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Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with "Zebra Chip," a New Potato Disease in Southwestern United States and Mexico

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ABSTRACT A new defect of potato, *Solanum tuberosum* L., "zebra chip," so named for the characteristic symptoms that develop in fried chips from infected potato tubers, has recently been documented in several southwestern states of the United States, in Mexico, and in Central America. This defect is causing millions of dollars in losses to both potato producers and processors. Zebra chip plant symptoms resemble those caused by potato purple top and psyllid yellows diseases. Experiments were conducted to elucidate the association between the psyllid *Bactericera cockerelli* (Sulc) (Homoptera: Psyllidae) and zebra chip by exposing clean potato plants to this insect under greenhouse and field conditions. Potato plants and tubers exhibiting zebra chip symptoms were tested for phytoplasmas by polymerase chain reaction. Potato psyllids collected from infected potato fields also were tested. Results indicated that there was an association between the potato psyllid and zebra chip. Plants exposed to psyllids in the greenhouse and field developed zebra chip. In the greenhouse, 25.8 and 59.2% of tubers exhibited zebra chip symptoms in the raw tubers and fried chips, respectively. In the field, 15 and 57% of tubers showed symptoms in raw tubers and chips, respectively. No zebra chip was observed in tubers from plants that had not been exposed to psyllids, either in the greenhouse or field. No phytoplasmas were detected from potato plants or tubers with zebra chip symptoms, suggesting that these pathogens are not involved in zebra chip. Of the 47 samples of potato psyllids tested, only two tested positive for the Columbia Basin potato purple top phytoplasma.

KEY WORDS *Bactericera cockerelli*, zebra chip, potato, phytoplasma

A new defect of potato, *Solanum tuberosum* L., named "zebra chip" (ZC), has recently been documented to occur in commercial potato production fields throughout southwestern United States, Mexico, and Guatemala (Secor and Rivera-Varas 2004). 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; this necrosis becomes more prominent when chips from infected tubers are fried (Fig. 1). The disease was first documented in potato fields around Saltillo, Mexico, in 1994, and it was first identified in the United States 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 other states, including Nebraska, Colorado, Kansas, New Mexico, Arizona, Nevada, and California (Gerhard Bester, personal communication). This potato defect was sporadically important economically until the 2004, 2005, and 2006 growing seasons when it caused millions of dollars in losses to both potato producers and processors in numerous locations in the United States and Mexico, often causing the abandonment of entire potato fields (Flores et al. 2004, Secor and Rivera-Varas 2004, Hernández-García et al. 2006, Salas-Marina et al. 2006).

ZC continues to be economically important in the fresh and processing potato producing areas of Mexico, particularly in the states of Coahuila and Nuevo Leon in northeastern Mexico, where it is called "papa manchada" (stained potato). Since 2003, ZC incidence in this region has been as high as 100% in some fields (Flores et al. 2004, Secor and Rivera-Varas 2004, Hernández-García et al. 2006, Salas-Marina et al. 2006); this area borders the winter potato production area in Texas where ZC was first found in the United States. The disease is also serious in potato production areas of Guatemala where it is named "papa rayada" (striped potato), and it causes a serious problem of market potatoes, subsistence gardens, and processed potatoes (Secor and Rivera-Varas 2004). A complete survey to determine the geographical extent of the disease has not been conducted.

ZC-infected potato plants exhibit a range of plant symptoms that resemble those of the potato purple top wilt syndrome caused by the Columbia Basin potato purple top phytoplasma and transmitted by the beet

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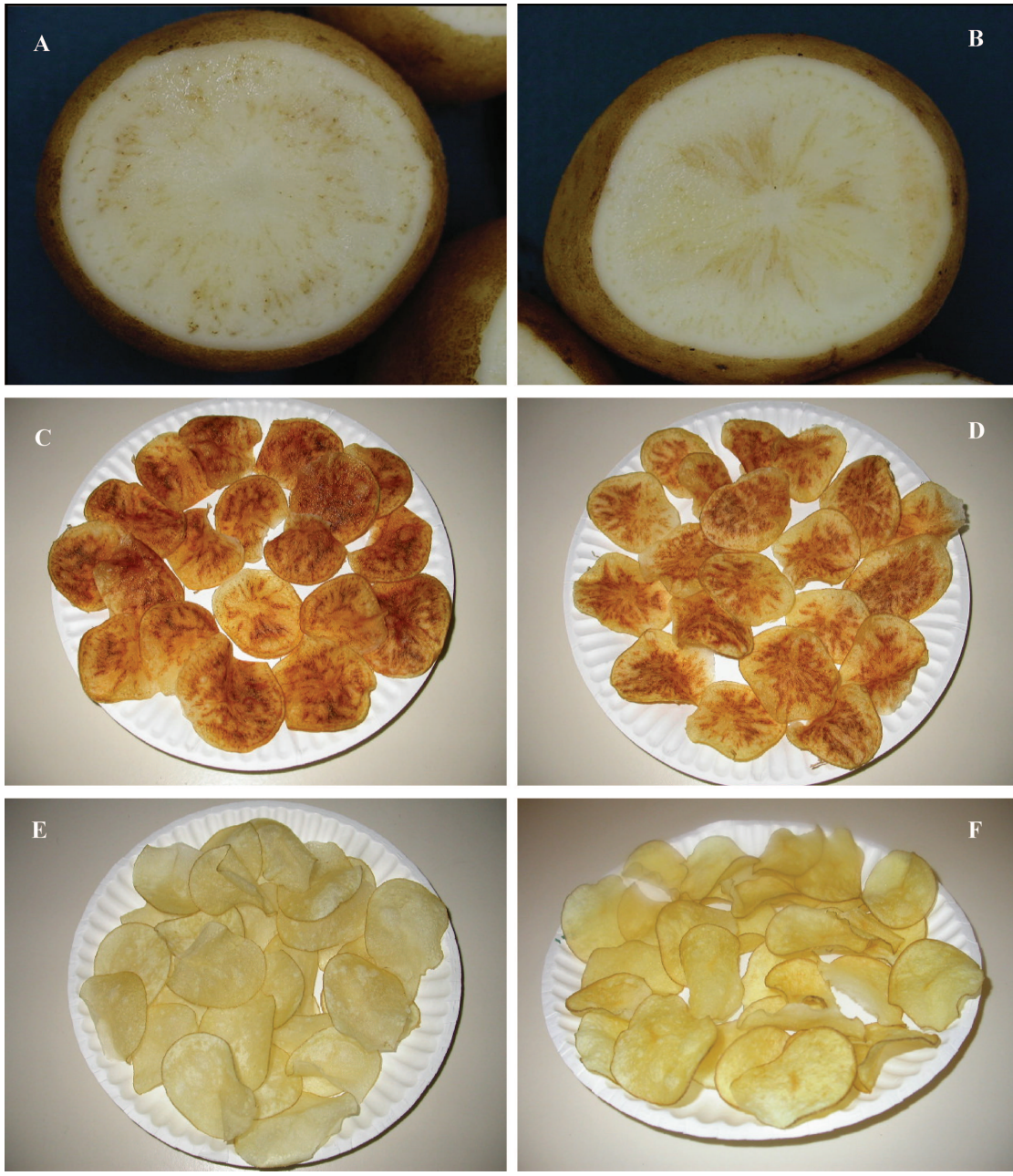

