Impact of Potato Psyllid Density and Timing of Infestation on Zebra Chip Disease Expression in Potato Plants

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Abstract


The impact of vector density and timing of infestation on potato were investigated. Healthy potato plants at different growth stages (4, 5, and 7 weeks after germination) were exposed separately to four different B. cockerelli densities (0, 5, 20, and 40 psyllids per cage) in field cages and Zebra chip (ZC) symptoms, leaf photosynthetic rates, tuber yield, and total nonstructural carbohydrate accumulation in leaves and tubers of healthy and B. cockerelli-infested plants were monitored. Potato psyllid nymph and egg populations reached a seasonal peak at 6 weeks after the exposure to insect. Younger plants at 4-week growth stage after germination were more susceptible to B. cockerelli infestation and ZC expression than older plants. As few as five B. cockerelli adults were enough to transmit the ZC pathogen and cause ZC expression both in foliage and tuber. At the density of 20 psyllids per cage, more than 50% of plants showed ZC symptoms in tubers. Furthermore, B. cockerelli infestation reduced leaf photosynthesis rates (Pn), resulting in less starch and more reducing sugars in tubers, and hence reduced tuber weight and yield, especially when psyllid infestation occurred at the early growth stages. The results indicate that early B. cockerelli infestation of younger plants was associated with more severe ZC expression in both foliage and tubers, leading to earlier dead plants. The data suggest that strategies for controlling B. cockerelli during early potato crop development could thus lessen the severity of ZC development.

Keywords: Bactericerca cockerelli; carbohydrates; reducing sugars; glucose; fructose; sucrose; starch

Zebra chip (ZC) disease is a relatively new and economically important disease which has been observed in processing as well as fresh market potato (Solanum tuberosum L.) varieties in the United States, Mexico, Central America, and New Zealand (Secor & Rivera-Varas 2004; Munyaneza et al. 2007a; Rubio-Covarrubias et al. 2011). And this disease has been documented on carrot in North Europe and the Mediterranean Region (Munyaneza 2010, 2012; Munyaneza et al. 2011; Alfarón-Fernández et al. 2012), and on celery crops in Spain (EPPO 2014). ZC is characterised by symptoms that develop in potato tubers, which consist of collapsed stolons, browning of vascular tissue, concomitant with necrotic flecking of internal tissues and streaking of the medullary ray tissues, all of which can affect the entire tuber. Upon frying, these symptoms become more pronounced and chips or fries processed from infected tubers show very dark blotches, stripes, or streaks, rendering them commercially unacceptable (Goolsby 2012). Supported by the National Nature Science Fund of China, Grant No. 31101491, and by the Texas Department of Agriculture through Texas AgriLife Research, Texas A&M University System, College Station, Texas, USA.

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et al. 2007; Miles et al. 2010; Munyaneza 2012). Symptoms on ZC infected plants include stunting, chlorosis, swollen internodes, and pinkish terminal leaves, which closely resemble those caused by psyllid yellow disease or purple top (Munyaneza et al. 2006, 2007a, b; Gao et al. 2009). It has been estimated that failure to control this disease could cause millions of dollars in losses to both potato producers and processors in affected areas, often causing the abandonment of an entire field (Secor & Rivera-Varas 2004; Gudmestad & Secor 2007; Munyaneza et al. 2007a, b; Liefting et al. 2008; Munyaneza 2010; Rubio-Covarrubias et al. 2011).

