Potato Progress

Research & Extension for the Potato Industry of Idaho, Oregon, & Washington

Andrew Jensen, Editor. ajensen@potatoes.com; 509-760-4859

www.nwpotatoresearch.com

Volume XIX, Number 6 | *13 May 2019*

Studies on the mysterious foliar damage in Northwest potato in recent years

Kylie D. Swisher Grimm, USDA-ARS

Carrie Wohleb & **Tim Waters**, Washington State University

In the 2017 and 2018 growing seasons, plants with disease-like symptoms appeared in July and August across the Columbia Basin of Washington and Oregon. Symptoms included leaf distortion with crinkled, warped leaves often found with small holes, purpling of upper terminal leaves, and stem damage seen as blisters and necrotic lesions (Figure 1). These symptoms were observed in organic potatoes, ruling out chemical damage as a cause. Most surprisingly, healthy plants without any leaf purpling, crinkling, or stem lesions were very difficult to find in these fields, suggesting that the symptom rate was 90% or higher. Despite these symptoms, tubers observed in early- to mid-August were of good size, no skin or internal symptoms were visualized, and reports after harvest indicated good tuber yields each year. To determine if a pathogen was associated with these mysterious disease-like symptoms, symptomatic tissue was subjected to molecular diagnostic and greenhouse grafting analyses, and field-grown tubers were analyzed for emergence of symptomatic tissue in the greenhouse.

Figure 1. Symptoms observed across the Columbia Basin during 2017 and 2018. Left, top and bottom: Leaf distortion. Middle, top and bottom: Purple terminals, negative for BLTVA phytoplasma. Right, top and bottom: Stem blistering and necrotic lesions.

Visual observations:

In 2017, symptoms were observed in several potato cultivars including Ranger Russet, Alturas, Umatilla Russet, Clearwater Russet, and Challenger. Initial observations suggested that symptom severity was variable depending upon cultivar, with Umatilla Russet foliage showing the most severe disease-like symptoms.

In 2018, an effort was made to compare symptom expression in different cultivars grown in the Columbia Basin. The differences in symptom timing and development across cultivars, despite field location, were readily apparent in the Columbia

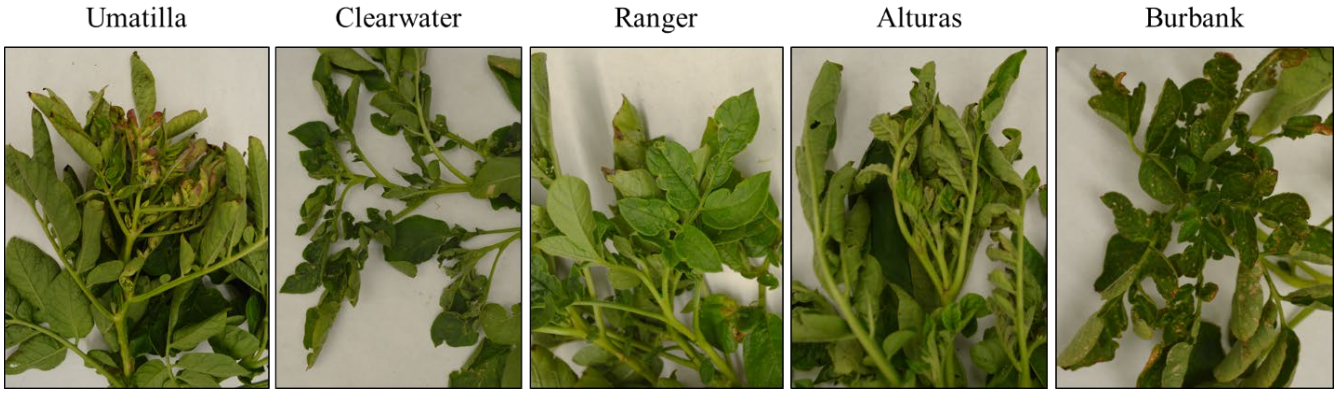

Figure 2. Symptoms varied across cultivars in the Columbia Basin in 2018 (end of July to early/mid-August). Umatilla was the most severely impacted cultivar, while Burbank showed the least amount of the mystery disease-like symptoms.

