AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Activating the potato immune system to control Potato Virus Y & Zebra Chip disease

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SUMMARY: In 2012, Oregon State harvested almost 17,000 hectares of potatoes with a production value of $172,838,000 making potatoes the seventh most important commodity for Oregon. Potato Virus Y (PVY) and Candidatus *Liberibacter* solanacearum, and Zebra Chip (ZC), are major potato pathogens affecting potato yield. Thiamine, a water-soluble B-complex vitamin (a.k.a. vitamin B1) has been shown in many crops to boost the plant's immunity, thereby increasing resistance against pathogens by inhibiting disease progression and reducing pest populations. Our objectives for this study were to test the effect of thiamine application on potato resistance to PVY and ZC, two diseases vectored by aphids and potato psyllids, respectively. We conducted Screenhouse studies to determine whether thiamine provided resistance against aphids/PVY and against psyllids/ZC. In independent studies, we tested different densities of aphids and psyllids (0, 1, 5, 10/plant) on potatoes using four treatments of thiamine at different concentrations (0 mM, 1 mM, 10 mM, 50 mM). On plants with disease-positive insects ("hot" insects), we monitored disease symptoms and progression by collecting leaves and performing ELISA and PCR. On disease-free plants ("clean" insects), we monitored insect feeding symptoms and damage to the plant by counting insect progeny to determine reproductive success on the host. PVY and ZC were tested in year one; the second year only PVY was studied.

OBJECTIVES: To test the effect of thiamine application on potato against PVY and ZC, two diseases vectored by aphids and potato psyllids, respectively.

PROCEDURES: This study was conducted in two Screenhouse on site at the Oregon State University-Hermiston Agricultural Research and Extension Center in Hermiston, OR during the 2014 season. In 2015, only PVY was studies. In 2014, one Screenhouse was used for the ZC study and a second one was used for the PVY study. Potatoes were planted by hand on 15 May following standard agronomic practices. Treatments were applied at the vegetative stage. Five treatments: CONTROL, T1= Tween 80, T2= Tween 80 + 1 mM thiamine, T3= Tween 80 + 10mM thiamine, and T4= Tween 80 + 50 mM thiamine were tested in combination with three insect densities (LO-1, MED-5, HI-10 insects/plant). Tween was used as a surfactant. The plots were assembled in a randomized block design in 3 replicates for a total of 40 plots per Screenhouse. There were 4 replicates of the control (no thiamine, no aphids). Thiamine was applied with a backpack sprayer 24h before insect release. Each plot was set up with two "clean" plants and two "hot" plants. In 2015, treatment rates were modified.

ZC/Psyllid Screenhouse:
The potato psyllids, *Bactericera cockerelli* Sülc*.,* were collected from the laboratory colonies fed on tomatoes and potatoes. The psyllids were tested using conventional PCR (Crosslin et al. 2011) prior to release to make sure "clean" colonies were negative for ZC, and "hot" colonies were positive for ZC. The insects treatments were counted out as: LO-1 insect, MED-5 insects, HI-10 insects, and placed in clip

cages. The clip cages (see picture, right) containing clean insects (two clip cages per plant) were brought to the Screenhouse and placed on their respective plants. “Hot” psyllids, or psyllids positive for ZC, were placed on the designated “hot” plants in each plot in the ZC Screenhouse and “clean” psyllids, psyllids negative for ZC, were released on the designated “clean” plants in the ZC Screenhouse. Psyllids were left to feed on plants for one week, sufficient time to transmit the bacteria.

PVY/Aphid Screenhouse:
Aphids were collected from the laboratory-reared colony of *Myzus persicae* L. grown on turnip and roses (both plants tested negative for PVY). The insects were counted out at specific densities: LO-1 insect, MED-5 insects, HI-10 insects, and placed in clip cages. Aphids were released on clean plants and left to feed for one week. This is sufficient time for aphids to transmit PVY since they can transmit the virus within seconds. “Clean” refers to plants that would not be receiving disease and only insects, so that we could observe the effect of thiamine application on the insect vector’s feeding and mortality. The “hot” plants were mechanically inoculated with PVY strain N:O (isolate Alt) in the PVY Screenhouse. The inoculum was made with $PVY^{N:O}$ positive tobacco leaves that were hand grinded with phosphate buffer. The inoculum was applied with a sponge after carborundum was applied to leaves to wound the plant and allow the virus to be absorbed through the broken leaf tissue.

