Progress in epidemiological research on plum pox virus

Most of 2012, both winter and summer, was spent in surveying commercial orchards for plum pox virus (PPV) infected peach trees for use in our studies. Permission was obtained from growers, and 18 orchard blocks with a previous history of PPV were extensively surveyed (11,740 trees). Of these, 11 trees at one site and 6 at a second site were found to be infected and were used in our study. Healthy and infected trees at each site are being monitored for the impact of PPV on growth, tree viability, yield, and fruit quality over the next four years. The distribution of PPV within the study trees is also being mapped each year to determine how virus spreads within the tree. This information is particularly useful when sampling orchard trees for virus detection. Preliminary studies suggest that when the virus is introduced by aphids feeding on leaves, the virus first travels into the rootstock and then in subsequent years upwards into the tree canopy. One question that remains unanswered is how long it takes from the time of aphid infection of the tree to when infection can be reliably diagnosed. Additionally, each orchard will be surveyed each year to give us a better understanding of the distances that aphids typically can spread this disease.

The impact of early infection of newly planted trees by PPV is largely unknown, and could significantly affect future production and viability of orchards. While other tree fruit viruses have been shown to stunt and kill young trees, little is known about the impact of the local Dideron strain of PPV on trees that are infected early in their development. A screen house was modified and certified by the Canadian Food Inspection Agency (CFIA) for PPV use, and 75 two year old trees of 5 peach varieties were established. Ten trees of each variety were inoculated with PPV in September 2012. Trees will be monitored for the impact of PPV on growth, yield, and fruit quality over the next four years.

To investigate the effects of tree vigour on the susceptibility of peach to PPV infection, trees in replicated plots were provided with normal amounts, twice normal amounts or no nitrogen fertilizer for three years. The detached leaf method developed at AAFC was then used to inoculate detached leaves collected at regular intervals throughout the season. Results helped verify that levels of resistance increased in summer, but there were no statistical differences between treatments. Related studies utilizing the detached leaf technique to determine possible differences in the onset and degree of seasonal resistance are being conducted for canning and fresh peaches and early versus late maturing varieties. These studies will be completed by this fall. Future research includes investigations of the effects of plant growth regulators and micronutrients on levels of seasonal resistance.

Insecticides are generally recognized as being ineffective for the management of aphid-borne non-persistent plant viruses. However, a number of recently registered aphicides that act rapidly or disrupt aphid feeding are being investigated in the laboratory for their ability to interfere with virus transmission. A model system using the green peach aphid to transmit turnip mosaic virus is being developed that will allow for the rapid evaluation of these materials.

Foliar oil sprays have been shown to inhibit the transmission of PPV and other non-persistent plant viruses and to also help control mites and soft bodied insects such as

aphids . In agreement with industry findings (PetroCanada), we were unable to show any detrimental effects on tree growth or fruitfulness following repeated applications of oil up to the end of June. We are currently looking at possible effects of oil on fruit maturity or quality following sprays of oil applied during fruit development. Studies were also completed to investigate any potential inhibitory effects of horticultural oil residues on leaves on the detection of PPV by serological or molecular based assays, an important consideration in field detection surveys. Our results clearly demonstrated that foliar oil residue did not impede virus detection. As a complement to earlier laboratory studies on the effectiveness of oil sprays for the inhibition of aphid transmission of PPV, a time-course study was conducted in the laboratory to determine the persistence of the oil applied to peach leaves. The persistence of oil under field conditions is planned for the summer of 2014.

In 2012 the black peach aphid, (*Brachycaudus persicae*) was found during the winter on peach trees in NOTL and surveys were made to determine its prevalence. As was shown elsewhere, this aphid is an effective vector of PPV. The degree to which this species contributes to the natural spread of PPV will be determined by population levels and periods of flight activity. An ongoing aphid monitoring program should help provide information on the biology of this species in southern Ontario.