Fig. 1. Potato tubers with ZC symptoms showing necrotic flecking (A) and streaking of medullary ray tissue (B), potato chips processed from ZC-infected tubers (C and D), and chips from ZC-free potato tubers (E and F).

leafhopper, *Circulifer tenellus* (Baker), recently found in Washington and Oregon (Lee et al. 2004, Crosslin et al. 2005, Munyaneza 2005, Munyaneza and Upton 2005, Munyaneza et al. 2006). The symptoms also resemble those caused by psyllid feeding on potatoes (Richards and Blood 1933, Wallis 1955, Cranshaw 1994). Plant symptoms include stunting, chlorosis, swollen internodes of the upper growth, proliferation of axillary buds and aerial tubers, browning of the vascular system in belowground portions of stems, leaf scorching, and early plant decline.

ZC tuber symptoms 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 (Fig. 1). These necrotic symptoms affect the entire tuber from the stem end to the bud end. Chips made from tubers of infected potato

plants have a severe dark brown streaking defect; hence, the name zebra chip (Fig. 1). 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 infected plants. Furthermore, tubers infected with ZC generally do not sprout, or if they do, produce hair sprouts or weak plants.

To date, the exact causal agent(s) and vector(s) of this disease are unknown. However, a preliminary survey of insects associated with the potato crop in affected areas of the southwestern United States indicated that the psyllid *Bactericera* (=*Paratrioza*) *cockerelli* (Sulc) (Homoptera: Psyllidae), was the most common and abundant insect in all the ZC-infected potato fields (J.E.M., unpublished data). Moreover, similar observations have been made in Mexico and this insect is strongly suspected to play a major role in this potato disease outbreak in this country (Hernández-García et al. 2006, Salas-Marina et al. 2006; J.E.M., unpublished data). Furthermore, recent observations indicated that high incidences of ZC in potato tubers were particularly reported in patches that were heavily infested with potato psyllids in Dalhart, TX (J.E.M., unpublished data).

It is well documented that feeding by the potato psyllid (nymphs in particular) causes injury to potatoes referred to as "psyllid yellows disease." This disease results in a dramatic loss of potato yield that consists of the production of a large number of tubers of small size, unmarketable, and of poor quality (Eyer and Crawford 1933, Richards and Blood 1933, Eyer 1937, Wallis 1955, Arslan et al. 1985, Cranshaw 1994). Losses or abandonment of entire potato fields due to sporadic *B. cockerelli* outbreaks have previously been reported in some central and western states of the United States, including Nebraska, Colorado, Wyoming, Montana, New Mexico, Utah, Nevada, Arizona, and Texas (Eyer and Crawford 1933, Richards and Blood 1933, Wallis 1955, Arslan et al. 1985, Cranshaw 1994). Similarly to ZC, early plant symptoms caused by psyllids to potato include erectness and mild chlorosis, basal upward cupping, and a progression of purple or yellow coloration of new leaves. As the disease intensifies, other symptoms may include an upward rolling of leaves on all parts of the plant; shortened and thickened terminal internodes that result in a rosette growth pattern; enlarged nodes; the formation of aerial tubers or axillary branches; pronounced chlorosis; and a cessation of new growth (Richards and Blood 1933, List and Daniels 1934, Carter 1939, Wallis 1955). Additional abnormalities caused to potato by psyllid feeding include phloem necrosis that occurs in stems, stolons, roots, and lateral rootlets, with this necrosis being most severe in stems and stolons (Eyer and Miller 1938). Necrosis of the underground stem and collapsed stolons is also characteristic of ZC severely infected potato plants. Various tuber symptoms have been associated with psyllid yellows, including reduction in tuber size and increased tuber set mentioned above. Other tuber symptoms include flabbiness, rough periderm, and disrupted dormancy that results in tubers sprouting in the ground before harvest or early in the storage. When psyllid-infected tubers are planted, a poor stand of weak stems or multiple hair sprouts are obtained, if any (Eyer and Crawford 1933; Snyder et al. 1946a,b; Wallis 1955; Arslan et al. 1985). Most of these tuber symptoms described above are similar to those caused by ZC.

