

## Further Evidence that Zebra Chip Potato Disease in the Lower Rio Grande Valley of Texas is Associated with *Bactericera cockerelli*

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### ABSTRACT

**Zebra chip (ZC) is an important and emerging potato disease that is causing millions of dollars in losses to both potato producers and processors in the southwestern United States, Texas in particular. This disease is characterized by symptoms that develop in fried chips from infected potato tubers and that consist of a striped pattern of necrosis in tuber cross-section. Zebra chip plant symptoms resemble those caused by potato purple top and psyllid yellows diseases. To increase the understanding of the role of the potato psyllid (*Bactericera cockerelli* Sulc) and phytoplasmas in the expression of ZC, controlled exposure and exclusion field experiments using cages were conducted in the Lower Rio Grande Valley of Texas, where the psyllid is common and abundant and the disease is very damaging. Also, potato tubers exhibiting ZC symptoms were tested for phytoplasmas by PCR. Results indicated that there was a strong association between the potato psyllid and ZC. Plants exposed to psyllids developed typical ZC symptoms in both raw tubers and fried chips. At harvest, potato plants exhibiting ZC symptoms in raw tubers averaged 79.2, 37.5, and 48.6% for uncaged plants, caged plants exposed to Texas field-collected psyllids, and caged plants exposed to laboratory-reared psyllids, respectively. Incidence of ZC increased when the harvested tubers were processed into fried chips. No single potato plant in the cages without psyllids (controls) showed ZC symptoms in raw tubers or fried chips, suggesting that the observed ZC symptoms were due to psyllids. No phytoplasmas were detected in tubers with ZC symptoms, suggesting that these pathogens are not involved in ZC.**

*Additional Index Words:* zebra chip, potato, phytoplasma, potato psyllid, Lower Rio Grande Valley.

Zebra chip (ZC), a new disease of potato (*Solanum tuberosum* L.), has recently been documented to occur in potato fields throughout southwestern United States, Mexico, and Central America (Secor and Rivera-Varas 2004, Secor et al. 2006, Goolsby et al. 2007, Munyaneza et al. 2007). This potato disease is characterized by symptoms that develop in potato tubers from infected plants and that consist of a striped pattern of necrosis in tuber cross-section (Munyaneza et al. 2007). In the United States, the disease was first identified in 2000 in commercial potato fields in Pearsall and Lower Rio Grande Valley in Texas (Secor and Rivera-Varas 2004). Since that time, ZC has been observed in a number of other states, including Nebraska, Colorado, Kansas, New Mexico, Arizona, Nevada, and California (Munyaneza et al. 2007). This potato disease is causing millions of

dollars in losses to both potato producers and processors in affected areas, often causing the abandonment of entire potato fields (Goolsby et al. 2007, Munyaneza et al. 2007).

ZC-affected potato plants exhibit a range of plant symptoms that resemble those caused by potato purple top and psyllid yellows diseases (Wallis 1955, Cranshaw 1994, Lee et al. 2004, Crosslin et al. 2005, Munyaneza 2005, Munyaneza and Upton 2005; Munyaneza et al. 2006, 2007). Plant symptoms include stunting, chlorosis, swollen internodes of the upper growth, proliferation of axillary buds and aerial tubers, browning of the vascular system in below-ground portions of stems, leaf scorching, and early plant decline (Figs. 1A-C). ZC symptoms in tubers include enlarged lenticels of the underground stem, collapsed stolons, and brown discoloration of the vascular ring

and necrotic flecking of internal tissues and occasionally streaking of the medullary ray tissues (Figs. 1D-E). These necrotic symptoms affect the entire tuber from the stem end to the bud end. Chips made from tubers of affected potato plants have a severe dark brown streaking defect, hence the name 'zebra chip' (Figs. 1F-G). This severe dark brown streaking defect has become a serious problem for fresh and processing potatoes produced in the areas where ZC occurs, causing rejection of fresh potatoes and chips made from tubers of affected plants (Munyanza et al. 2007). Furthermore, tubers affected with ZC generally do not sprout, or if they do, produce hair sprouts or weak plants (Munyanza et al. 2007).

