How Important is Zebra Chip Infected Seed or Volunteers as a Source of this Disease in the Columbia Basin?

Philip B. Hamm, Jordan Eggers, Silvia Rondon, and Alexzandra Murphy
Oregon State University, Hermiston, OR

Candidatus Liberibacter solanacearum (Lso) is transmitted by the potato psyllid *Bactericera cockerelli* (Sulc) and is known to cause zebra chip (ZC) disease in potato. Potato plants develop symptoms of the disease, typically 3 weeks or so after being infected. Initial symptoms include yellowing and purpling of leaves, up-curled leaves, swollen nodes, shortened internodes, aerial tubers and elongated axial buds. Eventually the leaves scorch, the whole plant turns yellow (chlorotic) and then dies. Typical ZC symptoms in tubers can begin to develop around the time leaf margins start turning brown. Infected tubers can have dark yellow to reddish brown flecks in all tuber tissues. Symptoms first appear in the stem end and near the vascular ring of the tuber but symptoms can vary from sporadic flecking in random parts of the tubers to a fairly uniform distribution of symptoms throughout. Frying ZC infected tubers for potato chips or French fries results in dark streaks and spots in the finished products. This disease can cause considerable economic losses in commercial potato fields through reduced yield and quality.

This disease was first confirmed in the Columbia Basin in the summer of 2011 (Crosslin et al. 2012). Foliar symptoms of ZC were first observed in commercial fields in mid-July, 2011. The first ZC symptomatic tubers were noticed in processing plants during the week of 22 August. Back dating (the time required for symptoms to occur in tubers following plant infection) suggests that there were psyllids with Lso in the Columbia Basin as early as approximately 15 June 2011; however the first potato psyllid was not detected in traps until 25 August. Significant infection levels, nearly 50%, occurred in several fields in 2011, though most fields had far less or little to no infection. The incidence and severity of the 2011 ZC epidemic in these highly infected fields has been linked to several factors. Most notably was the situation that low populations of several common potato insect pests during the early to mid-part of the 2011 growing season did not result in high enough insect populations to warrant insecticide applications. Timely application of some products that would typically be used to control pests such as aphids, potato beetles, leafhoppers, etc. would also have reduced or controlled potato psyllids. The long intervals between insecticide applications allowed psyllids carrying Lso to colonize these fields, reproduce, and spread the pathogen within that field and possibly to other fields. Weather also contributed to the Lso outbreak. Psyllids have reduced fecundity at high temperatures and those infected with ZC transmit the bacterium at reduced rates or not at all at temperatures at or above 32°C. The summer of 2011 was unusually cool, which allowed potato psyllids to reproduce and vector the disease efficiently during a greater amount of the growing season. Lastly, the repeated use of pyrethroids in these fields, once ZC was confirmed, likely had little effect to reduce their population.

Information is lacking as to the importance of infected tubers that either overwinter in the field and become volunteer potato plants the following year, or are planted as seed to be initial sources of Lso. The objectives of this study were to 1) return to fields in 2012 that had heavy ZC infestations in 2011 and scout for Lso symptomatic volunteers and 2) follow tubers collected from Lso infected plants in the fall of 2011 through storage, planting and plant development to determine how many plants emerge and were Lso infected. Objective two was executed in the screen houses at the OSU Hermiston Research and Extension Center in 2012 to prevent the movement of Lso to the surrounding area.

Methods

Volunteer survey

An initial survey for ZC-symptomatic volunteer potato plants in commercial fields in the Columbia Basin was conducted 11 May 2012. Two Columbia Basin fields planted with the cultivars Umatilla Russet and Ranger Russet in 2011 that had a high incidence of ZC infection (nearly 50%) were inspected for the presence of ZC-symptomatic potato volunteers. Both fields had been planted to corn in the spring of 2012 and the corn plants were approximately 6-8 inches tall at the time of the survey. Potato volunteers exhibiting typical ZC symptoms such as stunted growth, chlorotic or purple leaf color, and stunted and or upward cupped leaves were photographed and then collected to confirm the presence of Lso using standard PCR protocols. A total of 35 symptomatic plants were collected from the Umatilla field and 30 symptomatic plants were collected from the Ranger field. An additional three healthy plants adjacent to diseased-looking plants were collected from each field as negative checks. The number of volunteer potato plants in a 4 m^2 area around each symptomatic plant was recorded. The same fields were again inspected for ZC-symptomatic volunteers in late June, late August, and mid-October. A third field planted with the cultivar Alturas that had severe ZC infestation in 2011 also was inspected in the spring of 2012. This field had been planted to winter wheat in the fall of 2011. Fifteen symptomatic plants from this field were collected and tested but data used to estimate number of volunteers were not collected, due to the difficulty of finding plants among knee-high wheat.