The putative causal agent of ZC was unknown until 2008, although ZC was first documented in potato fields in Mexico as early as 1994 (Secor & Rivera-Varas 2004; Gudmestad & Secor 2007; Hansen et al. 2008). More recent research has implicated a new bacterium, Candidatus Liberibacter solanacearum (referred to as “Lso” hereafter), as a pathogen causing ZC disease (Hansen et al. 2008; Liefting et al. 2008; Butler & Trumble 2012). Furthermore, the potato psyllid [Bactericera cockerelli (Sulc) (Hemiptera: Psyllidae)] is now known to be a major vector of the ZC disease causal agent (Munyaneza et al. 2007b; Gao et al. 2009; Sector et al. 2009; Butler & Trumble 2012). The potato psyllid is a well-known economically important pest of potatoes, tomatoes, and other solanaceous crops in the western United States, southern Canada, Mexico, Central America, and New Zealand (Munyaneza et al. 2007a). B. cockerelli has an extensive range of acceptable hosts, including species in 20 plant families, but solanaceous species (tomatoes, potatoes, nightshade) are preferred (Wallis 1955). Crosslin et al. (2010) reported a spread of ZC across geographic regions by dispersing B. cockerelli which helped maintain the bacterium in these regions during the insect’s overwintering period. Munyaneza et al. (2011) reported yield losses due to B. cockerelli and ZC damage ranging from 43% to 93% in several commercial potato varieties under controlled field cage conditions in Texas and Washington State. Effective monitoring and control of B. cockerelli are essential strategies in order to better manage ZC in potatoes.

Gao et al. (2009) reported that the timing of B. cockerelli infestation and ZC development was important because of its potential influence on the size of actively photosynthesising leaves at different growth stages. Also important with regards to ZC vector management is the question of how many B. cockerelli can cause ZC symptoms and expression in potato leaves and tubers. These questions have implications for B. cockerelli management strategies aimed at controlling the incidence and severity of ZC disease. Hence, a better understanding of the timing of infestation, vector density, and population dynamics of B. cockerelli is important to reduce incidence of ZC and yield losses in potatoes.

The objectives of this study were: (1) to determine which growth stage of potato plants is more susceptible to B. cockerelli infestation and ZC expression; (2) to determine if there is a threshold of vector density to cause ZC expression in potato leaves and tubers; and (3) to characterise the effects of B. cockerelli infestation on leaf carbon assimilation (photosynthesis) and carbohydrate (starch, sucrose, and reducing sugars) dynamics in leaves and tubers, as well as tuber yield and quality after B. cockerelli infestation.

**MATERIAL AND METHODS**

**B. cockerelli.** Adults of B. cockerelli used in all experiments were collected from potato plants maintained in large screened Plexiglas® cages (70 × 60 × 60 cm) located in an air-conditioned insectary laboratory (25 ± 2°C, 50–70% relative humidity, 14:10 h light/darkness) at Texas AgriLife Research & Extension Center at Weslaco, Texas (26°09′26.36″N, 97°57′48.56″W; 24 m a.s.l.). These B. cockerelli specimens were tested to be infected with Liberibacter.

**Field conditions.** Field cage experiments were conducted at the Texas AgriLife Research & Extension Center at Weslaco, using a commercial processing potato variety Atlantic. Two potato tubers were individually seeded in each cage (1.0 × 1.5 × 1.5 m) covered with organdy screen (80 mesh) on January 22, 2009, and were watered thereafter as needed. During the course of the experiments, no pesticides were used.

**Experimental design.** All potato plants were planted at the same time and psyllids were added to the plants at different growth stages. Three plant growth stages were investigated, namely the vegetative stage, tuber initiation, and the tuber bulking stages, corresponding with B. cockerelli introduction to plants at 4, 5, and 7 weeks after seed germination. Hence there were three B. cockerelli introduction treatment times. In each treatment, four psyllids densities were used: 0, 5, 20, and 40 adult B. cockerelli per cage. Each treatment was replicated five times. A total of 60 exclusive cages (two plants per cage) were used in this experiment.
Field observation and measurement. During the growing season, the numbers and stages of *B. cockerelli* on the upper, middle, and lower leaf of each potato plant were recorded about every two weeks until all plants died. Meanwhile, ZC symptoms, leaf photosynthesis rates ($P_n$), and total non-structural carbohydrate (TNC) – glucose, sucrose, and starch accumulation in leaves of healthy and *B. cockerelli*-infested plants were monitored and recorded using the methods previously described by Gao et al. (2009). The four observation times are March 5, March 25, April 14, and May 5. Plants were harvested on June 19, 2009, the tubers weighed and visually inspected for ZC symptom by making a cross-section cutting near the stem end. The tubers were then sliced into chips and fried to check for chip discoloration or ZC symptom as described in Gao et al. (2009). Freshly harvested tuber samples were also retained for total non-structural carbohydrate analysis using methods described in Gao et al. (2009).