To date, the exact causal agent(s) and/or vectors of ZC are unknown. However, a survey of insects associated with the potato crop in Texas indicated that the potato psyllid, *Bactericera* (= *Paratrioza*) *cockerelli* (Sulc) was the most common and abundant insect in all of the ZC-affected potato fields (Goolsby et al. 2007). Furthermore, a recent study by Munyanza et al. (2007) showed that there was a strong association between this potato disease and the potato psyllid. However, this study was conducted in the greenhouse and field under Washington State conditions where the potato psyllid is uncommon and ZC has so far not been documented (J.E.M., unpublished data). Moreover, the environmental conditions in the Pacific Northwest are different from those in the southwestern United States and may influence the expression of the disease symptoms and severity. In fact, ZC symptoms observed during this study conducted in Washington were less severe than those usually observed in Texas.

The main objective of the present study was to increase the understanding of the role of the potato psyllid in the expression of ZC by conducting controlled field exclusion and exposure experiments in the Lower Rio Grande Valley of Texas which is a native range for *B. cockerelli* (Wallis 1955) and where this potato disease causes serious losses to both potato producers and processors (Goolsby et al. 2007, Munyanza et al. 2007). In addition, since ZC symptoms resemble those of the potato purple top disease, investigation of potential ZC causal agents was conducted by testing tubers exhibiting typical ZC symptoms for the phytoplasmas that cause potato purple top disease.

## MATERIALS AND METHODS

The experiments were conducted at the USDA-ARS Research Farm in Weslaco, TX. Certified clean potato seed of three potato chipping varieties (Atlantic, FL1879, and FL1867) were obtained from J. Wallace

Farms, Edinburg, TX, and Black Gold Farms, Grand Forks, ND. The potatoes were hand-planted in field plots that consist of 3.6 m of a single row each on January 18, 2007 (Fig. 2). The plots were arranged in a complete randomized design. Twelve potatoes (four for each variety) were planted in each plot. There were four treatments: caged plants infected with psyllids from the laboratory colony, caged plants infected with Texas field collected psyllids, caged plants without psyllids, and uncaged plants. Each cage consisted of a 4.6 m tent-like narrow model cage designed to cover a single row of potato plants and was made of PVC pipes, Agribon fabric (Polymer Group Incorporated, Charlotte, NC), heavy nylon twine, and tent stakes (Fig. 2). Treatments with caged plants receiving psyllids were replicated six times each whereas control treatments consisting of caged plants without psyllids and uncaged plants were replicated four times each (for a total of 20 plots). In the treatment involving laboratory-reared psyllids, the insects used were from a colony that had been established at the USDA-ARS Laboratory at Wapato, WA, since late fall 2005, from psyllids originally collected from a potato field severely affected by ZC in Dalhart, TX; these psyllids had been maintained on potato and eggplant for several generations. In the treatment involving 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 plots. In both treatments, approximately 300 psyllid nymphs were introduced in each cage; the insect release was made on March 13, 2007, when the plants in the cages were in the pre-bloom stage (Fig. 2B) and psyllids had been observed on the uncaged plants.

Plots with cages were covered on January 30, 2007, before potato emergence and the bottoms of the cages were buried in the ground 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 the fertilizer was delivered through this irrigation as desired. 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 in the uncovered plots by counting psyllid eggs and nymphs on 60 leaves (20 leaves for each potato cultivar) weekly. The plants in the cages were checked for psyllid establishment on April 12, 2007, by briefly opening the cages and visually documenting presence or absence of the psyllids on the plants. A regular inspection throughout the growing season was not conducted to avoid possible intrusion of unwanted insects in the cages. Uncaged plants were also monitored for ZC symptoms. Potatoes were harvested



**Fig. 1.** Potato plants exhibiting observed zebra chip (ZC) symptoms (A-C), raw potato tubers exhibiting observed ZC symptoms (D and E), fried chips from harvested ZC infected potato tubers (F and G), and fried chips from a ZC-free potato tuber (H).



**Fig. 2.** Experimental potato plots with or without cages (A), growth stage of the potato plants when potato psyllids were released in the cages (B), caged potato plants (C), and potato plants being inspected for potato psyllids (D).