Although the potato yield loss due to potato psyllid has extensively been documented, little is known on the effects of the potato psyllid damage on the potato tuber internal defects or processing quality. Sanford and Grimble (1944), Snyder et al. (1946a), and Sanford (1952) reported an internal necrosis of the potato tuber caused by the potato psyllid. Snyder et al. (1946a) described this tuber necrosis as consisting of discontinuous dark flecks that were distributed in cross section from the main vascular ring to near the center of the tuber and extended throughout the length of the tuber; these flecks were somewhat more prominent at the stem end. These described internal tuber symptoms strongly resemble those observed in the raw tubers from plants severely infected by ZC; however, these authors did not process the tubers to check for chip fry discoloration. It is possible that, if these tubers had been processed into chips, symptoms consistent with ZC would have been observed.

The main objective of this study was to elucidate the association between the potato psyllid and ZC potato disease by exposing clean potato plants to psyllids in controlled experiments under greenhouse and field conditions to determine whether ZC symptoms in fried chips were due to this insect. In addition, plants and tubers exhibiting typical ZC symptoms were tested for phytoplasmas to determine whether observed ZC plant symptoms were associated with potato purple top disease causal agent. Furthermore, the field association of potato psyllid and phytoplasmas was investigated.

Materials and Methods

Greenhouse Study. The experiments were conducted at the USDA–ARS Laboratory, Wapato, WA. Potato mini-tubers (from tissue culture) of four chipping varieties (Atlantic, FL 1879, FL 1867, and FL 1833) used in the current study were obtained from CSS Farms, Colorado City, CO (FL varieties are Frito Lay Inc. proprietary potato varieties that are processed into chips). They were planted in 14-liter pots (Classic Nursery Supplies, McMinnville, OR) in the greenhouse maintained at 29 ± 2.6°C, 50 ± 3.5% RH, and a photoperiod of 16:8 (L:D) h. The growth media consisted of a mixture of 86% sand, 13.4% peat moss, 0.5% Apex time release fertilizer (J. R. Simplot Co., Lathrop, CA), and 0.1% Micromax micronutrients (Scotts Co., Marysville, OH). The growth media pH was adjusted to 6.8 through the addition of dolomite lime to optimize tuber germination and plant growth.

A potato psyllid colony was established in the laboratory at the USDA–ARS facility at Wapato by using psyllids originally collected from a potato field se-

verely affected by ZC in Dalhart, TX, late fall in 2005. The insects were reared on a mixture of potato plants and eggplant, *Solanum melongena* L., in a controlled environmental room for several generations; the room was maintained at 29 ± 0.8°C, 50 ± 1.2% RH, and a photoperiod of 16:8 (L:D) h. Potato psyllids voucher specimens are deposited in the Insect Collection at the USDA–ARS Laboratory, Wapato.

Psyllid exposure studies were conducted in spring 2006 in a small (4 by 3 m) greenhouse room maintained at 29 ± 2.6°C, 50 ± 3.5% RH, and a photoperiod of 16:8 (L:D) h; the room was lit by a single 1,000-W metal halide light. Forty potted potato plants (10 for each variety used in the study), at bloom stage, were randomly placed on a greenhouse bench, and ≈300 potato psyllid adults were released in the room on 14 April 2006 and left free to reproduce for several generations on the potato plants. The plants were monitored weekly for the presence of psyllid nymphs and adults and observed for psyllid feeding symptoms. Another group of 40 potato plants was held in a similar greenhouse room and under similar environmental conditions. Special care was taken to prevent psyllids from invading this greenhouse room; these potato plants served as controls. The potato plants in both greenhouse rooms were harvested on 30 June 2006. Pending chip processing, the harvested tubers were properly stored in a cold room maintained at 10 ± 0.5°C and 98 ± 1.5% RH. Three tubers were collected from each harvested plant (total of 30 tubers per variety) and visually checked for ZC symptoms by making a cross-section cutting near the stem end. The tubers were then sliced into chips and fried to check for chip discoloration. Because potato plant symptoms caused by psyllid feeding are similar to those caused by phytoplasmas, samples of plants exhibiting psyllid foliar symptoms were collected and tested for phytoplasmas by using polymerase chain reaction (PCR) assay (see Materials and Methods). Also, samples of tubers exhibiting typical ZC symptoms were tested for phytoplasmas by using PCR. The phytoplasma testing was performed at both USDA–ARS laboratories at Prosser, WA, and Wapato.

Field Study. The experiments were conducted at the USDA–ARS Field Experiment Station at Moxee, WA. Mini-tubers from three potato chipping varieties (Atlantic, FL 1879, and FL 1867) were obtained from the same source at CSS Farms and planted in preestablished large field cages on 15 May 2006. Five plants of each variety were grown in each cage (15 plants in total); they were planted in three rows of five plants each inside the cage. Each cage was 3 by 5 m in width and 1.5 m in height. The frame of the cage was made of 2.5- by 5-cm lumber, which was held firmly together by screws; 30 cm of each leg was buried in the soil. Agribon fabric (Polymer Group Incorporated, Charlotte, NC) was wrapped around and firmly stapled to the cage frame, and the bottom of the fabric was buried in the soil to prevent the escape of the psyllids or introduction of unwanted insects. Plants in the cages were irrigated using subsoil drip irrigation. There were 12 cages in total, eight with potato psyllids and four without psyllids (serving as controls). The psyllids were released in the cages on 7 July 2006, when the plants were in bloom stage. Approximately 300 psyllid adults, collected from the same laboratory colony described above, were released in each psyllid assigned cage. Similarly to the greenhouse study, the plants were monitored for the presence of psyllid nymphs and adults and observed for psyllid yellows symptoms.

