Potato Progress

Research and Extension for Washington's Potato Industry
Published by Washington State Potato Commission **www.potatoes.com**
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Volume III, Number 3 *April 7, 2003*

Evidence That The Beet Leafhopper-Transmitted Virescence Agent Caused the 2002 Epidemic of Potato Yellows Disease in The Columbia Basin

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Introduction

A serious epidemic of a yellows type disease of potato occurred in many fields throughout the Columbia Basin in 2002. Early symptoms resembled those caused by current season potato leafroll virus infection. In later stages, plants developed yellow and purple discoloration, leathery leaves, thickened nodes, short internodes, aerial tubers, and a general bushy, stunted appearance. The cause of the disease could not be established with any degree of certainty, as described recently by Hamm et. al.(Potato Progress, Feb. 10, 2003). Although the symptoms resembled those caused by a phytoplasma, three different laboratories failed to detect either the aster yellows phytoplasma or the beet leafhopper-transmitted virescence agent (BLTVA) in symptomatic tissues. Later, two additional laboratories detected the BLTVA in some plants. The symptoms also resembled psyllid yellows, a condition caused by a toxin released into plants by feeding of potato psyllid nymphs (Potato Progress, Aug. 29, 2002).

Over the past 15 years, psyllid yellows has routinely appeared in late-planted experimental potato plots in the Yakima Valley. The symptoms generally did not develop in early-planted plots since the plants were already in senescence before psyllid yellows began to develop in vigorously growing late season potatoes. Psyllid yellows symptoms did not appear in plots protected by insecticide. The presence of psyllids was associated with the yellows symptoms we observed. In contrast to the late season development of psyllid yellows in our plots, the yellows disease symptoms of the 2002 epidemic began to appear in late June and July. Perhaps psyllid yellows appears only late in the fall in the Columbia Basin because of the long journey required from psyllid overwintering grounds along the Mexican border.

A second key distinction between psyllid yellows and symptoms observed in the 2002 yellows epidemic is that psyllid yellows affected all plants in our plots. The psyllids apparently did not selectively infest some plants and exclude neighboring plants. In contrast, plants in yellows-diseased fields in 2002 either expressed distinct symptoms or expressed no symptoms. This is to be expected with a transmissible disease. Individual plants are either infected and express symptoms or they are not infected and do not express symptoms.

The beet leafhopper (*Circulifer tenellus*) is the only known vector of BLTVA (although further study may find other species of leafhoppers are also vectors). This, together with the fact that 2002 also saw an epidemic of beet leafhopper-transmitted beet curly top virus (BCTV) in other crops, suggests that beet leafhopper may have been important in the potato yellows epidemic. The BCTV epidemic could not have occurred without an abundant supply beet leafhopper. We previously confirmed BLTVA infection in potatoes during years when BCTV was prevalent. The disease was prevalent only in experimental plots that had received no insecticide treatments. It was observed occasionally in commercial fields. The symptoms observed in the 2002 epidemic were the same as those we had associated with BLTVA in the past. For these reasons, we have thought from the beginning that the yellows disease was probably caused by BLTVA.

We have conducted studies during the past fall and winter that implicate BLTVA as an important, if not the only, factor involved in the 2002 potato yellows disease epidemic. These experiments are described below, with further discussion.

Experimental Methods

A number of fields with varying incidence rates of the potato yellows disease (PYD) were visually examined in the Paterson region of Washington and in the Boardman region of Oregon. These fields had received various insecticidal treatments prior to the appearance of the disease. The disease was also observed in experimental field plots at the Irrigated Agriculture Research and Extension Center (IAREC), Prosser, Washington. These plots received no insecticidal treatments either prior to or after appearance of the disease. All plants in these plots expressed the disease.

To conduct studies on the cause of the disease, we harvested tubers and foliage from 20 symptomatic and 20 asymptomatic Ranger plants in an Oregon circle where infection incidence was about 80%. Tubers were tested for their ability to sprout without a dormant period in greenhouse soil pots. Greenhouse cuttings were started from foliage of each plant, and greenhouse plants were generated from tubers of each plant after dormancy was broken in cold storage. Foliage from cuttings started in the greenhouse, and foliage produced on plants generated from tubers were analyzed by ELISA and PCR for infection with major potato viruses (PVA, PVS, PVM, PVX, PVY-N strain, PVY-O strain, PLRV), and the beet curly top virus (BCTV). Each of these foliage sources were also extracted and subjected to broad spectrum PCR analysis for phytoplasmas and to specific analyses for BLTVA. In addition, foliage of four symptomatic plants was mailed to Bruce Kirkpatrick, UC Davis, for PCR analysis. The cuttings from diseased plants did not survive well in the greenhouse, but 17 of the original 20 produced sufficient new growth for virus and phytoplasma analysis.

A second study was conducted with tubers harvested from the experimental plots at IAREC. A plant was generated in the greenhouse from a tuber harvested from each plant in the plots. All plants were examined for symptoms and tested for potato virus infection as described above. Selected plants were analyzed for BLTVA.

Tests to confirm our PCR results by Bruce Kirkpatrick are underway. Transmission studies, using both grafting and beet leafhoppers to transmit BLTVA from plants that tested positive by PCR, are also underway to further confirm a BLTVA etiology of the yellows disease.