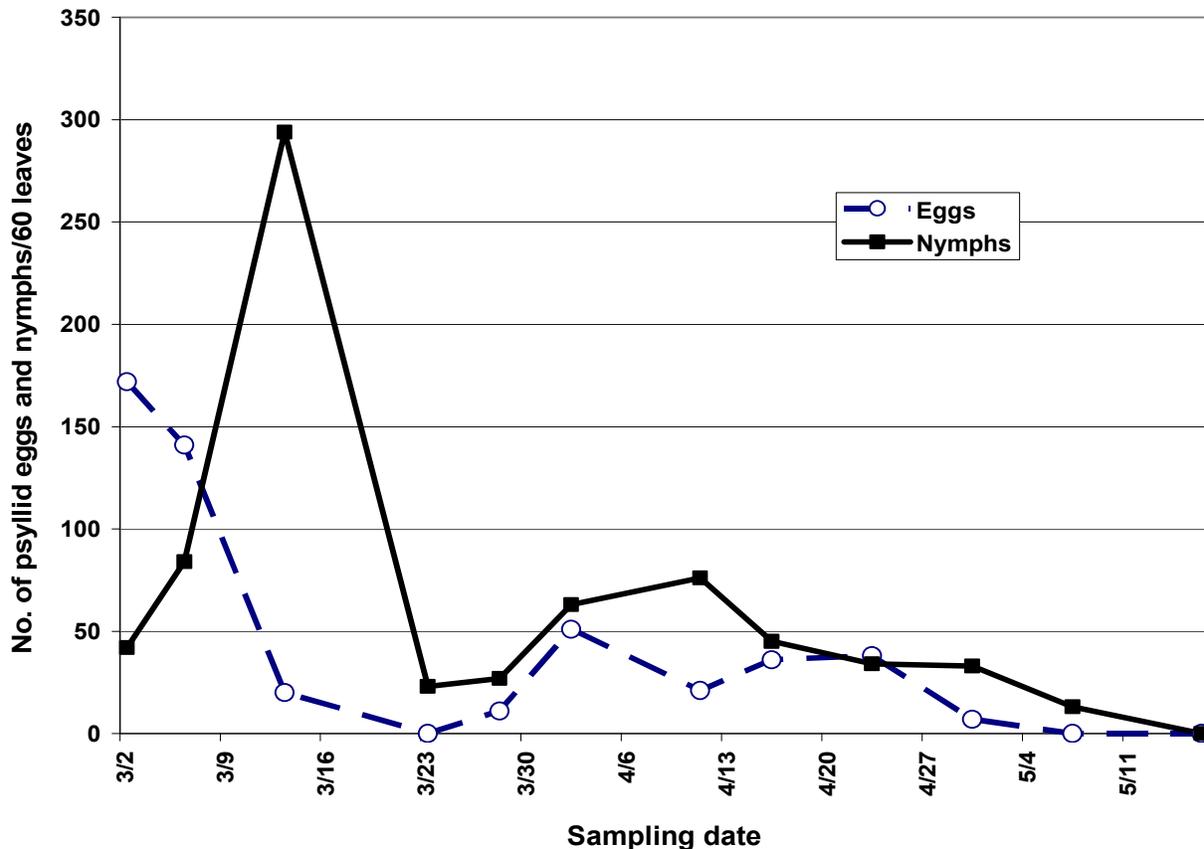
on May 25, 2007. Potato tubers from each individual plant in each treatment were collected in paper lunch bags and shipped to the USDA-ARS Laboratory in Wapato, WA, for processing. The raw tubers were checked for ZC symptoms by making a cross-section cutting near the stem end. The tubers were then sliced into chips and fried according to Munyaneza et al. (2007) to check for discoloration and ZC symptoms. Due to the resemblance of ZC symptoms and those caused by the potato purple top disease, tubers exhibiting typical ZC symptoms were randomly collected from 46 potato plants (18 Atlantics, 19 FL1879s, and 9 FL1867s) at harvest and tested for phytoplasmas using a nested polymerase chain reaction (PCR) assay with universal primer pairs P1/P7 and fU5/rU3 (Crosslin et al. 2006). The testing was performed at USDA-ARS Laboratory in Prosser, WA.

Percentages of plants with ZC symptoms in raw tubers and fried chips were calculated for each treatment and potato variety used in the study. Data were analyzed by using SAS general linear models procedures (SAS Institute 2003). Analysis of variance

(PROC GLM) was performed following transformation of percentage data using  $\arcsin \sqrt{x}$ . The level of significance was set at  $P=0.05$  and the least significant difference (LSD) test was used to separate means.