The tubers from the test plants were harvested by hand on 25 September 2006, and, similarly to the tubers from the greenhouse, they were stored in a cold room maintained at 10 ± 0.5°C and 98 ± 1.5% RH, pending chip processing. Two tubers were then collected from each harvested plant, visually inspected for ZC symptoms by cross cutting them, and then processed for chip discoloration and ZC symptoms by frying tuber slices; 10 tubers per variety per cage in total were collected. To determine whether the symptoms were due to phytoplasma infection, samples of plants exhibiting psyllid symptoms and tubers with typical ZC symptoms were collected and tested for phytoplasmas using PCR.

Phytoplasma Testing. To verify whether the observed plant symptoms were not due to potato purple top disease, 20 potato plants in total exhibiting psyllid feeding symptoms and 35 tubers with typical ZC symptoms were collected from both the greenhouse and field experiments and tested for phytoplasmas by using PCR. An additional 15 plants and 15 tubers without psyllid or ZC symptoms were tested for phytoplasmas. Moreover, samples of 30 potato plants and 32 tubers with typical ZC symptoms were collected from ZC-infected commercial potato fields in McAllen, Pearsall, and Dalhart, TX, and tested; another asymptomatic 15 plants and 25 tubers were also tested. Furthermore, 47 samples of potato psyllids (a group of five insects per sample) collected from ZC-infected commercial potato fields in Texas and Mexico also were tested for phytoplasmas to determine whether there was an association between these psyllids and phytoplasmas. Nucleic acid extractions and PCR were conducted according to Crosslin et al. (2006).

Midveins, petioles, or tuber tissue was excised with a new razor blade (≈200–500 mg per sample) and placed into a mesh grinding bag (Agdia, Inc., Elkhart, IN). Total nucleic acid was extracted using the method of Presting et al. (1995), with minor modifications. The tissue was triturated with 1.2 ml of buffer (100 mM Tris-HCl, pH 8.0, 500 mM NaCl, 50 mM disodium ethylenediamine tetraacetic acid, and 10 mM 2-mercaptoethanol) by using a large pestle. Six hundred microliters of the solution was placed in a microcentrifuge tube containing 60 μl of 10% sodium dodecyl sulfate, mixed, and incubated 10 min at 65°C. Two hundred microliters of acidified 5 M potassium acetate was then added, mixed by vortexing, and the solution was incubated on ice for 10 min. Debris was pelleted by centrifugation at 14,000 × *g* for 10 min, and 600 μl of the clarified supernatant was transferred to a new microcentrifuge tube. After addition of 300 μl of isopropanol, the solution was mixed, held on ice for 10

min, and centrifuged for 10 min. The nucleic acid pellet was washed once with 70% ethanol, air-dried, and resuspended in 300 μl of sterile distilled water.

Nucleic acids were extracted from insects by using the hexadecyltrimethylammonium bromide (CTAB) extraction method of Zhang et al. (1998) but without grinding in liquid nitrogen. Briefly, the insects (in groups of five) were washed once with CTAB buffer and then ground in 600 μl of fresh buffer by using a micropestle and processed as described above for the plant materials. Nucleic acids were resuspended in 100 μl of sterile distilled water. In total, 47 sample sets of five insects were tested.

First-round reactions of nested PCR used phytoplasma universal primers P1 and P7. Reaction mixtures contained 5 μl of 10× PCR buffer (Promega, Madison, WI), 0.5 μl of 10 mM (each) dNTP mixture, 1 μl each 20 μM primer solution, 37.3 μl of sterile distilled water (dH_2O), and 0.2 μl (1 U) of *Taq* polymerase (Promega). Five microliters of DNA extracts varying in concentration were added, the reactions were overlaid with mineral oil, and the reactions were incubated 2 min at 94°C, then 30 cycles of 94°C for 15 s, 55°C for 90 s, 72°C for 90 s, followed by a final extension of 5 min at 72°C, then held at 4°C. For the nested reaction, 5 μl of the P1/P7 reaction was removed, diluted with 95 μl of sterile dH_2O, and 2 μl was used in reactions as described above, except 5 μl of Rediload (Invitrogen, Carlsbad, CA) and primers fU5/BLTVA-int were included in the reaction mix and the amount of water was adjusted as necessary. The nested primer pair fU5/BLTVA-int specifically amplifies group 16SrVI phytoplasmas. For phytoplasma group-nonspecific amplification, the nested primers used were fU5/rU3. Amplification conditions were as described above. Ten microliters of the reactions were subjected to electrophoresis in 1.5% agarose gels, stained with ethidium bromide, and visualized with UV light. The fU5/BLTVA-int and fU5/rU3 amplicons are ≈1,200 and 880 bp, respectively.

Potato Chip Processing. After collection, potato tubers were washed to remove any dirt or mud. Individual tubers were cut into half transversally. One half-tuber was sliced (cross section) by using an OXO Good Grips Mandoline Slicer (OXO International, New York, NY) into 1.05-mm-thick slices. The potato slices were placed in a beaker with clean, room temperature water, and stirred for 10 s to remove excess starch. The slices were removed from the beaker, placed on a paper towel, and blotted to remove excess water. The slices were placed in the basket of a deep fryer (Hamilton Beach/Proctor-Silex, Inc., Southern Pines, NC) and fried for 3 min at 180°C in canola oil. The fried chips were then checked for color and ZC symptoms.