Building on earlier studies involving artificial inoculation of potted trees or detached peach leaves using the green peach aphid as the virus vector, our research has shown that trees become more resistant to natural infection by aphids as the growing season progresses. Trees appear to be most susceptible in the spring and early summer which also coincides with peak numbers of aphids. A possible mechanism for increasing resistance in summer might be RNA silencing, which is a natural plant antiviral resistance mechanism. In herbaceous hosts, RNA silencing is known to be more active when plants are grown at higher temperatures. However, this has not been tested in woody plants. Samples of healthy and infected leaves, collected over the spring, summer and fall, are being examined to identify any RNA silencing mechanisms that may be induced over the warmer months of the summer. Understanding how such silencing might operate to make trees more field resistant to infection might become an important tool in breeding programs. Analysis of these genetic markers might also help determine when trees are no longer susceptible to infection and actions to prevent the spread of virus, such as the use of oil sprays, are no longer required. Peach trees are being tested for the expression of key peach silencing genes (such as Argonaute genes) during the course of the season. The real time reverse transcriptase polymerase chain reaction assay (Q-RT-PCR) has been optimized and suitable peach reference genes have been identified that show stable expression during the course of the season and in PPV-infected or healthy trees. Preliminary results suggest that the expression of Argonaute 1 is not significantly altered during the course of the growing season. These results will be validated by analysis of a new set of samples that will be collected during the 2013 growing season. The expression of additional silencing genes (e.g., other Argonautes, Dicers and RNA-dependent RNA polymerases) will also be tested using samples collected in 2012 and 2013.

http://www.omafra.gov.on.ca/english/crops/hort/news/tenderfr/tf1705a3.htm

Fig 1 PPV foliar symptoms, fruit symptoms, and symptoms of blossoms of symptomatic varieties

Fig 2 Leaf samples collected from orchards are macerated in buffer and dispensed into multiwell plates for testing.

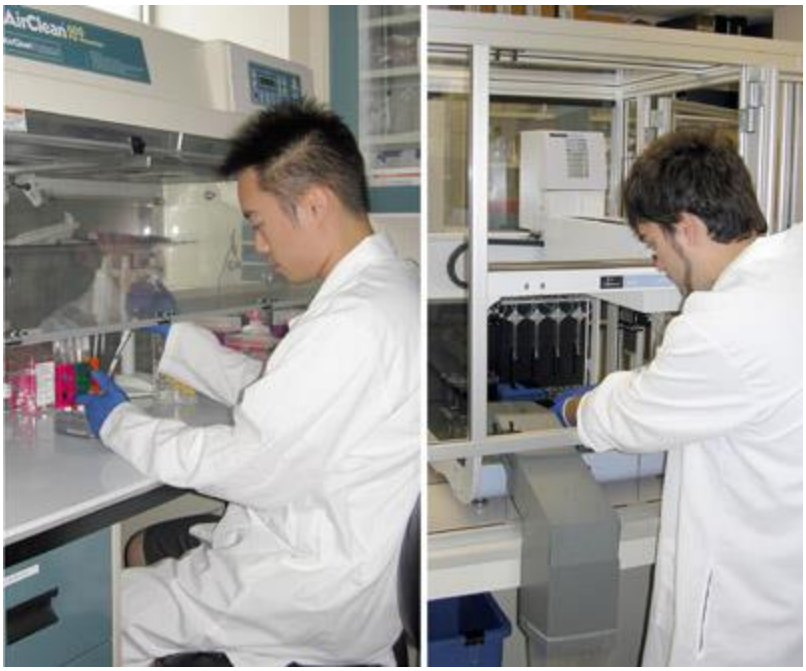

Fig 3 Left: RNA is isolated from the plant macerates, Right: Setting up the liquid handling robot for adding reagents to the extracted plant RNA samples and dispensing into assay plates for analysis.

For more information:

Toll Free: 1-877-424-1300 FREE
E-mail: **ag.info.omafra@ontario.ca**

Author: Dr. Lorne Stobbs, Dr. David Hunter, Dr. Rana Samara, Agriculture and AgriFood Canada, Vineland, and Dr.Tom Lowery and Dr.Hélène Sanfaçon, Agriculture and AgriFood Canada, B.C. Technical assistance by Neva Greig, Patricia Vickers, Lori Bittner, and Cheryl Collucci (Vineland) and Rajita Karran (Summerland)

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