Statistical analysis. Data were analysed using the General Linear Model (GLM) procedures of SAS 9.2 (SAS Institute Inc., 2013). Glucose, sucrose, and starch (TNC) concentrations in leaves and tubers, leaf photosynthetic rate ($P_n$), and tuber yield were analysed using the analysis of variance (Two-way ANOVA), with potato plant age and density of adult *B. cockerelli* as sources of variability, respectively. Treatment means were compared using Student-Newman-Keuls multiple-range (SNK) test at $P < 0.05$.

RESULTS

ZC symptom expression. Control potato plants not exposed to *B. cockerelli* did not show any ZC symptoms in both foliage and harvested tubers; whereas the potato plants on which adult *B. cockerelli* were infested at different ages with different adult *B. cockerelli* densities exhibited different responses to infestation and expression of ZC disease (Table 1).

<table>
<thead>
<tr>
<th>Density</th>
<th>4 weeks</th>
<th>5 weeks</th>
<th>7 weeks</th>
<th>4 weeks</th>
<th>5 weeks</th>
<th>7 weeks</th>
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<tbody>
<tr>
<td>0</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.13 ± 2.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.39 ± 3.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.00 ± 5.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.58 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.00 ± 2.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>36.36 ± 5.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.18 ± 3.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.28 ± 2.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.67 ± 6.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.85 ± 5.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.00 ± 0.94&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>40</td>
<td>54.54 ± 8.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.36 ± 5.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.00 ± 3.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.85 ± 8.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.67 ± 5.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.18 ± 2.17&lt;sup&gt;a&lt;/sup&gt;</td>
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Different letters in the same column indicate significant differences among treatments at $P < 0.05$ level by SNK test.

ZC disease incidence was relatively high in potato plants exposed to *B. cockerelli* during the first 4 weeks after plant emergence and infection declined thereafter. Psyllid exposure at 4 weeks, even at low densities, still resulted in ZC incidence in nearly 50% of tubers. At higher psyllid densities (> 20 per cage), ZC incidence was nearly 90%. At 5 weeks, ZC incidence represented about a 50% damage in tubers, especially when psyllid density was 20 per cage. At 7 weeks, ZC incidence declined, but the potato plants could still be infected even at adult *B. cockerelli* density of 5 per cage.

Population dynamics. The trend of *B. cockerelli* nymph population was at the peak on April 14 during the plant growth development stage. But the amount of nymphs in the 4-week treatment was much higher than in the other two treatments. *B. cockerelli* egg population dynamics had a similar trend as the nymph population dynamics (Figure 1).

Leaf physiology and biochemistry responses

Photosynthesis. Leaf photosynthetic rates ($P_n$) generally declined with plant age. On April 14, leaf photosynthetic rates of plants without *B. cockerelli* were significantly higher than of those exposed to *B. cockerelli* at the 4- and 5-week growth stages. No significant differences in leaf $P_n$ were found among plants exposed to *B. cockerelli* at the 7-week growth stage, indicating that early infection could significantly reduce the $P_n$ ($P < 0.05$; Figure 2).

Leaf TNC concentrations. Leaf sucrose content did not change significantly under adult *B. cockerelli* infestation at the 4-, 5- or 7-week growth stages. Psyllid density had no significant effects on leaf sucrose contents except for the 5-week infestation stage where leaf sucrose of plants infested with adult *B. cockerelli* was significantly higher than that of plants without *B. cockerelli* infestation ($P < 0.01$; Figure 2).

Leaf starch concentrations were significantly increased after *B. cockerelli* infestation in all treatments.
compared to the plants without *B. cockerelli*. Additionally, leaf starch was significantly influenced by the timing of *B. cockerelli* infestation, with leaves of plants infested at the 4-week growth stage generally having higher starch accumulation in leaves than those from the latter infestation stages (Figure 2).