Zebra Chip and PVY treatments were covered with additional netting. This netting was a fine mesh held up by fiberglass poles and buried in the soil to prevent unwanted insects from getting into the plots (see picture, left). Two leaflets were collected per plant every week from insect release until harvest and visual assessments of foliar symptoms were conducted weekly. Each plant was individually hand-harvested in October 2014 and all tubers were weighed individually per treatment per plant. Specific gravity was also measured for each plant. For the ZC trial, all tubers were cut at each end to determine presence or absence of ZC symptoms and their severity using the Texas raw tuber rating scale (0-no ZC symptoms, 1-mild, 2-severe, 3-very severe). Plant DNA was extracted using the Dellaporta extraction buffer protocol and then high fidelity PCR was used to determine ZC presence in weekly leaf samples. For the PVY trial, all tubers were checked for external and internal symptoms of Potato Tuber Ring Necrotic or PTNRD. Enzyme-linked immunosorbent assay (ELISA) is ongoing to determine PVY presence in the weekly leaf samples. Leaflets were freeze-dried and ground with mortar and pestle for ELISA testing. Differences among treatments were determined using a Tukey’s least significant means test at a significance level of $\alpha = 0.05$ and One-Way ANOVAs.

SIGNIFICANT ACCOMPLISHMENTS: For the ZC trial, our control plots had the least percentage of ZC infected tubers, but no significant differences were found among treatments. There were, however, significantly more tubers with a 0 ZC rating (no visible ZC symptoms) than any other rating; perhaps thiamine decreased the amount of the bacteria titer or prevented the disease from moving to the tubers. In general, there were no significant differences between treatments for yield, weight of tubers, or ZC incidence. The ZC diseases does not move evenly in the plant and makes it difficult to quantified molecularly.

For the PVY trial, we analyzed clean and hot plants separately because we only released insects on “clean” plants and mechanically inoculated “hot” plants, so density of insects was not a factor for “hot” plants. For “clean” plants, the control plots yielded significantly more tubers than T1-MED, T3-LO, and T4-HI. Looking at hot plants only, the control plots yielded significantly more tubers than all treatments. This could be because the control plots were the only plots that were not inoculated with PVY, a disease known to reduce yield. When all plants were pooled by treatment (“clean” and “hot” plants), T4 (50 mM thiamine) was the only treatment with a similar yield to the control. All other treatments (T1, T2, and T3) had significantly lower mean yields compared to the control. However, it is

possible that thiamine application delayed disease expression. ELISA testing is ongoing. Foliar symptoms in all treatments were mild; moreover, plants were still very green and upright when we harvested plots in early October 2014. These are preliminary results for the first attempt of thiamine as a control measure on potatoes that were repeated during the 2015 season with similar results.

BENEFITS & IMPACT: This is the first attempt to control diseases of potato with thiamine. Our preliminary results are promising and encourage further study. If thiamine treatment delays disease progression, growers could combine this technique with other management practices to avoid yield loss from PVY and ZC. If thiamine is effective at enhancing the plant's own defenses and priming plants against future infection from pathogens, it could be added into a pesticide management program. Treating plants with thiamine could decrease insecticide input, because the plants will be healthier and potentially less insecticide applications will be needed to control for vectors such as aphids and psyllids.

ADDITIONAL FUNDING RECEIVED: None so far, since we are using this preliminary data to apply for federal funding. One grant has been submitted to the National Potato Council; a second one for the North Western Consortium. Our results have been presented at the 2015 Annual Entomological Society Meeting, Insect Pest Management Pacific Northwest Meeting, and in field days.

Reference Cited

Crosslin, J.M., H. Lin, and J.E. Munyaneza. 2011. Detection of Candidatus *Liberibacter* solanacearum in the potato psyllid, *Bactericera cockerelli* (Sulc) by conventional and rea-time PCR. Southwestern Entomol. 36: 125-135.