NE1006:

ERADICATION, CONTAINMENT AND/OR MANAGEMENT OF PLUM POX DISEASE (SHARKA)

Abstracts from the
Annual Meeting of NE1006
November 17-18, 2003
US Fish and Wildlife
National Conservation Training Center
Shepherdstown WV
http://www.lgu.umd.edu/project/home.cfm?trackID=33

NE1006 Plum Pox Virus Annual Meeting

US Fish and Wildlife National Conservation and Training Center
Shepherdstown, West Virginia
November 17-19, 2003

Welcome – Dariusz Swietlik, Director, USDA-ARS Appalachian Fruit Research Station, Kearneysville WV

Session 1: Determining the distribution and incidence of plum pox virus in North America. (Ruth Welliver, moderator)

Blake Ferguson – *Canadian survey summary*
Don Albright – *United States survey summary*
Nancy Richwine – *Pennsylvania survey summary*
John Halbrendt – *Non-Prunus surveillance and survey in Pennsylvania*

Session 2: Filling gaps in knowledge about PPV survival and spread through basic and applied research, Part 1: Hosts, Strain Characterization, and Detection. (John Halbrendt, moderator)

Dan Thompson – *An update on PPV host range studies in Canada*
Vern Damsteegt – *Potential Prunus host range of PPV-PENN isolates by aphid transmission*
Kara Maraden – *Ultrastructure and immunolabelling of PPV in herbaceous and woody hosts*
D'Ann Rochon – *Sequence studies of Canadian PPV isolates and development of a polyclonal antibody for detection of PPV*
Bill Schneider – *Molecular analysis of plum pox populations in Pennsylvania*
Chris Wallis – *Impacts of host shifting and mode of transmissions on PPV microevolution*

Session 3: Survey and Detection: Perspective from States, Provinces, and Independent Testing Labs. (Ruth Welliver, moderator)

Mike Tiffany – *Summary of activities performed during the 2003 USDA-APHIS National Plum Pox Virus Survey*
Simon Scott – *Work in South Carolina*
Panel Reports: Kathy Kosta, CA; Jennifer Dominiak, MD; Richard Kaitany, MI; Grace O'Keefe, VA

Session 4: Filling gaps in knowledge about PPV survival and spread through basic and applied research, Part 2. Epidemiology, Survival, and Spread. (Fred Gildow, moderator)

Fred Gildow – *Risk of PPV transmission by peach fruit*
Thomas Lowery – *Host range and transmission of the Canadian isolate of PPV*
Deena Erampalli – *Detection and distribution of plum pox virus in peach*
Tim Gottwald – *Update on PPV epidemiology*
Greg Krawczyk – *Survey of potential aphid vectors of PPV in Pennsylvania stone fruit orchards and ornamental Prunus trees*

Guest Speaker "Overview of Plum Pox Virus in Europe" Dr. Michel Ravelonandro

Session 5: Developing PPV management strategies: transgenic resistance, pest exclusion. (Ralph Scorza, moderator)

Ralph Scorza – *Stability of gene silencing-based PPV resistance*
Hélène Sanfaçon – *Progress towards engineering resistance to the Canadian isolate of plum pox virus in model plants*
Pamela Hughes-Watson – *Evaluation of methylation of the plum pox virus coat protein transgene in mature field-grown plum pox virus resistant plum trees*
Charles Divan – *PPV as a select agent in the US*
Bill Howell – *How secure is our defense infrastructure?*

Session 6: Developing traditional and innovative delivery systems for information transfer to stone fruit researchers and extension personnel, fruit growers, and fruit industry representatives on current knowledge of plum pox virus. (Mike Celetti, moderator)

Mike Celetti – *Extension programs on PPV in Ontario*
Wayne Roberts – *Prunus certification in Ontario - Getting grower buy-in*
Ruth Welliver – *Stone fruit certification in the US: Excellence in patches*

Summary of the 2004 Canadian PPV Survey

Blake Ferguson
Horticulture, Grains and Field Crops Specialist
Ontario Program Network
Canadian Food Inspection Agency
1200 Commissioners Road East, Unit19
London, ON N5Z4R3
Government of Canada
www.inspection.gc.ca
(519) 691-1306 ext. 142
fergusonb@inspection.gc.ca
Facsimile (519) 691-1314

The 2003 Canadian Plum Pox survey consisted of intensive sampling (individual trees at the sampling rate of 12 leaves per tree) in all orchards in the isolated quarantine areas, in blocks of mother trees or budwood source trees and in Niagara quarantine area blocks which had a at least one historical positive find. Hierarchical survey methods were used in all non-quarantined areas of Nova Scotia and Ontario as well as in previously negative orchards in the Niagara area.