Statistical Analysis. Percentages of ZC-infected tubers and plants with psyllid feeding symptoms were calculated for each potato variety used in both the greenhouse and field studies. Data were analyzed by using SAS general linear models procedures (SAS Institute 2003). Analysis of variance (ANOVA) was performed after transformation of percentage data by using arcsine $\sqrt{(x)}$. The level of significance was set at $P = 0.05$, and the least significant difference (LSD) test was used to separate means.

Table 1. Potato plants, tubers, and chips exhibiting psyllid yellows and ZC symptoms resulting from potato plant exposure to *B. cockerelli* under greenhouse conditions

Potato variety	% plants with psyllid yellows symptoms	% raw tubers with ZC symptoms	% fried chips with ZC symptoms
Atlantic	90	36.7 ± 2.1a	70.0 ± 0.4a
FL 1879	70	33.3 ± 0.9a	63.3 ± 1.1a
FL 1833	60	20.0 ± 1.8a	46.7 ± 0.9a
FL 1867	50	13.3 ± 0.2a	56.8 ± 0.8a

No plant or tuber ZC symptoms were observed in the *B. cockerelli*-free greenhouse room. Means followed by the same letter within columns are not significantly different ($P > 0.05$; LSD).

Results

Greenhouse Study. Potato psyllids reproduced and developed on the potatoes in the greenhouse room and nymphs and adults were present on the plants throughout the experiment. Two weeks after release until the end of the experiment, the psyllid density averaged 22.5 ± 8.6 and 44.9 ± 18.2 (mean ± SEM) adults and nymphs per plant, respectively. Plants started exhibiting symptoms resembling those of psyllid yellows or phytoplasma infection ≈3 wk after the psyllid release. Initial plant symptoms consisted of a rolling upward of the top leaves, developing into a basal cupping of the leaflets, accompanied with yellowish and reddish discoloration. Later, the symptoms included proliferation of axillary buds, shortened internodes, swollen nodes, leaf scorching, and early plant decline. By the end of the experiment, symptoms were observed in 67.5% of the plants exposed to psyllids, ranging from 50% for FL 1867 to 90% for Atlantic (Table 1). Of the 120 tubers collected from the plants exposed to psyllids, 25.8% of the raw tubers exhibited ZC symptoms and ranged from 13.3% for FL 1867 to 36.7% for Atlantic, whereas 59.2% of fried chips showed typical ZC symptoms and ranged from 46.7% for FL 1833 to 70% for Atlantic (Table 1). There were no significant differences in raw tubers (ANOVA: $F = 1.25$; df = 3, 39; $P < 0.3097$) or fried chips (ANOVA: $F = 0.60$; df = 3, 39; $P < 0.6195$) exhibiting ZC symptoms between the different potato varieties tested (Table 1). No psyllid feeding or ZC symptoms were observed in plants or tubers from the control greenhouse room where the plants had not been exposed to psyllids.

Field Study. Due to very high summer temperatures (≈37°C) that prevailed in the field cages shortly after the psyllids were released, the psyllid population in the cages was very low at first, and the insects were hard to find 2 wk after release, but they increased considerably late in the summer when the temperature started to cool down. From mid-August until the end of the experiment in September, the density of the psyllids in the cages averaged 13 ± 6.9 and 29 ± 11.7

Table 2. Potato plants, tubers, and chips exhibiting psyllid yellows and ZC symptoms resulting from potato plants exposure to the *B. cockerelli* under field conditions

Potato variety	% plants with psyllid yellows symptoms	% raw tubers with zebra chip symptoms	% fried chips with zebra chip symptoms
Atlantic	52.5 ± 3.2a	21.3 ± 1.2a	57.5 ± 1.8ab
FL 1879	45.0 ± 1.9a	17.5 ± 1.7ab	66.3 ± 0.9a
FL 1867	30.0 ± 2.2a	6.3 ± 0.5b	47.5 ± 1.3b

No plant or tuber ZC symptoms were observed in the *B. cockerelli*-free field cages. Means followed by the same letter within columns are not significantly different ($P > 0.05$; LSD).

adults and nymphs per plant, respectively. Symptoms of psyllid damage in the cages were visible by late August; the symptoms were similar to those observed in the greenhouse, in addition to numerous aerial tubers that were produced on the infected plants. At the end of the experiment, 42.5% of plants exhibited symptoms of psyllid damage, ranging from 30% for FL 1867 to 52.5% for Atlantic (Table 2). Of the 240 tubers collected from the harvested plants in the psyllid-exposed cages, 15% of raw tubers showed ZC symptoms, ranging from 6.3% for FL 1867 to 21.3% for Atlantic, whereas 57% of tubers exhibited ZC symptoms in the fried chips and ranged from 47.5% for FL 1867 to 66.3% for FL 1879 (Table 2). Although there was no statistically significant difference in plant symptoms between the three varieties tested (ANOVA: $F = 1.97$; df = 2, 23; $P < 0.1765$), differences in ZC symptoms in raw tubers (ANOVA: $F = 3.61$; df = 2, 23; $P < 0.0384$) and fried chips (ANOVA: $F = 4.93$; df = 2, 23; $P < 0.0240$) between varieties were significantly different (Table 2). No psyllids were observed in the four control cages and no plants or tubers in these cages showed any psyllid damage or ZC symptoms.