Results and Discussion

Among the four plants sent to Bruce Kirpatrick for analysis, none tested positive using a standard test designed to detect a broad spectrum of phytoplasmas, but one reacted positively in a PCR test specific for BLTVA. Similarly, in our laboratory, none of the DNA extracts from the 17 surviving symptomatic plants or 20 asymptomatic plants tested positive for any phytoplasma in standard, broad spectrum PCR analyses. None reacted positively in standard, BLTVA specific PCR analyses, not even the one that had produced a positive BLTVA test in Bruce Kirkpatrick's laboratory. However, 12 of the 17 extracts produced distinctly positive tests for BLTVA using the more sensitive, nested PCR procedure specific for BLTVA. By the time these tests were completed, only three of the five plants that remained negative for BLTVA by the nested PCR procedure were still alive in the greenhouse. These plants were extracted a second time and tested again. All three were positive for BLTVA in the second nested PCR trial. Thus, BLTVA was detected in a total of 15 of the 17 cuttings started from diseased plants. It could have been present in the remaining two plants that were no longer available for a second analysis. Extracts from asymptomatic plants from the same field all tested negative for BLTVA.

These results provide strong evidence that BLTVA was at least one, if not the only, cause of the potato yellows disease epidemic. We do need to confirm that our plants are infected with BLTVA by testing for transmission with beet leafhoppers and grafting to diagnostic hosts.

The success of the more sensitive nested PCR procedure over the failure of standard PCR procedures to detect BLTVA in potato could reflect low phytoplasma concentrations in infected plants. Alternatively, it is known that inhibitors of enzymes that control the PCR process are sometimes present in extracts from some plant species under certain conditions. The dilution of the extracts inherent in the nested PCR procedure might decrease the inhibitory effect of such enzymes sufficiently to achieve more reliable detection of phytoplasmas. The fact that a second DNA extraction was required to detect BLTVA in three of the plants suggests that phytoplasma concentration in plants or enzyme inhibitors in plants may vary according to environmental factors. Our success in detecting BLTVA in diseased plants could reflect the fact that we used foliage from greenhouse cuttings rather than field-produced foliage for DNA extraction.

Three of the symptomatic plants tested positive for PLRV and one was positive for PVY^N. Among asymptomatic plants, two were positive for PLRV and two were positive for PVY^N. None of the other potato viruses (A,M,S,X) nor BCTV were detected in any plants. The inconsistency in virus infection among the plants indicates that these were not associated with the yellows disease.

Greenhouse plants derived from field-harvested seed pieces of 20 symptomatic and 20 asymptomatic plants from the Oregon circle were all free of yellows symptoms and all were PCR negative for BLTVA. About 10 % of the plants generated from tubers from the experimental field plots at IAREC tested positive for BLTVA. Some of the tuber-generated plants from the field plots did not survive long enough in the greenhouse to undergo analysis. The reason for this lack of survival is not known, but BLTVA might have been a factor. These results indicate that BLTVA is inconsistently tuber-borne.

Some have speculated that a second phytoplasma, in addition to BLTVA, was involved in the yellows epidemic of 2002. If so, it is likely that the second phytoplasma is also transmitted by the beet leafhopper or else it coincidentally appeared in epidemic proportion in the same year as BLTVA appeared. In addition, it would have to be a phytoplasma that is not readily detected by broad spectrum PCR analysis nor readily tuber borne. It seems unlikely that any other phytoplasma would meet all of these criteria.

Tubers from psyllid yellows affected plants normally sprout without a dormant period. The fact that tubers did not sprout without a dormant period is further evidence that the disease was not caused by psyllids.

If we are correct that the yellows disease of 2002 was caused by BLTVA, then we should expect intensity of the disease to vary from year to year with similar intensity to BCTV. This is true because for both pathogens the beet leafhopper is the only known vector. We know that intensity of BCTV infection varies according to the numbers of beet leafhoppers that survive winter. We observe at least a low incidence of BCTV disease each year, and intense epidemics occur sporadically.

So, why haven't we seen the potato yellows epidemic in past years of intense BCTV infection? Recent years have seen some remarkable changes in insecticides and their use to control PLRV. The pesticides we use today may not be as effective in controlling the beet leafhopper as those used in the past. Also, last year may have been the first year of intense beet leafhopper exposure since new pesticides have been adopted. For example, we observed side-by-side circles, one with about 5% yellows infection, the other with more than 50% infection. Both had been treated with imidacloprid, the 5% circle with a foliar application in early July, the other with an in-furrow application at planting. In addition, the circle with only 5% infection received two Vydate treatments not received by the other, on May 28 and June 14.

With funding from the potato commission, a working group of researchers has begun research on this problem.

Washington Potato Seed Lot Trial

In the North Basin, two seed "drop-offs" have been established. One is at the Bob Holloway storage (east end of the eastern-most storage) just north of Road 3 NW and east of Dodson Road. The second is at CW Potato Services, south of I-90 about six miles east of Moses Lake (just east of the Moses Lake Simplot Soilbuilders). Samples need to be at these locations by 2:00 pm the day before each planting date to be included.

The remaining planting dates for 2003 are:

2nd	**April 10**
3rd	**April 24**
4th (Late)	**May 8**

The 2003 WSU Potato Field Day is scheduled for Friday, June 27. This project is sponsored by the Washington State Potato Commission, the Washington State Potato Foundation, and Washington State University.