Screenhouse experiment

The effects of tuber-borne ZC infection were evaluated by collecting, in the fall of 2011, Alturas, Ranger Russet, and Umatilla Russet tubers from plants that had died from natural ZC infection in commercial fields and tubers from Russet Norkotah, Russet Burbank and Umatilla Russet from hills where plants had already died down from Lso infection or natural causes. An additional set of tubers was collected from Alturas (Alturas Green) plants that were infected with Lso but were still alive at tuber collection. The tubers were stored in a commercial seed storage and were planted in two screen houses at the Hermiston Agricultural Research & Extension Center on 8 May 2012. There were 7 seed treatments for each cultivar: under 4 oz uncut, 4-8 oz bud end, 4-8 oz stem end, 4-8 oz uncut, over 8 oz bud end, over 8 oz stem end, over 8 oz uncut. Tubers were cut 3 days prior to planting and allowed to suberize. Internal symptoms were not recorded nor were seed tubers tested for Lso prior to planting. A healthy seed lot representing each cultivar, from a commercial seed grower, was planted as a negative control. Seed piece size for the negative controls was 1.5-2.0 oz and was always from the stem end of the tuber. Treatments were planted in a randomized complete block design with 10 seed tubers per replicate, 4 replicates per treatment. Plants were maintained with regular water and fertilizer programs similar to standard management practices. Yellow sticky traps were placed in the screen houses and inspected weekly for the presence of potato psyllids. Insecticides (Admire Pro at 8.7 oz/acre in furrow at planting, Asana at 9.6 oz/acre on 6/20/12, Lannate at 3 pts/acre on 7/16/12, Agri-Mek at 16 oz/acre and Fulfill at 5.5 oz/acre on 7/26/12, Agri-Mek at 16 oz/acre and Fulfill at 5.5 oz/acre on 8/13/12, Fulfill at 5.5 oz/acre on 9/14/12) were applied to help ensure no psyllid activity in the screenhouse. Plant emergence dates, the presence or absence of ZC symptoms at emergence, number of sprouts at emergence, plant height, and date of mortality were recorded for each plant that emerged. Leaves from each plant were sampled up to three times during the course of the experiment, depending on when a plant emerged and how long a plant survived. The first leaf sample was collected 10-15 days after a plant emerged and the second and third samples were collected on 26 July and 12 September. Symptomatic plants were tested for the presence of Lso using standard PCR protocols.

Results

Zebra Chip Volunteer Survey

Symptomatic plants were easily found in all three fields that had high levels of ZC in 2011 (Figure 1). Of the ZC-symptomatic volunteer plants tested, 48.6% of the Ranger Russet and 53.3% of the Umatilla tested positive for Lso. None of the healthy-appearing volunteers tested positive for Lso. The percentage of plants positive for Lso, when both symptomatic and asymptomatic plants were counted within the 4 m^2 area around each symptomatic plant, was 7% and 9% for the Ranger and Umatilla field, respectively. Using these numbers to calculate plants/acre the possible maximum projected number of Lso infected volunteers was 491 and 607 plants/acre for the Ranger and Umatilla fields, respectively. No ZC-symptomatic plants were observed during the late June, late August, and mid-October inspections of the Ranger and Umatilla fields. Six of the fifteen Alturas volunteers tested positive for Lso.

Screenhouse Experiment

Of the 1,680 field tubers planted, 53% sprouted and produced plants. Emergence for control (healthy) plants was 99%. No psyllids were found in either screen house and no negative control plants (healthy seed from a commercial supplier) developed ZC symptoms through the duration of the experiment. Of the plants that emerged from field tubers, only 10.4% showed foliar ZC symptoms at or shortly after emergence. Overall, 58% of symptomatic plants tested positive for Lso. All asymptomatic plants survived until harvest.

In general, plants from seed from fields with Lso tended to sprout slower, have lower percent emergence, fewer sprouts at emergence, shorter plant heights, more symptomatic plants, and live fewer days after emerging than control plants. Both symptomatic (those with symptoms of Lso) and asymptomatic (those appearing healthy) plants grown from field tubers took longer to emerge, had shorter vines, and lived fewer days after emergence than healthy controls (Table 1). Asymptomatic plants took significantly longer to emerge than symptomatic plants. Asymptomatic and healthy control plants had significantly more sprouts at emergence, were taller, and lived longer than symptomatic plants (Table 1).

A total of ten plants had delayed ZC symptom development (plants that did not show foliar ZC symptoms at emergence and initially grew normally but developed ZC symptoms later). This occurred with random plants throughout the duration of the experiment. The ten plants that had delayed ZC symptom development were very similar to asymptomatic plants with respect to emergence speed, number of sprouts, and plant height, but lived fewer days after emergence (data not shown). However, plants with delayed ZC symptom development had significantly more sprouts at emergence, had taller vines, and survived longer after emergence than plants that initially emerged with ZC symptoms.