Basin (Figure 2). Umatilla Russet consistently showed the most severe symptoms, with leaf distortion, terminal leaf purpling and stem lesions evident in mid-August. Interestingly, compared to the same time in 2017, symptom development appeared milder, with less overall purpling and plant senescence. Despite this, in both years of this study, Umatilla was the most impacted cultivar. In mid-August, Clearwater Russet plants generally looked healthy, though leaf distortion and the initiation of leaf purpling was evident across all fields observed. In both the Alturas and Ranger Russet cultivars, clear leaf distortion and stem lesions were present in late July and mid-August, respectively, but very little purpling was observed. Russet Burbank was surprising, showing very low levels of leaf distortion and stem lesions in mid-August. Most Russet Burbank plants generally appeared healthy, despite showing visible damage from thrips. Additionally, symptomatic leaf tissue of the Lamoka cultivar was received from the Klamath Basin, which also showed purpling terminal tissue, minor leaf distortion, and stem blisters and lesions.

In 2018, observations were made on commercial Umatilla plants caged with and without the artificial release of lygus bugs throughout the season (Tim Waters, ongoing research). Plants in the lygus-free control cages showed mostly green, healthy leaf tissue with only minor amounts of leaf distortion and minor amounts of reddening along leaf margins (Figure 3). Very minimal levels of stem blistering were observed, with a few necrotic stem lesions present. In contrast, plants in the lygus-infested cage showed severe leaf distortion on terminals, with some terminals showing minor reddening along the margins and others showing significant yellowing and purpling (Figure 3). Stem blisters and necrotic lesions were readily visible on these plants as well.

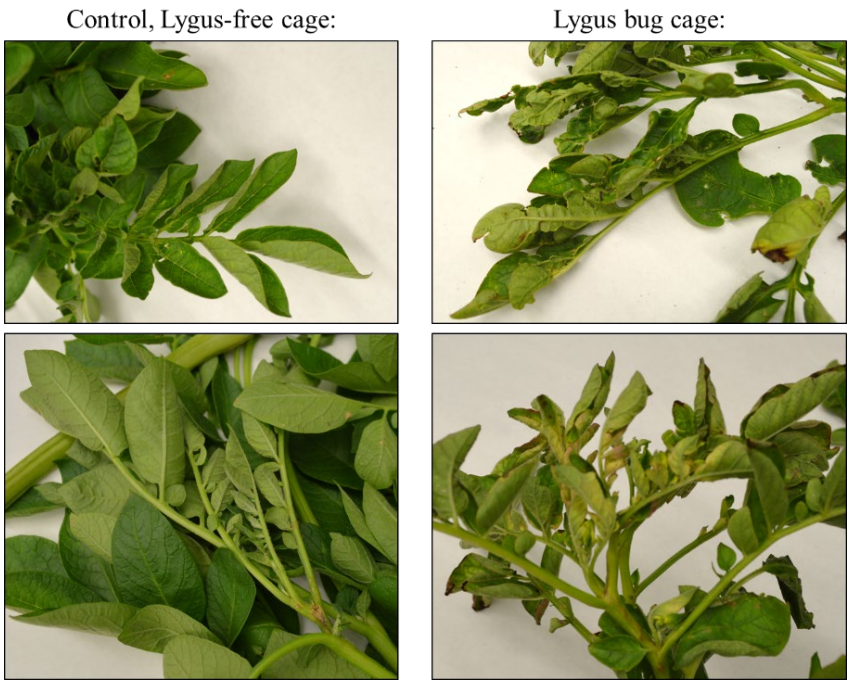

Figure 3. Symptoms observed on plants collected from control, lygus-free cages (Left) and on plants collected from cages artificially infested with lygus bug (Right).