As a result of this intensive sampling, over 408,000 samples were collected and tested during the 2003 Canadian survey. This represents over 40% of all the samples taken in Canada since 2000. The number of orchard blocks with infection levels over 6.5% has continued to decrease due to the forced removal of blocks above this disease threshold. However, due to the more intensive sampling method used this year, as well as a streamlined approach to confirmation of suspect samples, the number of blocks infected at less than the 1% level of disease incidence rose and some new infected blocks were found.

The current PPV action plan ends on March 31, 2004. In the coming months the survey results will be analyzed. Options for dealing with the disease in future years will be presented to decision makers as soon as possible in order that planning for next year's survey can begin. These options will take into account the recommendations from our National PPV Task Force and from a panel of foreign experts contracted by the CFIA to provide advice and direction.

2003 Plum Pox Virus (PPV) Survey Report for the United States

Don I. Albright, USDA-APHIS-PPQ, PPV Program
Nancy S. H. Richwine, Pennsylvania Department of Agriculture

National PPV Survey Notes:
The National PPV Survey for 2003 was setup to maintain surveillance in those states with the highest risk based upon proximity to infected areas in the US and Canada, historical nursery trade practices, and the volume of untested host materials. There were six states, other than Pennsylvania that participated in the survey – Maryland, New Jersey, New York, Delaware, Michigan, and California. 157,005 laboratory leaf samples were processed from these states and found negative for PPV. The breakdown by state is:

State	# lab samples processed	Results
California	23,750	Negative
Delaware	1956	Negative
Maryland	24,342	Negative
Michigan	29,132	Negative
New Jersey	17,966	Negative
New York	13,957	Negative
Other	45,902 leaf 16,349 fruit	Negative Negative

Additional testing was conducted in 4 states as a result of aphid transmission study findings by scientists at Ft Detrick, MD, in 2002. During the study, peach fruit was obtained from local markets in Frederick, MD, to be used as negative controls. Prior to using the fruit, ELISA tests were performed to establish that the fruit was indeed negative for PPV. One fruit showed an elevated background in ELISA. Follow-up testing by PCR produced positive results. The remainder of the fruit was tested using PCR and 4 other samples tested positive. Additional fruit was obtained from local stores as a follow-up to the initial testing with an emphasis on maintaining the fruit's origin identity by noting the markings on the product code stickers that were placed on the fruit during packing. These stickers included information with the product code that identified the origin of the fruit to State, packer, and brand. Further testing revealed that fruit from 4 of 5 states tested positive for PPV by either ELISA or PCR, or both. Extensive testing was done to rule out contamination as the source of positive test results. Since the lab was running out of original material, PCR product was cloned and sequenced to see if this material was distinct from in house isolates.

When the USDA ARS scientists were confident that they had indeed found infected fruit from non-regulated states, officials at USDA APHIS PPQ were informed of their findings at meeting held at Ft Detrick the end of January, 2003. Since the findings were not able to be independently confirmed by APHIS, no regulatory action could be taken. The information was turned over to APHIS Investigative and Enforcement Services, IES, to do trace backs through the distribution chain for the fruit from the markets to the producer. Once completed, the grower or source information was given to the regulatory officials in each state identified by the research and follow-up investigation. Discussion and planning sessions were held and a survey was designed to try to replicate Ft Detrick's findings. Survey and laboratory PCR protocols were developed for fruit and additional funding was supplied to the affected states. The results are as follows:

State	# Growers	Area	# Leaf Samples	# Fruit Samples	Results
MD	1	3000 trees	4,484	616	negative
VA	1	6 blocks	1,470	3644	negative

SC	1	49 acres	1008	2982	negative
NJ	1 Coop (15 growers)	1500 acres +	38,940	9,107	negative

All ELISA and PCR results were negative to date. Some follow-up/confirmation tests are being completed on apparently contaminated PCR samples. All of the retests have remained negative so far.

PPV Survey in 2004 is again being planned in each of the states that participated in either of this year's survey. Additionally, a small amount of detection survey work remains to be completed on the West Virginia panhandle area to complete 3 years of detection survey for the state. All of this work is contingent upon adequate funding which has still not occurred.

Pennsylvania 2003 PPV Survey notes:
This year's survey in Pennsylvania narrowed the focus and intensified the sampling scheme. Orchard survey was completed in 11 counties using the following sampling protocol in relation to where the orchards were located:

- Q-area – 100% of the trees were sampled at 8 leaves per tree, 1 tree per sample
- Q-5 mi – 100% of the trees were sampled at 4 leaves per tree, 2 trees per sample
- Balance – 25% of the trees were sampled at 4 leaves per tree, 2 trees per sample

This survey encompassed 3700 acres in 876 orchard blocks that produced 164,201 laboratory samples. Processing of these samples detected 3 positive trees in 2 orchard blocks that covered 17 acres, the 500 meter exposed zone for these 2 blocks encompassed 6 additional blocks totaling 18 acres, giving a total of 35 commercial orchard acres removed in 2003. This brings the total acreage destroyed due to Plum Pox Virus in the United States to 1440 acres. The location of 1 of the 2 positive blocks was just (100 meters) outside of the quarantine. This resulted in a small expansion of the regulated area in Menallen Township, Adams County, which was already partially regulated.