Phytoplasma Testing. None of the potato plants or tubers with psyllid damage or typical ZC symptoms collected from the greenhouse or field cages tested positive for phytoplasmas by nested PCR; the same results were observed with the asymptomatic plants tested. Also, from all the plants and tubers exhibiting typical ZC symptoms collected from ZC-infected commercial potato fields in Texas, only one plant from McAllen tested positive for the Columbia Basin potato purple top phytoplasma; the asymptomatic plants tested negative for phytoplasmas. Of the 47 sample sets of five potato psyllids tested, only two samples tested positive for phytoplasmas in the nested PCR by using universal primer pairs P1/P7, fU5/rU3, and fU5/BLTVA-int.

Discussion

ZC is an important and emerging disease that has the potential to cause serious losses to the potato industry in the United States and other affected countries. Ultimately, a good understanding of this disease is essential to develop effective management strategies; therefore, it is imperative that the disease vectors and mechanisms by which these vectors transmit this potato disease be correctly identified.

Results from the present greenhouse and field studies clearly indicated that the potato psyllid plays an important role in this potato disease. The results showed that 59.2 and 57% of potato tubers collected from the greenhouse room and field cages that had been exposed to potato psyllids, respectively, produced chips with typical ZC symptoms when fried. In contrast, no ZC symptoms were observed when potato tubers collected from plants grown in the greenhouse room and field cages that had not been exposed to psyllids were processed into chips. In addition, only plants produced in the greenhouse and field cages that had been exposed to psyllids exhibited plant symptoms similar to those caused by potato psyllid damage or phytoplasma infection. However, no phytoplasmas were detected in symptomatic plants or tubers with PCR testing, suggesting that the observed ZC symptoms in fried chips were due to psyllid feeding by injecting a toxin or transmitting an unknown pathogen.

To date, the true nature of psyllid yellows has not been fully understood. Psyllids are well known to inject toxins into the plants when feeding, resulting in disturbances of the plant metabolism and development (Eyer and Crawford 1933, Eyer 1937, Eyer and Miller 1938, Carter 1950, Wallis 1955, Abernathy 1991, Purcell et al. 1997, Liu and Trumble 2004, Liu et al. 2006). It also has been reported that some psyllid species transmit plant pathogens, including phytoplasmas, bacteria, and viruses (Davies et al. 1992, Carraro et al. 1998, Blomquist and Kirkpatrick 2002, Tedeschi and Alma 2004, Coletta-Filho et al. 2005, Salazar 2006). A study by Binkley (1929) suggested that the psyllid yellows disease is caused by a virus, rather than the mere feeding of the psyllid nymphs. However, Richards (1931) and Richards and Blood (1933) showed that the severity of the symptoms is proportional to the psyllid nymphal population, and they supported the theory that it is caused by a toxin injected by nymphs while feeding. Moreover, it has been reported that potato plants with psyllid yellows can recover when psyllids are removed from them or by insecticides, suggesting that the material injected into the potato plants by the nymphs is a toxin and not a plant pathogen (Blood et al. 1933, Richards and Blood 1933, List and Daniels 1934, Eyer 1937, Carter 1950, Sanford 1952, Daniels 1954, Wallis 1955, Arslan et al. 1985, Abernathy 1991).

Symptoms of plants infected by ZC resemble those caused by phytoplasmas, particularly the Columbia Basin potato purple top phytoplasma (Lee et al. 2004; Munyaneza 2005, 2006b; Crosslin et al. 2005; Munyaneza et al. 2006). Recent studies indicated that a very low incidence of three phytoplasmas was detected in plants and tubers infected by ZC; these phytoplasmas are the aster yellows phytoplasma (16SrI-A), the clover proliferation phytoplasma (16Sr VI), and a previously unknown phytoplasma in the stolbur phytoplasma group (16SrXII) that was recently named "*Candidatus* phytoplasma americanum" (Lee et al.

2006; Secor et al. 2006). However, in the current study, only one plant of 117 ZC symptomatic plants and tubers tested was positive for phytoplasmas. In addition, the current study indicated that phytoplasmas (specifically the Columbia Basin potato purple top phytoplasma) were detected in only two samples of potato psyllids collected from ZC-infected potato fields in Texas and Saltillo, Mexico. Furthermore, although some chips processed from tubers collected from positively tested Columbia Basin potato purple top phytoplasma-infected potato plants showed some defects, the symptoms did not resemble those caused by ZC (Munyaneza 2006a). All this evidence suggests that there is no correlation between phytoplasmas and observed ZC symptoms. The low incidence of ZC symptomatic plants infected with phytoplasmas observed in ZC-infected commercial fields is probably due to other insect vectors, including leafhoppers. Leafhoppers are well known to be major vectors of phytoplasmas and several species were found to be very common and abundant in several of ZC-infected commercial fields that were inspected (J.E.M., unpublished data). Although the Columbia Basin potato purple to phytoplasma was detected in two samples of potato psyllids during the current study, so far, there is no conclusive evidence that these insects are capable of transmitting this plant pathogen to potato.

It has recently been shown that ZC can successfully be transmitted by grafting (Gary Secor, personal communication), suggesting that this disease is caused by a plant pathogen. Daniels (1954) also reported that experiments with plants heavily laden with psyllid toxin had showed that tissue grafts into healthy plants carried over the phytotoxic effect in sufficient amount to cause psyllid yellows; however, this author indicated that subsequent grafts resulted in a gradual recovery in the form of a reversible reaction of the potato plants. In addition, this researcher indicated that vegetative propagation experiments had demonstrated further the subsequent dissipation of psyllid phytotoxic effect and gradual recovery of the potato plants, suggesting that psyllid yellows may not be the result of a plant pathogen infection. Unfortunately, to date, the nature of the toxin transmitted by the potato psyllid has not been identified and cannot be detected using current modern molecular techniques such as PCR; this makes it difficult to conclusively determine whether the potato psyllid induces ZC by merely injecting toxins or enzymes in the plants or whether a plant pathogen is involved.