Greenhouse grafting:

In both years of this study, a subset of the plant tissue collected from commercial fields and research plots was grafted to healthy greenhouse-grown minituber plants at the USDA-ARS Prosser research station (Secor et al. 2009). In 2017, a total of 48 grafted plants were generated in the greenhouse from each of the five cultivars sampled, each with two or three scions originating from the commercial or research field plants.

In 2018, a total of 51 grafted plants were generated in the greenhouse from each of the six cultivars sampled, each with two scions. Among these grafted plants in 2018 were scions generated from plants collected from commercial Umatilla lygus bug and control (lygus-free) cages. Control plants consisting of greenhouse-grown scions grafted to greenhouse-grown recipient plants, were also grafted every time field-collected samples were grafted. The grafted controls served as indicators of greenhouse-related symptoms that could occur independently of field sample symptoms. All grafted plants were observed on a weekly basis for symptoms of terminal leaf purpling, leaf distortion, and stem lesions or blisters. Visualization of these symptoms on healthy leaf tissue of the recipient plants would indicate that symptoms were transmissible, and therefore that a pathogen was responsible for the disease-like foliar symptoms seen across the Columbia Basin.

Generally, plants with the mysterious disease-like symptoms of leaf distortion, terminal leaf purpling, and stem blistering or necrotic lesions did not transmit from the grafted scions to the recipient plants, as no symptoms were seen that correlated across all plants. As with the control plants, nearly all recipient plants grafted with field-collected scions showed normal, healthy plant growth. Similarly, in 2018, scions grafted from the control (lygus-free) and lygus bug cages did not induce symptoms in the recipient plants.

In both years of this study, a very small subset of recipient plants (two plants in 2017, four plants in 2018) did show purpling of leaves and stems and developed aerial tubers (Figure 4). In 2017, two plants were grafted with scions originating from a commercial field, while in 2018 all four plants were grafted with scions from plants collected from an Umatilla research plot located at the USDA-ARS research farm in Moxee, WA. Interestingly, these plants that showed symptom transmission all tested positive for beet leafhopper transmitted virescence agent (BLTVA) phytoplasma, the causal agent of purple top disease, by molecular analysis.

Figure 4. Recipient plants grafted with BLTVA-infected scions showed symptom development, including purpling terminal leaves and aerial tuber formation. Red arrows point to scions grafted onto the healthy plants. White arrows point to purpling and aerial tuber formation. Images were taken 6 ½ weeks after grafting.

In 2017, some additional observations were made on recipient plants that did not appear normal or healthy. One plant showed possible leaf deformity, but a control plant showed similar leaf deformity suggesting that the symptom was not be due to the field sample. In 2018, a couple additional observations were made on recipient plants that did not appear normal or healthy. Specifically, one plant showed aerial tuber formation at the site of grafting but showed no other visible symptoms. It is assumed that the aerial tuber developed as a result of the grafting process which causes disruption of the phloem tissue. One additional plant grafted with a symptomatic Clearwater scion showed a single leaflet with possible leaf distortion. This is the only plant that displayed the leaf distortion symptoms, indicating that it is unlikely to have been caused by graft transmission of the mysterious disease-like symptoms.

Molecular Diagnostics:

In both 2017 and 2018, leaf tissue from each field sample was subjected to molecular diagnostic assays to identify any pathogen(s) present in the samples. Two samples were taken from the leaf tissue of each plant for diagnostics to ensure that detection of a pathogen would not be missed. Nucleic acids were extracted from each terminal leaf sample using the standard Dellaporta protocol (Crosslin and Hamlin, 2011). Extracts were then subjected to standard polymerase chain reaction (PCR) analysis or reverse transcription-PCR analysis for the detection of phytoplasmas, '*Candidatus* Liberibacter solanacearum' (Lso), *Tobacco rattle virus* (TRV), *Potato mop top virus* (PMTV), *Alfalfa mosaic virus* (AMV), *Tomato spotted wilt virus* (TSWV), *Potato leafroll virus* (PLRV), and *Potato virus Y* (PVY; Crosslin et al. 2006, Crosslin and Hamlin, 2011, Cating et al. 2015). In 2017, samples were also tested by PCR for fungal pathogens using a universal target (Toju et al. 2012).