Another key element of the PPV eradication project is the homeowner survey. This year plans were made to visit 50,000 properties in a 300 square mile area collecting samples from any Prunus plant within that area. These plants were sampled at 8 leaves per tree, 1 lab sample per plant. GPS readings were taken for each property with Prunus and property information along with sample collection data was associated with each GPS site. The data was entered into handheld computers and downloaded into a database. The Pennsylvania Department of Agriculture and PPQ hired a total of 25 technicians to complete this work. These workers managed to visit a total of 42,524 properties of which 14,102 had Prunus plants. A total of 36,530 samples were collected for testing of which 8 samples tested positive for PPV on 6 newly infected properties. All of these sites were within the current regulated areas. These six positive property sites resulted in the removal of Prunus from an additional 56 properties within 500 meters of their locations.

Another activity that has direct bearing on the PPV project is the Pennsylvania State Nursery Certification Program. This involves the testing of certified budwood source trees, unregistered budwood source trees, and nursery row stock for several pathogens which includes PPV. This year PDA tested 616 certified budwood source trees, 2473 unregistered budwood source trees, and 3399 nursery row trees. Since every project needs one surprise per year, this year was no exception. All 6488 samples tested negative for PPV using ELISA except for 1 sample that had a slightly higher background but not high enough to be considered positive. Since there was some question, PDA ran the sample through RT-PCR testing and got a positive. Through a series of more intense sampling schemes, PDA was able to identify 2 positive Santa Rosa plum trees that were part of the original suspect ELISA test sample. Further intensive field testing located another 4 plum trees and 2 Sunhigh peach trees as positive. All of the trees were 2 years old and grafted onto Pumiselect rootstock from Oregon.

There was only 1 location at 1 nursery in Adams County that was found infected and it was 5 miles outside of the quarantine area and 6 miles from the closest PPV positive site. The source of the infection was investigated by having the rootstock mother trees in Oregon and the budwood source trees resampled with both sources testing negative. That would leave aphid transmission of the virus as the most likely source with the mode of movement being wind, hitchhiking, infected fruit from a cull pile, or an unknown reservoir. This positive detection resulted in the establishment of a new quarantine area in Southwestern Butler Township, Adams County.

Shipping records from the nursery for the past 5 years are currently being entered into a database to facilitate trace forwards and survey planning as a follow-up. There is little chance of the virus having been spread since the trees in the infected nursery block would not have been moved until the fall of 2003 but other trees have been moved from the general area previous years and we want to be sure that there was no infected stock moved.

In summary, great progress has been made in eliminating this virus from the US and in particular Pennsylvania. This has been accomplished by steadily increasing the sampling volume and intensity, and by promptly removing any infected material and exposed plant hosts within 500 meters. Research on PPV in the US, Canada, and Europe has been closely monitored and findings adapted to survey protocols and project activities. All activities are based upon recommendations made by the Scientific Issues Group. In order to confirm that the virus has been removed from the orchard environs, a sentinel tree program has been established along with wild species sampling to affirm that there are no wild alternate hosts that the virus is established in, and a bait plant project is being run by Penn State University in cooperation with PDA to quickly detect virus movement by aphids in the core area. The following table summarizes sampling activities for the past 4 years in the 4 county area where PPV has been detected.

Lab Samples from the 4-County Area

Year	Orchard Samples	Homeowner Samples	Other Samples	Total Samples	Total Positives	% Pos.
2000	51,429	547	586	52,562	399	0.776
2001	80,012	5,556	1,326	86,894	27	0.034
2002	90,388	15,748	1,913	108,049	7	0.008
2003	164,201	36,530	6,845	207,576	11	0.005

Plum Pox Virus (PPV) Weed Survey and Surveillance Projects in Pennsylvania

John M. Halbrendt
The Pennsylvania State University
Fruit Research and Extension Center
Ph. (717) 677-6116 ext.#3; email jmh23@psu.edu

2003 Plum Pox Virus Weed Survey:
The host range for PPV is known to include many herbaceous plants including a number of common weeds that may serve as virus reservoirs. There are also many plants including some native tree species that have never been tested for susceptibility to PPV. Several European studies have supported the idea that weeds play an important role in the perpetuation of the disease. This project will determine if common weeds or native trees have become infected with PPV.

The weed collecting sites were located on the boarders of orchards that were known to have high levels of plum pox virus based on the results of the Pennsylvania commercial orchard survey. The sites were situated in the transition zone between orchard and woodland and thus contained diverse vegetation including herbaceous plants, brambles and deciduous trees.