In brief, although the exact causes of ZC are still unknown, results of the current study indicate that there is a strong association between the psyllid *B. cockerelli* and this emerging and devastating potato disease. Despite that ZC plant symptoms strongly resemble those caused by the potato purple top disease, evidence suggests that phytoplasmas seem not to be involved in ZC. However, it remains unclear whether *B. cockerelli* induces ZC symptoms by injecting toxins or transmitting plant pathogens other than phytoplasmas or both. Results of the current study also showed that relying on ZC symptoms in raw tubers by making cross-section cuttings into them to estimate damage caused by ZC will more likely underestimate the incidence of this potato disease in tubers; the best way to accurately evaluate ZC symptoms in potato tubers is by properly processing the tubers into chips and frying them.

To effectively manage ZC, it is imperative that mechanisms by which *B. cockerelli* induces this potato disease be determined. The information from the current study will make it possible for potato producers to focus monitoring and controlling efforts on *B. cockerelli* that should lead to a reduction in the incidence of ZC and serious losses that this disease causes to the potato crop.

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References Cited

Abernathy, R. L. 1991. Investigation into the nature of the potato psyllid toxin. M.S. thesis, Colorado State University, Fort Collins, CO.

Arslan, A., P. M. Bessey, K. Matasuda, and N. F. Oebker. 1985. Physiological effects of psyllid (*Paratrioza cockerelli*) on potato. Am. Potato J. 62: 9–22.

Binkley, A. M. 1929. Transmission studies with the new psyllid yellows disease of solanaceous plants. Science (Wash., D.C.) 70: 615.

Blomquist, C. L., and B. C. Kirkpatrick. 2002. Frequency and seasonal distribution of pear psylla infected with the pear decline phytoplasma in California pear orchards. Phytopathology 92: 1218–1226.

Blood, H. L., B. L. Richards, and F. B. Wann. 1933. Studies of psyllid yellows of tomato. Phytopathology 23: 930.

Carraro, L., R. Osler, N. Loi, P. Ermacora, and E. Refatti. 1998. Transmission of European stone fruit yellows phytoplasma by *Cacopsylla pruni.* J. Plant Pathol. 80: 233–239.

Carter, R. D. 1950. Toxicity of *Paratrioza cockerelli* (Sulc) to certain solanaceous plants. Ph.D. dissertation, University of California, Berkeley, CA.

Carter, W. 1939. Injuries to plants caused by insect toxins. Bot. Rev. 5: 273–326.

Coletta-Filho, H. D., M. A. Takita, M.L.P.N. Targon, and M. A. Machado. 2005. Analysis of 16S rDNA sequences from citrus huanglongbing bacteria reveal a different "*Ca.* Liberibacter" strain associated with citrus disease in São Paulo. Plant Dis. 89: 848–852.

Cranshaw, W. S. 1994. The potato (tomato) psyllid, *Paratrioza cockerelli* (Sulc), as a pest of potatoes, pp. 83–95. *In* G. W. Zehnder, M. L. Powelson, R. K. Hansson, and K. V. Raman [eds.], Advances in potato pest biology and management, APS Press, St. Paul, MN.

Crosslin, J. M., J. E. Munyaneza, A. S. Jensen, and P. B. Hamm. 2005. Association of beet leafhopper (Hemiptera: Cicadellidae) with a clover proliferation group phytoplasma in Columbia Basin of Washington and Oregon. J. Econ. Entomol. 98: 279–283.

Crosslin, J. M., G. J. Vandemark, and J. E. Munyaneza. 2006. Development of a real-time, quantitative PCR for detec-

tion of the Columbia Basin potato purple top phytoplasma in plants and beet leafhoppers. Plant Dis. 90: 663–667.

Daniels, L. B. 1954. The nature of the toxicogenic condition resulting from the feeding of the tomato psyllid *Paratrioza cockerelli* (Sulc). Ph.D. dissertation, University of Minnesota, St. Paul, MN.

Davies, D. L., C. M. Guise, M. F. Clark, and A. N. Adams. 1992. Parry's disease of pears is similar to pear decline and is associated with mycoplasma-like organisms transmitted by *Cacopsylla pyricola.* Plant Pathol. 41: 195–203.

Eyer, J. R. 1937. Physiology of psyllid yellows of potatoes. J. Econ. Entomol. 30: 891–898.

Eyer, J. R., and R. F. Crawford. 1933. Observations on the feeding habits of the potato psyllid (*Paratrioza cockerelli* Sulc.) and the pathological history of the "psyllid yellows" which it produces. J. Econ. Entomol. 26: 846–850.

Eyer, J. R., and M. Miller. 1938. A study of the pathological anatomy of psyllid yellows with special references to similar changes in sugar beets affected with curly top. Phytopathology 28: 669.

Flores, O. A., M. G. Gallegos, and M. O. García. 2004. Memorias del simposio punta morada de la papa. Universidad Autónoma Agraria Antonia Narro, Saltillo, Coahuila, Mexico.

Hernández-García, V., A. Sánchez-Arizpe, G. A. Frías-Treviño, and E. Padrón-Corral. 2006. Factores abióticos y su relación con el síndrome de punta morada de la papa, p. C-17. *In* Memoria de XXII Congreso de la Asociacion Latinoamericana de la Papa, 30 Julio–4 Agosto 2006. ALAP, Toluca, Mexico.

