4.7 $\pm$ 0.4ab	0.6 $\pm$ 0.2cd	108.8 $\pm$ 12.3d	33.0 $\pm$ 10.2e

<sup>a</sup> Means followed by the same letter within columns are not significantly different ( $P > 0.05$ ; RGEWQ).

In addition, results from the Kruskal-Wallis test, with data from the controls excluded, indicated that there was no evidence that treatments affected ZC symptom severity in raw tubers or fried chips (Table 2). The overall results showed that potato plants in the plots that were exposed to psyllids that induce ZC produced commercially unacceptable chips.

Only one of the 70 tubers with typical ZC symptoms collected and tested for phytoplasmas by nested PCR using the universal primer pairs P1/P7 and fU5/rU3 (Crosslin et al. 2006) was positive for these plant pathogens.

### DISCUSSION

Results of the present study are consistent with previous research conducted by Munyaneza et al. (2007a,b) and conclusively implicate the potato psyllid as a vector of the agent(s) causing this emerging and damaging potato disease. Several of the potato psyllid

colonies used in the study readily induced typical ZC symptoms in potato plants, tubers, and chips. Interestingly, three of the psyllid colonies did not cause the disease, suggesting that some populations of potato psyllid are not infective.

Not only does the potato psyllid cause ZC, this insect also affects the potato yield. During this study, all the populations of the potato psyllid significantly reduced the number of commercially acceptable potato tubers per plant, including psyllids from populations that did not induce ZC. Up to 93% yield loss was observed in potato plants that were exposed to psyllids. Moreover, early tuber sprouting was observed in plants that were colonized by psyllids. These results suggest that this insect is economically important and that even populations that are not infective will cause substantial yield loss to potatoes. In addition, early tuber sprouting was observed in a large number of plants that had been exposed to

**Table 2.** Impact of different potato psyllid colonies on tuber weight and zebra chip (ZC) symptom severity in raw tubers and fried chips.

Treatment (Psyllid Colony)	Tuber weight (g) Mean +/- SEM (Range)	ZC severity in raw tuber Mean +/- SEM (Range)	ZC severity in fried chip Mean +/- SEM (Range)	Correlation coefficient values
Control	117.63 ± 18.24 (5.63-279.10)a	0.00 ± 0.00	0.00 ± 0.00	---
Washington Field	43.85 ± 7.10 1.63-109.67)c	1.26 ± 0.17 (0-3)	1.33 ± 0.25 (0-4)	r= 0.57; P= 0.0064* (r= 0.66; P= 0.0010) **
Texas Field	58.68 ± 9.21 3.03-121.89)bc	0.79 ± 0.19 (0-3)	1.41 ± 0.34 (0-4)	r= 0.27; P= 0.2962 (r= 0.80; P< 0.0001)
South California	53.21 ± 9.07 (1.07-156.25)bc	2.20 ± 0.34 (1-4)	2.22 ± 0.29 (0-4)	r= 0.47; P= 0.0372 (r= 0.54; P= 0.0147)
Munyaneza Lab	60.30 ± 9.72 (1.02-135.76)bc	1.90 ± 0.25 (0-3.5)	1.75 ± 0.27 (0-4)	r= 0.65; P= 0.0020 (r= 0.48; P= 0.0328)
Horton Lab	32.51 ± 6.42 (4.30-102.63)c	1.60 ± 0.23 (0-3)	1.60 ± 0.35 (0-4)	r= 0.81; P< 0.0001 (r= 0.69; P= 0.0008)
Mixture	87.42 ± 15.07 (5.0-321.78)ab	2.27 ± 0.27 (0-4)	2.32 ± 0.30 (0-5)	r= 0.18; P= 0.4237 (r= 0.76; P< 0.0001)
	P< 0.0001***	P= 0.0601	P= 0.5395	

†Controls were excluded from comparison analysis between treatments because severity scores were all zeroes

\*Correlation between tuber weight and ZC severity in fried chips; \*\*Correlation between ZC severity in raw tuber and fried chips

\*\*\*Comparison between treatments using either ANOVA (tuber weight) or a Kruskal-Wallis test (severity scores); means followed by the same letter within columns are not significantly different (P> 0.05)

psyllids. The results are consistent with previous reports of the production of a large number of abnormally small and unmarketable potato tubers which sprout without a dormant period due to the potato psyllid damage (Wallis 1955, Cranshaw 1994).

Results of the present study showed that ZC symptom severity in fried chips was positively correlated to ZC symptom severity in raw tuber material. However, there were also observations of a

wide range of ZC symptom severity between tubers of the same plant, with some tubers showing no visible ZC symptoms, but yet exhibited typical symptoms in fried chips. These results are consistent with previous observations (Munyaneza et al. 2007a) that led to the conclusion that accurate estimation of ZC incidence in potatoes requires frying. Results of the present study also indicated that ZC symptom severity in fried chips increases with increasing tuber weight. On the other

hand, undersized tubers are unmarketable, increasing the economic loss due to ZC and potato psyllid. Furthermore, potato tubers affected by ZC and potato psyllid usually do not sprout and cannot be used as seed (Wallis 1955, Cranshaw 1994, Munyaneza et al. 2007a).

To date, mechanisms by which the potato psyllid induces ZC are not well understood. However, pathogens and/or toxins are suspected to be involved in the development of ZC symptoms in potato (Munyaneza et al. 2007a,b). Despite the fact that ZC foliar symptoms strongly resemble those of potato purple top disease, phytoplasmas that cause this disease were very rarely detected in ZC symptomatic tubers during the present study. These results are consistent with previous conclusions reached by Munyaneza et al. (2007a,b) and suggest that these plant pathogens are not involved in ZC. Recent testing for other plant pathogens by PCR however has revealed the presence of a previously undescribed species of the bacterium “*Candidatus Liberibacter*” in potato tubers showing typical symptoms of ZC (Liefing et al. 2008; Lin et al. 2008; USDA-APHIS 2008). In addition, this bacterium has been detected in potato and tomato plants exhibiting symptoms of potato psyllid damage; in addition to potato psyllids (Hansen et al. 2008; Liefing et al. 2008; Lin et al. 2008). However, despite the association of this bacterium with ZC-infected material and the potato psyllid, the role of this putative causal agent in ZC symptom expression is not yet clear.

In conclusion, results of the present study provided conclusive evidence that the potato psyllid is the vector of the agents that cause ZC and support previous findings by Munyaneza et al. (2007a,b). Results also showed that not all potato psyllid populations induce ZC. However, mechanisms by which this insect induces this potato disease are not well understood and more studies are needed to increase the understanding of the epidemiology of this disease. During the present study, exposing potato plants to psyllids not only produced ZC and reduced potato processing quality, but also caused substantial potato yield loss. ZC symptom severity in chips was not only positively correlated to ZC symptom severity in raw tuber material but also to the tuber weight. Effective monitoring and management strategies for the potato psyllid are needed to minimize damages caused by this emerging potato disease and the impact of this insect pest on the incidence and severity of the disease and potato yield.

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