

# Assessments of the Edge Effect in Intensity of Potato Zebra Chip Disease

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## Abstract

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Zebra chip is a newly emerging potato disease which imparts dark colorations on fried chips, rendering them unmarketable. The disease is associated with the phloem-limited proteobacterium '*Candidatus Liberibacter solanacearum*', vectored by the potato psyllid *Bactericera cockerelli*. First reported from Mexico in the mid-1990s, the disease was observed for the first time in Texas in 2000 and is now prevalent in several potato-producing regions of the United States. In this study, we were interested in investigating whether there are edge effects in zebra chip intensity that can be assessed as a "foot print" of the associated insect vector. In 2009, we conducted studies in three fields in the Texas Panhandle in paired plots of 10 by 20 m around the field edges and 100 m infield in which symptomatic plants were counted just before harvest. The number of plot pairs (edge and infield) ranged from 15 to 18 depending on the size of the fields. In a separate study, temporal disease progress was assessed in two fields around the edges of the center-pivot circle in approximately 10-by-450-m areas. In 2010, the

paired plot studies were repeated in 10 potato fields in Texas, Kansas, and Nebraska. Zebra chip intensity data from the paired-plot studies for both years were analyzed using the Wilcoxon's signed-rank test, a nonparametric equivalent of the classical (parametric) paired *t* test. In the 2009 study in all three fields, the edge plots had significantly greater zebra chip intensity than the infield plots ( $P < 0.05$ ). Edge plots in the 2010 study also had greater zebra chip intensity in all fields and the differences were significant in the majority of fields ( $P < 0.05$ ). In the diseases progress study in both fields, weekly zebra chip intensity on the edges reached its maximum after the third week of its first detection, and the disease progress curves were best fitted with the second-degree polynomial (quadratic) for both fields. The 2-year study clearly demonstrated that zebra chip intensity in potato fields was greater on the edges than in the infields. This finding has significant implications for psyllid management because greater emphasis in psyllid control strategy can be directed toward the edges for better results.

Potato zebra chip (ZC) is a newly emerging disease which has caused widespread concerns among growers and potato industries. Initially described in Mexico in 1994 and then in Texas in 2000 (41,42), the disease is now prevalent in several potato-producing regions of the United States (49). Although Koch's postulates have not been fulfilled, because of the fastidious nature of the putative pathogen, there is convincing evidence that the phloem-restricted proteobacterium '*Candidatus Liberibacter solanacearum*' (syn '*Ca. L. psyllaurosus*'), vectored by the potato psyllid *Bactericera cockerelli* (Sulc), is associated with the disease (1,15,23–25,29,42). Affected plants exhibit a variety of foliar and stem symptoms such as proliferation of aerial tubers, shortened internodes, and leaf curling and yellowing followed by scorching (Fig. 1A) (28,29). These symptoms may be confused with those caused by other factors which restrict carbohydrate transport, including psyllid yellows, believed to be caused by the psyllid toxin (7,8,35,43). However, tubers from plants affected with psyllid yellows lack internal symptoms characteristic to ZC (43). Raw tubers from ZC-symptomatic plants display internal necrosis (Fig. 1B) which, when fried, become unacceptably dark (Fig. 1C), rendering the chips unmarketable (28). Affected tubers frequently don't sprout and those which do fail to produce normal plants (18).

Potato ZC can cause considerable losses in marketable yield due to the dark colorations it imparts to fried chips (26). Currently, there are no commercially available resistant cultivars, although cultivars may differ in susceptibility. Therefore, the main management strategy is regular insecticide applications to reduce psyllid numbers. Various combinations and sequences of insecticides are generally used for control of the psyllids and these can vary from grower to grower and region to region. For the Texas Panhandle,

insecticide regimens for control of the insect are similar to that described by Zens et al. (51).