**Tuber yield and biochemistry**

*TNC concentrations.* Tuber glucose, fructose, and sucrose contents were significantly higher in plants exposed to *B. cockerelli* at the 4-week growth stage than those at the 5- and 7-week stage when *B. cockerelli* density was up to 20 (*P* < 0.05; Figure 3). Within each exposure time, psyllid density was positively associated with higher tuber glucose and sucrose contents. On the contrary, tuber starch contents declined with increasing psyllid density. Within each psyllid density group, tuber starch contents at the 4-week infestation time were significantly lower than that at the 5- and 7-week stages. Within each infestation stage, there were no significant differences in starch content among *B. cockerelli* densities except the 4-week infestation time, where significantly lower starch contents were found in plants infested with even 5 adult *B. cockerelli* if compared to non-infested plants (*P* < 0.05; Figure 3).

**Tuber yield and weight.** Tuber yields from plants exposed to 20 or 40 adult *B. cockerelli* were significantly lower at the 4-week growth stage than at the 5- and 7-week stage (*P* < 0.05), indicating that the 4-week growth stage was the crucial stage for impacts on potato yield. Within the 4-week stage, yields were reduced significantly when density was up to 20 (*P* < 0.05; Figure 4).

**DISCUSSION**

The current data clearly demonstrated that early *B. cockerelli* infestation of younger plants was associated with more severe ZC expression in both foliage and tubers. In Texas, potatoes are generally planted in January, and *B. cockerelli* encounter all ages of potato. Munyaneza (2012) reported that all plant growth stages of potato were susceptible to ZC infection. Buchman *et al.* (2012) released twenty *B. cockerelli* at the potato tuber initiation
stage for one week, and reported that it takes about three weeks after Lso inoculation for ZC symptoms to develop in potato plants and tubers, and plants exposed to Lso-infected *B. cockerelli* less than three weeks before harvest usually produce tubers without ZC symptoms. Levy *et al.* (2011) found that Lso was detectable in tomato and potato plants 2–3 weeks after plant exposure to Lso-infected *B. cockerelli*, most frequently in the upper- and middle-tier leaves. The current study not only indicates that younger plants at the 4-week growth stage after germination were more susceptible to *B. cockerelli* infestation and ZC infection, but also that it only took one week after *B. cockerelli* infestation for ZC symptoms to develop in potato plants and tubers. This probably was due to that the Lso bacterium was transmitted in *B. cockerelli* at a relatively high rate (Hansen *et al.* 2008), and Lso was transmitted to potato leading to ZC development rapidly by *B. cockerelli* activities (Sengoda *et al.* 2010; Buchman *et al.* 2012).

The current data also demonstrate that at 5 adult *B. cockerelli* per cage, ZC symptoms were observed in both leaves and tubers, and especially at high densities (> 20), more than 50% of plants showed ZC symptoms in tubers, compared to uninfested plants which remained disease-free. These observations are consistent with previous reports of Munyaneza *et al.* (2007a) who reported that *B. cockerelli* was a major vector of the pathogen causing ZC disease. Buchman *et al.* (2011) also showed that exposure of a healthy potato plant to 20 Lso-infected adult *B. cockerelli* for a period resulted in ZC symptom development. Furthermore, a single Lso-infected adult *B. cockerelli* was as damaging as 25 *B. cockerelli* adults per plant. However, the present results indicated that the severity of ZC disease expression varied depending on the density of *B. cockerelli* and the plant growth stage at which potato plants were exposed to *B. cockerelli* infestation. At an earlier age, plants exposed to more *B. cockerelli* developed the most severe ZC disease expression in both foliage and harvested tubers, while plants expressed ZC symptoms at low *B. cockerelli* density as much as at high *B. cockerelli* density at much advanced growth stages. The mecha-
Figure 3. Glucose, fructose, sucrose, and starch contents (mean ± SD) of potato tubers from caged potato plants that were exposed to potato psyllids at different growing stages (infested 4, 5, and 7 weeks after germination) with different densities in the field.