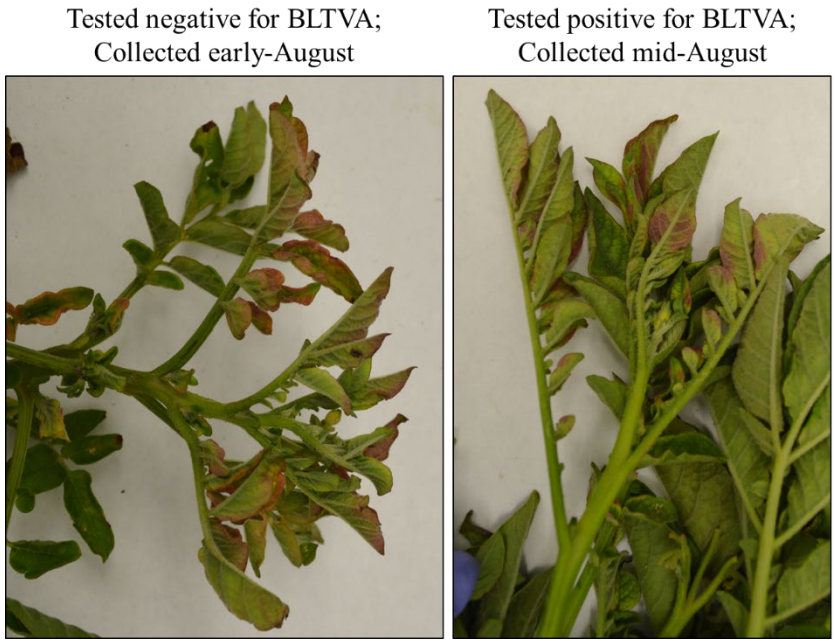

Figure 5. A comparison of purple terminals from a BLTVA-negative plant (left) and a BLTVA-positive plant (right).

In both years of this study, there were no pathogens identified by molecular analysis that correlated to the field symptoms. In 2017, two samples collected mid-July from a commercial field, and two samples collected at the beginning of August from a research plot, were positive for BLTVA phytoplasma. Two of these four samples were grafted in the greenhouse and produced the classic BLTVA symptoms, including purpling of leaf and stem tissue and aerial tuber formation. In 2018, four plants collected mid-August from the USDA-ARS Moxee research farm were positive for BLTVA phytoplasma. Tissue from these four plants was grafted in the greenhouse and produced classic purple top symptoms. No other field samples, many of which showed classic purple top symptoms, were positive for phytoplasma. In both 2017 and 2018, a visual comparison of purple terminals from BLTVA-infected and BLTVA-free plants was done, but there was no obvious distinction in symptoms (Figure 5).

No foliar samples were positive for Lso, TRV, PMTV, AMV, TSWV, or PLRV in 2017. Numerous foliar samples were positive for PVY, including PVY^{NTN} and $PVY^{N:O}$/$PVY^{N\text{-}Wilga}$ strains. However, there was no correlation between symptomatic samples with leaf distortion, purpling terminals, and stem blistering, and those positive for specific strains of PVY. Similarly, in 2018, no samples were positive for Lso, TRV, PMTV, AMV, TSWV, or PLRV. Of the 69 plants collected across the Columbia and Klamath Basins, 23 were positive for PVY.