Since ZC was first discovered, Texas growers have experienced repeated rejections of truckloads of potato tubers at chip-processing plants. In addition to losses from ZC, yield losses can occur from psyllid feeding (psyllid yellows), resulting in small-sized, malformed, poor-quality tubers (19,33,36,48). Potato psyllids overwinter in the southwest United States (Texas, New Mexico, and Arizona) bordering Mexico, in what has been termed as "winter breeding areas", on wild *Lycium* spp. (13,39,48), and are believed to migrate northward in the spring. It is speculated that migration begins when temperatures in the breeding areas exceed tolerable levels. Potato crops are planted in succession from south Texas (late fall and winter planting) to the northern states (spring planting) and it appears that the psyllids follow the planting succession in northward movement, arriving in northern regions in late July (27). In the Texas Panhandle, psyllids are generally observed beginning in April, a few weeks after planting begins.

Field edges may be defined as boundaries between cultivated fields and the surrounding, distinct ecological habitats (37), and are frontlines (gateways) through which organisms move back and forth between the two habitats (3,10,11,37,50). Edge effects are influenced by several factors, including preferences of the organisms, quality and structures of the edges, and the surrounding habitats (37). Organisms, including insects, differ in their responses to field edges. For some, population densities can be higher on the edges than in the center but, for others, it can be lower or similar (31,32,37,38).

In 1939, Jensen (20) reported the occurrence of psyllid yellows in border rows in Nebraska. However, the role of field edges in epidemiology of ZC is largely unknown and there is a need for greater understanding of the relationship between field edges and ZC intensity as a consequence of psyllid feeding behaviors. This is important from a management perspective because more emphasis in psyllid management tactics could be directed toward the field edges if edges were found to be greater in ZC intensity than elsewhere in the fields. The primary objectives of this study were to (i) determine whether edges of potato fields have greater ZC intensity

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than the infields and (ii) assess temporal ZC intensity progression on the edges.

## Materials and Methods

**Edge effect studies.** In 2009, ZC intensity on field edges and in the infields were compared in three potato fields in the Texas Panhandle (approximately 50 ha each). Fields 1 and 2 were located in Lamb County (near Olton) and were planted the third week of March. Field 3 was in Hartley County (near Dalhart, about 200 km N of Olton) and was planted on 11 May. The three fields were planted to 'FL 1867'. In each field, 10-by-20-m plots were established on the edges around the center-pivot circle at intervals of approximately 160 m giving, rise to 15 to 18 per field. Plots also were similarly established in the infield 100 m from the edges corresponding to each of the edge plots, resulting in a total of 30 to 36 paired plots per field. Plant spacing for chipping potato generally varies from 230 to 280 cm along the rows and 0.76 m between rows, giving rise to an estimated 922 to 1,126 plants per plot (depending on the spacing). The number of symptomatic plants in each plot was counted within 1 week prior to harvest (116 days after planting [DAP]). Tubers generally become ready for harvest in about 100 DAP. Tubers from symptomatic plants suspected to be caused by factors other than ZC were dug up, sliced, and inspected for the presence of the diagnostic ZC symptom.

The study was repeated in 2010 in 10 fields with slight modifications. Plot sizes and distances from edge to infield plots increased to 20 by 30 m and 200 m, respectively. Estimates of plant density per plot ranged from 2,765 to 3,380. The 2010 data were collected as part of a larger regional impact assessment study which, among others, included field locations in the Lower Rio Grande Valley (LRGV, 'Atlantic'); Pearsall (Atlantic); Olton and Dalhart, TX (FL 1867); Garden City, KS (1867); and Bridgeport, NE (FL 1879). Pairs of plots per field ranged from 7 to 16. Tubers from every symptomatic plant in each plot were dug up, sliced, and visually examined for the presence of diagnostic ZC symptoms (26). If one tuber from a plant was found to be symptomatic, the plant was considered ZC positive. Samples from questionable symptomatic tubers (along with ZC-positive and -negative subsamples), based on visual assessments, were taken into the laboratory and tested for the presence of '*Ca. L. solanacearum*' by polymerase chain reaction (49) for further confirmation.