Different lowercase letters within an infestation density indicate significant differences between treatments (SNK test: df = 2, 6; P < 0.05), different uppercase letters indicate significant differences between treatments within the same infestation times by potato psyllid (SNK test: df = 3, 8; P < 0.05).

Mechanisms for this response are unclear but it is probable that the physiologically mature shoot structures of older plants were less palatable to *B. cockerelli* for feeding and hence ZC pathogen transmission (Gao et al. 2009). Temporal monitoring of *B. cockerelli* could help predict whether populations of *B. cockerelli* present in a given area pose a significant risk of producing ZC, and therefore should be targeted vigorously for control. Previous studies reported that *B. cockerelli* populations were the highest at field edges initially, but if not controlled could eventually spread throughout the crop (Butler & Trumble 2012; Henne et al. 2012). In this study, *B. cockerelli* nymph and egg populations reached the seasonal peak at 6 weeks after being exposed to plants, indicating that *B. cockerelli* population increased rapidly, and this makes it possible for plants to get more severe ZC expression, leading to earlier plants death. Leaf bronzing, upward cupping, and chlorosis were also observed in the current study; these reactions to *B. cockerelli* exposure could directly reduce photosynthesis and carbohydrate flow in potato plants, leading to ZC symptoms in the tubers. These results were also in agreement with previous findings that insect injury to stem tissue causes reduced phloem translocation of photoassimilates (Nielsen et al. 1999), and that carbohydrate levels (including starch) increase on the leaves of injured plants (Pirone et al. 2005). Gao et al. (2009) and Buchman et al. (2011) showed that ZC infection resulted in overall high glucose and sucrose levels in tubers compared with uninfected tubers. A similar result was observed in the present study regarding the production of high levels of reducing sugars and less starch in tubers from infested plants than in healthy plants, which was consistent with ZC disease expression or dark stripes in infected tubers. This study also demonstrated that *B. cockerelli* infestation can reduce tuber quality and yield, especially during early infestation. Gao et al. (2009) observed that plants exposed to *B. cockerelli* had fewer tubers with reduced tuber size. Berry et al. (2011) reported that exposure of growing potato plants to Lso-free *B. cockerelli* at different densities and for different periods of time under controlled field conditions did not have a major influence on potato yield. Munyanza et al. (2011) reported yield losses due to *B. cockerelli* and ZC damage ranging from 43% to 93% in several commercial potato varieties under controlled field cage conditions in Texas and Washington. From 2009 to 2011, estimates of yield loss due to ZC and *B. cockerelli* in the southwestern and central United States ranged from 0.5% to 75% (Guenthner et al. 2011). These observations are
consistent with the present results that the tuber yield losses were from 7.89% to 66.7% when compared to healthy tuber. This could be due, in part, to reduced photoassimilate production and transport to developing tubers following infestation and a concomitant reduction in functional leaf area. This process is also associated with a reduction in starch accumulation in tubers, resulting in lower dry matter. In addition, during mid to late tuber bulking stage, early plant death subsequent to B. cockerelli injury could disrupt the conversion of sugars to starch (Gao et al. 2009).

In summary, the present study demonstrated that psyllid infestation during the early growth stages of potato plants increases the vulnerability of such plants to ZC disease, and that implementing early control of B. cockerelli may help reduce the incidence of this potato disease. In addition, low psyllid infestation densities were associated with reduced ZC expression in both foliage and tubers. B. cockerelli nymph and egg populations reached a seasonal peak at 6 weeks after being exposed to plants. Furthermore, B. cockerelli infestation leads to a reduction in Pn resulting in less starch and sugar in tubers and hence lowering tuber quality and yield. These observations indicate that to achieve an effective reduction in tuber yield and quality, strategies for controlling B. cockerelli must be implemented early in the growing season or upon detection of the insect in fields. In addition to insect control, other complementary management practices such as planting dates and cultivar selection could help reduce the severity of ZC disease on potato tubers.

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