## RESULTS

Potatoes began to emerge approximately four weeks after planting. By early March, the pressure of naturally occurring potato psyllids was very high on potato plants in uncovered plots (Fig. 3) and some of the plants had already started to exhibit psyllid damage symptoms. Plant symptoms included a rolling upward of the top leaves, developing into a basal cupping of the leaflets, accompanied with yellowish and reddish discoloration; proliferation of axillary buds, shortened internodes, swollen nodes, aerial tubers, leaf scorching, and early plant decline (Fig. 1A-C). By mid-April, these uncovered potato plants were already showing severe ZC symptoms in raw tubers (Fig. 1D-E), even in very young and small tubers. The brief



**Fig. 3.** Number of potato psyllid eggs and nymphs per 60 potato leaves on each sampling date at the USDA-ARS Research Farm in Weslaco, TX.

inspection conducted on the caged plants four weeks after psyllid release showed that psyllid nymphs and adults were present on the potato plants, indicating establishment of the released psyllids in the cages; however, the exact psyllid density in the cages could not be determined. Although the plants in the cages had been exposed to psyllids at an older stage than the uncovered plants (Fig. 2B), psyllid damage symptoms were readily visible on some of the plants at the time of the cage inspection.

At harvest, results showed that potato plants exhibiting ZC symptoms in raw tubers (Fig. 1D-E) averaged 79.2, 37.5, and 48.6% for uncaged plants, caged plants infected with Texas field collected psyllids, and caged plants infected with laboratory-reared psyllids, respectively (Table 1). Plants showing typical ZC symptoms in fried chips (Fig. 1F-G) averaged 87.5, 52.8, and 63.9% for uncaged plants, caged plants with Texas field collected psyllids, and caged plants with laboratory-reared psyllids, respectively (Table 1). No single potato plant in the cages without psyllids (controls) showed ZC

symptoms in raw tubers or fried chips (Table 1). Incidence of ZC in raw tubers was significantly different among treatments ( $F=12.69$ ,  $df=3, 16$ ;  $P=0.0007$ ). Also, there were significant differences in the incidence of ZC in fried chips between treatments ( $F=20.73$ ,  $df=3, 16$ ;  $P<0.0001$ ). Uncaged plants had significantly higher ZC incidence in both raw tubers and fried chips than potato plants in the cages (Table 1). However, no significant differences were observed in ZC incidence in raw tubers and fried chips between caged potato plants exposed to Texas field collected and laboratory-reared psyllids.

Although there was no significant difference in the incidence of ZC in raw tubers between different potato cultivars used in the study ( $F=2.45$ ,  $df=2, 13$ ;  $P=0.0997$ ), there was a significant difference in plants exhibiting ZC symptoms in fried chips among cultivars ( $F=4.05$ ,  $df=2, 13$ ;  $P=0.0253$ ), with both Atlantic and FL1879 showing higher disease incidence than FL1867 (Table 2).

None of the tubers with typical ZC symptoms collected from the selected 46 experimental potato

**Table 1.** Potato plants with zebra chip (ZC) symptoms in raw tubers and fried chips; varieties were pooled.

Treatment	Percentage of plants with ZC symptoms in raw tubers Mean $\pm$ SEM)	Percentage of plants with ZC symptoms in fried chips (Mean $\pm$ SEM)
Uncaged plants	79.2 $\pm$ 10.5c	87.5 $\pm$ 5.4c
Caged plants with Texas field collected psyllids	37.5 $\pm$ 8.8b	52.8 $\pm$ 5.6b
Caged plants with laboratory reared psyllids	48.6 $\pm$ 9.0b	63.9 $\pm$ 8.2b
Caged plants without psyllids	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a

Means followed by the same letter within columns are not significantly different ( $P > 0.05$ ; LSD).

plants tested positive for phytoplasmas by the nested PCR using universal primer pairs P1/P7 and fU5/rU3 (Crosslin et al. 2006).

### DISCUSSION

ZC is an important potato disease that is causing serious losses to the potato industry in the southwestern United States, Texas in particular. This disease has caused millions of dollars in losses to potato producers in the Lower Rio Grande Valley (Goolsby et al. 2007, Munyaneza et al. 2007). A good understanding of this disease is essential to develop effective management strategies to reduce damages caused by this disease in affected areas. This requires 1) a correct identification of the disease vectors, 2) mechanisms by which the disease is transmitted, and 3) the factors influencing disease symptoms expression and severity.