Lee, I.-M., K. D. Bottner, J. E. Munyaneza, G. A. Secor, and N. C. Gudmestad. 2004. Clover proliferation group (16SrVI) subgroup A (16SrVI-A) phytoplasma is a probable causal agent of potato purple top disease in Washington and Oregon. Plant Dis. 88: 429.

Lee, I. M., K. D. Bottner, G. Secor, and V. Rivera-Varas. 2006. '*Candidatus* phytoplasma americanum', a phytoplasma associated with a potato purple top disease complex. Int. J. Syst. Evol. Microbiol. 56: 1593–1597.

List, G. M., and L. B. Daniels. 1934. A promising control for psyllid yellows of potatoes. Science (Wash., D.C.) 79: 79.

Liu, D., and J. T. Trumble. 2004. Tomato psyllid behavioral responses to tomato plant lines and interactions of plants lines with insecticides. J. Econ. Entomol. 97: 1078–1085.

Liu, D., J. T. Trumble, and R. Stouthamer. 2006. Genetic differentiation between eastern populations and recent introductions of potato psyllid (*Bactericera cockerelli*) into western North America. Entomol. Exp. Appl. 118: 177–183.

Munyaneza, J. E. 2005. Purple top disease and beet leafhopper-transmitted virescence agent (BLTVA) phytoplasma in potatoes of the Pacific Northwest of the United States, pp. 211–220. *In* A. J. Haverkort and P. C. Struik [eds.], Potato in Progress: Science Meets Practice. Wageningen Academic Publishers, Wageningen, The Netherlands.

Munyaneza, J. E. 2006a. Impact of the Columbia Basin potato purple top phytoplasmas on potato tuber processing quality. Potato Country 22: 12–13.

Munyaneza, J. E. 2006b. Research update: potato purple top disease and beet leafhoppers in the Columbia Basin. Potato Country 22: 28–29.

Munyaneza, J. E., and J. E. Upton. 2005. Beet leafhopper (Hemiptera: Cicadellidae) settling behavior, survival, and reproduction on selected host plants. J. Econ. Entomol. 98: 1824–1830.

Munyaneza, J. E., J. M. Crosslin, and J. E. Upton. 2006. The beet leafhopper (Hemiptera: Cicadellidae) transmits the Columbia Basin potato purple top phytoplasma to potatoes, beets, and weeds. J. Econ. Entomol. 99: 268–272.

Presting, G. G., O. P. Smith, and C. R. Brown. 1995. Resistance to potato leafroll virus in potato plants transformed with the coat protein gene or with vector control constructs. Phytopathology 85: 436–442.

Purcell, M. F., J. K. Balciunas, and P. Jones. 1997. Biology and host-range of *Boreioglycaspis melaleucae* (Hemiptera: Psyllidae), potential biological control agent for *Melaleuca quinquenervia* (Myrtaceae). Environ. Entomol. 26: 366–372.

Richards, B. L. 1931. Further studies with psyllid yellows of the potato. Phytopathology 21: 103.

Richards, B. L., and H. L. Blood. 1933. Psyllid yellows of the potato. J. Agric. Res. 46: 189–216.

Salas-Marina, M. A., A. Flores-Olivas, A. Sánchez-Arizpe, O. García-Martínez, I. H. Almeida-León, and J. A. Garzón-Tiznado. 2006. Eficiencia de insectos vectores en la transmission de fitoplasmas de la punta morada de la papa, pp. O-1. *In* Memoria de XXII Congreso de la Asociacion Latinoamericana de la Papa, 30 Julio–4 Agosto 2006. ALAP, Toluca, Mexico.

Salazar, L. F. 2006. Emerging and re-emerging potato disease in the Andes. Potato Res. 49: 43–47.

Sanford, G. B. 1952. Phloem necrosis of potato tubers associated with infestation of vines by *Paratrioza cockerelli* Sulc. Sci. Agric. 32: 433–439.

Sanford, G. B., and J. G. Grimble. 1944. Observations on phloem necrosis of potato tubers. Can. J. Res. 22: 162–170.

SAS Institute. 2003. SAS user's guide: statistics, version 9.1. SAS Institute, Cary, NC.

Secor, G. A., and V. V. Rivera-Varas. 2004. Emerging diseases of cultivated potato and their impact on Latin America. Rev. Latinoamericana de la Papa (Suppl.) 1: 1–8.

Secor, G. A., I. M. Lee, K. D. Bottner, V. Rivera-Varas, and N. C. Gudmestad. 2006. First report of a defect of processing potatoes in Texas and Nebraska associated with a new phytoplasma. Plant Dis. 90: 377.

Snyder, W. C., H. E. Thomas, and S. J. Fairchild. 1946a. A type of internal necrosis of the potato tuber caused by psyllids. Phytopathology 36: 480–481.

Snyder, W. C., H. E. Thomas, and S. J. Fairchild. 1946b. Spindling or hair sprout of potato. Phytopathology 36: 897–904.

Tedeschi, R., and A. Alma. 2004. Transmission of apple proliferation phytoplasma by *Cacopsylla melanoneura* (Homoptera: Psyllidae). J. Econ. Entomol. 97: 8–13.

Wallis, R. L. 1955. Ecological studies on the potato psyllid as a pest of potatoes. U.S. Dep. Agric. Tech. Bull. 1107. Washington, DC.

Zhang, Y.-P., J. K. Uyemoto, and B. C. Kirkpatrick. 1998. A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. J. Virol. Methods 71: 45–50.

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