**Temporal disease progress.** In 2009, temporal ZC progress on field edges was studied in a field in the Dalhart area (planted 11 May) labeled here as field A. New symptomatic plants were identi-

fied weekly and marked by the date of observation in a 10-by-450-m area around the edges of the 50-ha center-pivot circle. Toward the end of the season (when no new symptomatic plants were observed), marked plants were categorized by dates and counted. The study was repeated in a separate field (field B), about 25 km north of field A, planted on 16 May. In this field, potato tubers from marked (symptomatic) plants were dug up and fried to further understand the relationship between visual symptoms and discoloration of fried chips.

**Data analyses.** To determine whether there were differences between the edges and the infields in counts of diseased plants, individual edge and infield plots facing the same direction (directional relationship) from the center points of the fields were paired for *t* test analysis. However, because the differences between the pairs of observations were not uniformly normally distributed across all the different fields, the Wilcoxon's signed-ranks test (a non-parametric equivalent of the classical, parametric, paired *t* test) was used instead (44). The null hypothesis that the difference between the pairs of observations (edge plots versus infield plots) in ZC intensity was zero was tested using the PROC UNIVARIATE in SAS (SAS Institute Inc., Cary, NC). In addition, we conducted correlation analysis for each field to determine the relationship between the ZC intensity in plots on the edges and those in the infield using PROC CORR in SAS. In the correlation analysis, each edge plot was paired with an infield plot perpendicularly (straight), one step ahead (diagonally forward), or one step behind (diagonally backward). Temporal disease progress was assessed by regressing counts of diseased plants against time, and the percentage of symptomatic fried chips was calculated from the total number of plants visually classified as ZC symptomatic.

## Results

**Edge effect.** In the 2009 study, edge plots in all three fields had greater ZC intensity than the infield plots. Field number 2 had the greatest diseases intensity (Table 1). Overall edge plots had two to three times greater ZC intensity than the infield plots and the differences were significant in all three fields ( $P < 0.05$ ). Results of the correlation analysis showed that there was no relationship between the edge plots and infield plots in ZC intensity in any of the three paired directions (straight, one step forward, and one step backward). In the 2010 study, the mean number of ZC-symptomatic plants per plot ranged among fields from 1 to 21.7 on the edges and 0.2 to 5.3 in the infields. As in the 2009 study, all fields had greater ZC intensity on the edges than within the field. The



Fig. 1. Images of A, a zebra chip-symptomatic plant; B, a symptomatic raw tuber showing internal necrosis (left) and a nonsymptomatic tuber (right); and C, fried chips from a symptomatic (left) and a nonsymptomatic tuber (right).

Table 1. Mean number of zebra chip symptomatic plants on the edges and the infields in studies conducted in three fields in 2009

Location	Field	N <sup>a</sup>	Edge	Infield	T <sub>s</sub>	Pr ≥  T <sub>s</sub>
Olton	1	17	4.53	2.53	36	0.0215
	2	15	9.87	3.60	45	0.0083
Dalhart	1	18	3.50	1.56	35	0.0442

<sup>a</sup> Number of pairs of plots in which symptomatic plants were counted.

differences were statistically significant in all fields except field number 2 in Pearsall and number 1 in LRGV (Table 2). Also, as in the 2009 study, there was no correlation in ZC intensity between the edges and the infield plots (*data not shown*).

In one of the fields by Garden City (KS), only 3 edge plots (of 32) had symptomatic plants. In addition, in the field at Bridgeport (NE), 11 of the 20 plants on the edges that were positive for ZC were detected in one plot, and there were only two plots in the infields with ZC (3 plants total). Even though the edge plots in these two fields had greater ZC intensity than the infield plots, we felt that the data were not suitable for the statistical methodology that we used for analysis of data from the other locations. Therefore, data from the two locations were not shown in Table 2.

**Temporal disease progress.** In field A, symptomatic plants were first observed 66 DAP and the number of newly observed plants increased weekly until the third week (80 DAP) and declined thereafter (Fig. 2A). In the second field (field B), symptomatic plants were first observed 75 DAP and, as in the first field, the number of newly observed symptomatic plants was at its maximum in the third week of observation (89 DAP; Fig. 2B). However, the overall count of symptomatic plants was greater in the first field (field A) than in the second (field B), in which there were 128 and 68 symptomatic plants for the first and second field, respectively. In field B, 89% of symptomatic plants had tubers with ZC symptoms which showed dark colorations when fried.