Numerous foliar samples collected in 2017 showed a PCR product when a universal fungal ITS region was targeted. Five of these samples (different cultivars and different symptom severity) were subjected to molecular cloning and sequencing of the unknown fungal pathogen(s). Sequencing of three clones from each sample did not identify any pathogen that was correlated with the symptoms of leaf distortion, purpling terminals, or stem blistering and necrosis. Common fungal pathogens were however identified, including Alternaria species and *Sclerotinia sclerotiorum*.

Tuber analysis and grow-out:

In 2017, tubers from three commercial fields across the Columbia Basin showing foliar symptoms of terminal leaf purpling, leaf distortion, and stem lesions, were cut and observed for internal symptoms. No internal defects or symptoms were seen. Tuber tissue from 36 individual tubers was tested for the presence of phytoplasmas, Lso, TRV, PMTV, AMV, TSWV, PLRV, and PVY. Seventy-five percent of the tubers were infected with PVY, but no other common viral or bacterial pathogen was identified. These tubers, as well as

ten others received from a research plot in the Columbia Basin were planted in the greenhouse at the USDA-ARS research station in Prosser, WA, to inspect emerged foliar tissue for symptom development. No symptoms of leaf distortion, purpling terminals, or stem lesions were observed on the emerged plants.

Conclusions:

The inability to detect graft transmission of the mysterious disease-like symptoms from field samples in the Columbia Basin in 2017 and 2018, and Klamath Basin in 2018, provides strong evidence that the disease-like symptoms are not caused by a pathogen. In support of this, molecular diagnostic techniques used to detect common bacterial and viral pathogens did not identify a pathogen that correlated with the observed field symptoms, and commercial tubers collected from symptomatic plants did not produce symptomatic foliage when grown in the greenhouse. According to field managers in the Columbia Basin, the mysterious disease-like symptoms did not reduce tuber yield or induce any tuber quality defects in 2017 or 2018, indicating that the visual above-ground symptoms did not translate into any delay in tuber development and bulking, or affect overall tuber health.

Observations of symptom development in plants collected from control (lygus-free) and lygus bug cages were striking, showing significantly more disease-like symptoms on plants artificially infested with lygus than the control plants. As with the other field-collected samples, grafting and molecular analyses of plant tissue from lygus-infested, caged plants did not show graft transmission of symptoms and did not identify any common pathogens associated with all symptomatic plants. The lack of pathogen associated with these caged symptomatic, lygus-infested plants, indicates that symptoms may be caused by the mechanical feeding damage caused by lygus bugs. Research is currently underway by Tim Waters to explore the effects of lygus bug feeding damage on potato. Findings from these experiments will improve our understanding of the mysterious disease-like symptoms in the Columbia Basin.

Literature cited:

Cating, R.A., C.N. Funke, N. Kaur, P.B. Hamm, and K.E. Frost. 2015. Multiplex reverse transcription (RT) high-fidelity PCR protocol for the detection of six viruses that cause potato tuber necrosis. American Journal of Potato Research 92: 536-540.

Crosslin, J.M., G.J. Vandemark, and J.E. Munyaneza. 2006. Development of a real-time, quantitative PCR for detection of the Columbia Basin potato purple top phytoplasma in plants and beet leafhoppers. Plant Disease 90: 663-667.

Crosslin, J., and L. Hamlin. 2011. Standardized RT-PCR conditions for detection and identification of eleven viruses of potato and potato spindle tuber viroid. American Journal of Potato Research 88: 333-338.

Secor G.A., V.V. Rivera, J.A. Abad, I.-M. Lee, G.R.G. Clover, L.W. Liefting, X. Li, and S.H. De Boer. 2009. Association of '*Candidatus* Liberibacter solanacearum' with zebra chip disease of potato established by graft and psyllid transmission, electron microscopy, and PCR. Plant Disease 93: 574-583.

Toju, H., A.S. Tanabe, S. Yamamoto, and H. Sato. 2012. High-coverage ITS primers for the DNA-based identification of Ascomycetes and Basidiomycetes in environmental samples. PLoS one 7: e40863.