Conclusions

Large numbers of ZC-symptomatic potato volunteers were found growing early in the season in commercial fields one year after the same fields had been severely impacted by ZC. Additionally, tubers collected from these fields the fall of 2012, when planted in the screenhouse, produced Lso positive plants. Both of these examples indicated that infected tubers/seed can result in Lso positive plants. However, information gathered from this study suggests that these plants would not be a significant initial source of Lso for the following reasons. First, infected volunteers were only observed in fields early in the season, when potato psyllids, either from local sources or brought in on prevailing winds from the south, are not present or not present in significant numbers. Secondly, managing volunteers with herbicide appeared to effectively eliminate Lso-infected plants from the field so that subsequent visits found no surviving Lso-infected plants. Infected plants were apparently more impacted by the herbicide, due to stress from ZC infection, given that healthy potato volunteers were present at the second and third field visits. Even if volunteers were not managed with herbicide, such as in the field planted with winter wheat, the screen house study suggests that Lso-infected plants would succumb to the disease before psyllids are present in significant levels. Third, even if infected plants survived until psyllids were present, the current season crop would likely have over grown the Lso infected plant. Certainly wheat over-grew the Lso plants in the one field during this trial. The same would have occurred in the corn field. In either case the presence of the existing crop plants significantly reduces the likelihood that a psyllid would find the stunted infected plant, acquire the bacterium, and spread it to a current season potato crop.

The forth reason why infected volunteer potatoes are not likely sources of Lso is the low number of infected volunteers that likely would be present in a year following normal control of potato psyllids. In 2011, only three fields with severe ZC infection were found. Many other fields had ZC, but not nearly to the level of these three fields. As stated previously, the high level of infection in these three fields was due to the lack of insecticide use for over two months, during the very time when potato psyllids were present and actively increasing in number. These fields represent the very worst case scenario for volunteers. Yet the percentage of Lso positive volunteers and plants arising from tubers collected and used as seed was still relatively low. Through communications with processors, the approximate overall incidence of ZC in Columbia Basin potato fields in 2012 was less than 1%. According to our data, if any of 2012's Lso-infected plants produced infected seed, less than half of them would likely sprout and produce plants, only about 10% of the plants that emerged would show symptoms, and only about half of the symptomatic plants would have detectable levels of Lso (the approximate accumulated % would be less than 0.025%). Therefore, given the low incidence in 2012, the likelihood that there would be a significant number of plants in 2013 that could act a reservoirs for the bacterium is very low. Furthermore, with what appears to be a reoccurring risk of ZC from year to year, fields will not be left unprotected in the future which means far fewer chances of infected ZC plants emerging from volunteers.

Other considerations also support this conclusion. Weather in 2011 was cooler and more favorable for psyllid reproduction and multiplication of ZC within the psyllids as well as adding in the transmission of the bacterium to potato plants. Prior to ZC occurrence in the Columbia Basin, there have been no reported outbreaks of psyllid yellows, the symptom that occurs in potato plants from feeding by the potato psyllid. That would seem to indicate that normal levels of potato psyllids are low in the Columbia Basin and do not normally rise to high levels. This would include resident populations (which have not arisen suddenly) as well as those that would normally be carried to the region on jet streams. Lastly, even with high numbers of ZC volunteers potentially present in 2012 from the 2011 outbreak, overall infection levels in 2012 were very low.

It would seem that all the possible factors that could contribute to an outbreak of ZC occurred in 2011; favorable weather for insect reproduction and bacterial transmission, long term lack of insecticide use, and repeated use of pyrethroids. This conclusion does not mean that research in the biology and epidemiology of ZC should be curtailed in the Columbia Basin. In particular, a more sensitive trapping system to detect psyllids earlier in the season when they are at lower numbers would be an invaluable tool for IPM of this disease. This would allow for psyllids to be tested for Lso much earlier in the season which would provide growers real-time information as to when and where Lso-infected psyllids are present, leading to more targeted and efficacious insect management.

Literature Cited

Crosslin, J.M., P.B. Hamm, J.E. Eggers, S.I. Rondon, V.G. Sengoda and J. E. Munyaneza. 2012. First report of zebra chip disease and "*Candidatus* Liberibacter solanacearum" on potatoes in Oregon and Washington State. Plant Disease 96: 452.

Figure 1. Healthy (left) and ZC infected russet ranger volunteer, 2012.

Table 1. Comparison of plants that emerged from test seed showing ZC symptoms to plants that did not show ZC symptoms at emergence. Healthy control plants originated from commercially produced seed free of ZC.

Plant Type	Emergence (DAP[1])[2]	% Total Plants[3]	# Sprouts at Emergence	Plant Height (cm)[4]	Mortality (DAE[5])[6]
Asymptomatic	42 a	89.6 a	2.5 a	81 b	92 b
Symptomatic	35 b	10.4 b	2.0 b	26 c	45 c
Healthy Controls	24 c	NA	2.7 a	108 a	103 a
P>f	0.0005	0.0019	0.0037	0.0007	<.0001

[1]DAP= Days after planting
[2] The number of days after seed was planted that sprouts emerged
[3] The percentage of all the plants that emerged from test seed that either had ZC symptoms or not. Control plants not included.
[4] Plant heights were determined by measuring the longest central vine of each plant, 72 days after planting.
[5] DAE= Days after emergence
[6] The number of days a plant survived after it emerged.