In both fields, progress of cumulative and weekly disease intensity was best described by the second degree (quadratic) polynomial ( $Y = -0.11995X^2 + 22.583X - 937.07$ ,  $R^2 = 0.99$  and  $Y = -0.0758X^2 + 10.985X - 360.67$ ,  $R^2 = 0.95$  for the cumulative and weekly ZC intensity progress, respectively, for the first field;  $Y = -0.0317X^2 + 7.6206X - 386.1$ ,  $R^2 = 0.99$  and  $Y = -0.029X^2 + 5.2303X - 218.85$ ,  $R^2 = 0.87$ , respectively, for the cumulative and weekly ZC intensity progress, respectively, for the second field).

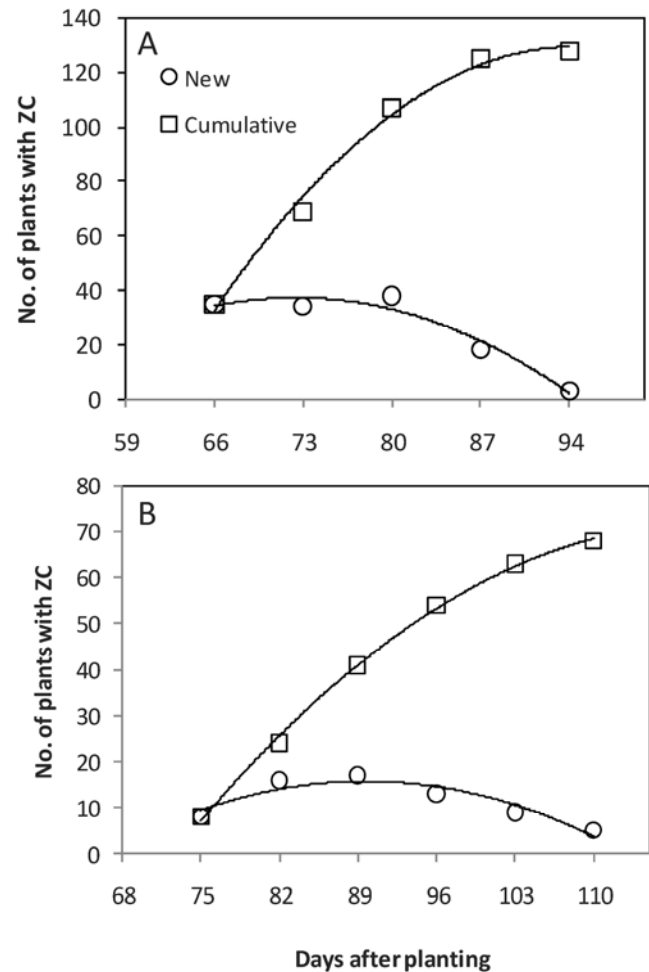
## Discussion

Edge effects exist when individual plant or animal species are denser on the edges than elsewhere in the field or vice versa (37,38). Edge effects have been extensively studied for many species of organisms, including birds, mammals, and insect populations, in both natural and cultivated habitats (37). One of the common themes among the findings in these studies is that organisms respond differentially to habitat edges (38). For example, some insect species respond positively (9,31,34,46) or negatively (9,10,14,31) while others are neutral (9,30,32).

In the current study, ZC intensity resulting from the bacteriferous potato psyllid feeding (26) was significantly greater on the edges than in the infields in a majority of fields studied over the 2-year period. Potato psyllids overwinter in the southwestern United States (13,48) and migrate northward in the spring but it is not clear how they end up causing greater damage on the edges than in the infields. Psyllids may be attracted to differences in adjacent habitat qualities contrasted at the edges due to (among other things) differences in height, thickness, or color, and land on the first visible host after long flights (6). Alternatively, they may rain down on a potato field at random and disperse to the edges in search of isolated plants (low density) or suitable microclimate

(4,5,21). Another possible scenario may be that they survive locally on wild solanaceous hosts (22) and move out into the spatially separated food source nearest to their survival habitats, which happen to be potato field edges (37). This may be especially true in LRGV and the Texas Panhandle, where some of the wild psyllid hosts are widely distributed, and some of them test positive for the pathogen (13,16,49). Furthermore, this last scenario may explain why two of the fields in the LRGV had substantially greater ZC intensity on the edges (Table 2) than in the infields. The fields (especially field number 2) were in close proximity to large areas of brush where some of the *Lycium* spp. (psyllid hosts) were reported to be prevalent (13), which suggests that the psyllids might have moved into the field in abundance from close proximity.