A recent study by Munyaneza et al. (2007) conducted in Washington showed that there was a strong association between ZC and the potato psyllid. However, since environmental conditions in the Washington potato growing area are different from those in Texas and the potato psyllid is uncommon in Washington, there was a need to conduct controlled psyllid exposure studies under ZC naturally occurring conditions in the Lower Rio Grande Valley of Texas to document the role of this insect pest in the

expression of ZC. The potato psyllid is well known to overwinter and reproduce in this region before spreading to the north or northwest with prevailing winds (Wallis 1955). This insect pest was also found to be the most common and abundant in potato fields infected with ZC in this region of Texas (Goolsby et al. 2007). Results of the present study are consistent with findings of the previous study by Munyaneza et al. (2007) and provide further evidence that ZC in the Lower Rio Grande Valley of Texas is strongly associated with the potato psyllid.

The results showed that none of the caged plants without psyllids produced tubers and fried chips with ZC symptoms. In contrast, an average of 87.5, 52.8, and 63.9% of the uncaged plants, caged plants with Texas field collected psyllids, and caged plants exposed to laboratory-reared psyllids, respectively, showed typical ZC symptoms in fried chips. These results strongly suggest that the observed ZC symptoms in tubers and fried chips were due to the damage caused by psyllids. The cause of the higher number of ZC infected potato plants in the uncaged plots compared to the caged plants (Table 1) is not clear but is probably due to a lower psyllid infestation that may have resulted from a poor psyllid establishment in the cages. Another explanation to this higher disease incidence could be attributed to the fact that the uncaged plants were exposed to psyllids much earlier (immediately after plant emergence) and at a

**Table 2.** Potato plants with zebra chip (ZC) symptoms in raw tubers and fried chips; only treatments with psyllids are included here.

Potato cultivar	Percentage of plants with ZC symptoms in raw tubers (Mean $\pm$ SEM)	Percentage of plants with ZC symptoms in fried chips (Mean $\pm$ SEM)
Atlantic	57.8 $\pm$ 3.8a	71.9 $\pm$ 3.6a
FL1879	59.4 $\pm$ 9.3a	75.0 $\pm$ 7.0a
FL1867	39.1 $\pm$ 6.1a	50.0 $\pm$ 5.7b

Means followed by the same letter within columns are not significantly different ( $P > 0.05$ ; LSD).

much younger growth stage than those in the cages (Fig. 2B). Also, FL1867 had a lower disease incidence in fried chips, suggesting that this potato cultivar is probably more tolerant of ZC than the other two varieties tested (Table 2). However, more investigation is needed before this conclusion can be reached. Similarly to Munyaneza et al. (2007), the disease incidence in fried chips was higher than in raw tubers (Tables 1 and 2), supporting the fact that to accurately estimate ZC incidence, it is important to process the potato tubers into fried chips.

Mechanisms by which the potato psyllid induces ZC symptoms in potato are still not known. However, it is suspected that this insect injects toxins or unknown plant pathogens in potato plants when feeding (Munyaneza et al. 2007). During the present study, we could not determine if this insect was causing ZC by transmitting toxins or pathogens. However, results of this study clearly showed that, although ZC symptoms in potato resemble those caused by potato purple top disease, phytoplasmas, which are the causal agents of this disease, were not involved in ZC. During this study, none of the tubers exhibiting typical ZC symptoms tested positive for phytoplasmas by a nested PCR using non-specific primer pairs P1/P7 and fU5/rU3. These results are consistent with conclusions reached by Munyaneza et al. (2007). It is also worth noting that psyllids that had been reared in the laboratory for several generations were able to successfully induce ZC symptoms as well as those naturally occurring in potatoes in the Lower Rio Grande Valley. This suggests that whatever ZC

causal agent(s) transmitted by the potato psyllid is not necessarily obtained from infected host plants directly but rather intrinsic to the insect.

In conclusion, results of the present study showed that ZC in the Lower Rio Grande Valley of Texas was strongly associated with the potato psyllid, supporting the findings of the previous greenhouse and field studies conducted in Washington State by Munyaneza et al. (2007). Information from this study will help potato growers in ZC affected areas minimize damages caused by this disease by developing effective monitoring and management strategies targeted against this insect pest. However, to develop effective management strategies for control, it remains imperative that mechanisms by which this potato psyllid induces ZC symptoms be further investigated.

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