The lack of correlation between ZC intensity in the edge plots and infield plots suggests that areas of the field edges with high or



**Fig. 2.** Temporal progression of zebra chip intensity (new symptomatic plants and cumulative) on the edges of two potato fields planted on **A**, 11 May and **B**, 16 May 2009 in the Dalhart (TX) area.

**Table 2.** Mean number of zebra chip symptomatic plants on the edges and the infields in studies conducted in multiple locations in 2010

Location	Field	N <sup>a</sup>	Edge	Infield	T <sub>s</sub>	Pr ≥  T <sub>s</sub>
Dalhart	1	15	1.00	0.20	18.0	0.0078
Garden City	1	16	2.00	0.50	28.0	0.0239
Olton	1	16	7.44	2.94	47.5	0.0045
Pearsall	1	15	4.20	1.80	27.0	0.0137
	2	12	4.08	3.58	3.50	0.7539
LRGV <sup>b</sup>	1	16	2.25	1.06	12.5	0.2227
	2	7	21.71	4.00	13.0	0.0313
	3	10	18.10	5.30	25.5	0.0059

<sup>a</sup> Number of pairs of plots in which symptomatic plants were counted.

<sup>b</sup> Lower Rio Grande Valley.

low disease intensity wouldn't necessarily have correspondingly similar ZC intensity levels in the infields. This shows that psyllids do not move in the field in a readily predictable manner. Disease intensity on the edges was not uniform all around but was observed in separated random clusters. For this reason, use of transects across the field from several directions failed to show consistent disease gradients from the field edges to the center field (17). In some of the fields (especially in the Texas Panhandle), edges facing south to southwest had greater ZC intensities than edges elsewhere, although large clusters were occasionally observed on other sides of the fields, including the northern edges.

In the disease progression study, there was a delay in first appearance of ZC-symptomatic plants in the field that was planted later (75 DAP) compared with the first planting (66 DAP). This may have led to the overall reduction in counts of diseased plants in the later planting compared with the first planting, which raises an interesting issue of whether delayed planting in the Dalhart area may reduce the disease intensity. However, because the two fields were at different locations, the role of local environmental factors on the outcomes may not be ignored. Further study is required on the effect of planting dates and environmental factors on ZC intensity.

The finding that potato field edges have greater ZC intensity than the infields is important to the growers because control tactics may be implemented in such a way that the edges would receive more attention than the infields. In addition, if psyllids first land on the field edges, establish, and then move into the rest of the field (which we did not cover in this study), early management action on the edges would substantially reduce the risk of severe losses to ZC. The width of the management zone may depend on the depth of the edge effect. In this study, ZC intensity was assessed 10 and 20 m into the fields from the edges in 2009 and 2010, respectively. Although we don't expect edge effect to extend much further than beyond 20 m from the edges (*unpublished observation*), further studies are required before specific recommendations are made because the width of the edge effect may be different for different locations and fields depending on the psyllid or ZC pressure.

In some insect-transmitted diseases, trap crops planted around the edges have been successful in reducing damage by the pathogens. This is especially true for insect vectors carrying nonpersistently transmitted viruses (12,19,47). The putative pathogen in ZC disease is presumed to be a secondary endosymbiont in the psyllid (45), which may suggest the existence of some permanent relationship with the vector. However, the bacterial titer level has been shown to be highly variable and can be affected by the vector environment and nutrition (40) and, in some cases, even to the degree of falling below detection level in the vector (2). Thus, the use of trap crops around the edges as a frontline contrast to lure the vector is a strategy that may need consideration for potato psyllid management in addition to pesticide applications.

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