The weeds were collected two to three times per week throughout the summer. Leaf samples were stored on ice in coolers in the field and transported to the Fruit Research and Extension Center in Biglerville, PA for testing by ELISA. Efforts were made to test a representative number of each plant but specific numbers of samples for each species were not assigned. The number of samples of each species varied according to the relative abundance of each species in the field. Therefore some relatively rare plants were collected only a few times while hundreds of the most common weeds were tested.

Results:
A total of 10,4212 samples were collected and tested by ELISA. None were positive for PPV. The weeds and native tree samples included 66 species from 34 plant families. Some weeds initially gave spurious positive results due to nonspecific binding of the antibody. All suspicious samples were compared with similar samples that were collected from locations about ten miles outside the quarantine zone to check if the result was typical for that species. All questionable samples were tested further until a definitive result was obtained either by additional ELISA tests or if necessary sent to the APHIS lab in Beltsville for confirmation by PCR.

Impact:
Short Range: This project will determine if PPV has become established in naturally occurring weed populations and identify the weeds that may serve as a reservoir of the virus.

Long Range: The success of the eradication program can only be determined by follow-up testing to verify that the virus does not reappear. Knowledge of natural weed hosts can aid in monitoring for the reappearance of PPV.

Plum Pox Virus (PPV) Surveillance Project:
The possibility of detecting reservoirs of PPV within the quarantine area may be improved by utilizing bait plants. The ideal bait plant would be both susceptible to PPV and attractive to its aphid vector. Bait plants used in this work included garden peas, elegant zinnia, cape marigold and red clover. By positioning bait plants at strategic locations within the quarantine there is increased opportunity that they will visited by aphids and become infected with PPV.

The bait plant locations have a previous history of PPV and are adjacent to undisturbed woodlands that may contain unknown reservoirs of PPV. Seedling bait plants were rotated in the field every two weeks throughout

the growing season. After an additional two weeks incubation in the greenhouse the plants were assed by ELISA.

Results:
A total of 5,508 bait plants were tested in 2003 and all were negative for PPV.

Impact:
Negative data is evidence of successful PPV eradication. Projects such as this help to provide the evidence needed to support the PPV eradication program in Pennsylvania.

Woody Host Range of Canadian PPV-D

Dan Thompson, CFIA

Studies were initiated in 2001 to determine the woody host range for the Canadian PPV-D isolate. Thirty-seven (37) Prunus species and seven (7) non-Prunus species were tested. Plants were bud-inoculated each spring and summer and then tested by ELISA and RT-PCR every two weeks during the growing season. To date, twenty-two (22) Prunus species have been confirmed as hosts. All except P. mexicana have previously been reported as PPV hosts. PPV was not found in any of the non-Prunus species (Euonymus, Juglans, Ligustrum, Rubus, Ribes, Morus).

2003 PPV Titre Results

In 2003, nine peach trees were sampled weekly from June 25 to October 8. Six (6) samples of twelve (12) leaves each were tested by ELISA using the Durviz antiserum. Overall, the average of the 6 samples tested Negative 28% of the time, Suspicious 25% of the time, and Positive 47% of the time. The period between late July and late September produced the lowest ELISA values. Using the highest ELISA value rather than the average, the samples tested Negative 8% of the time, Suspicious 17% of the time and Positive 75% of the time.

Potential Prunus Host Range of PPV-Penn Isolates by Aphid Transmission

Vern Damsteegt, Andrew Stone, Fred Gildow, William Schneider, and Douglas Luster

---

Since the confirmation of plum pox (Sharka) in Pennsylvania in 1999, there has been a concerted effort to eradicate the virus from the U.S.A. The causal virus is transmitted to a broad range of woody and herbaceous host species by at least 20 aphid species. The USDA-ARS Foreign Disease-Weed Science Research Unit in Frederick has been involved in determining the potential host range of the PA isolates mediated through aphid transmission. The virus isolates were obtained from different peach sources in PA and maintained in GF 305 and Lovell peaches in the BSL3-P containment facility at Fort Detrick. All transmission studies utilized *Myzus persicae* (green peach aphid) as vector and all woody species were started from seed. Seedlings of more than 20 *Prunus* species were inoculated with PPV, observed for symptoms, assayed by ELISA (Durviz kit) and RT-PCR, back-assayed to Lovell peach by healthy *M. persicae*, vernalized for 8 weeks at 4^0 C, regrowth observed for symptoms (systemic infection), and reassayed by ELISA. All commercial *Prunus* species except *P. cerasus* (sour cherry) were systemically infected in addition to several wild *Prunus* species. To date, important potential species shown to be susceptible include flowering almond, dwarf flowering almond, some Oriental flowering cherries, black cherry, choke cherry, bird cherry, sand cherry, and others. Research is continuing to compare potential host ranges by aphid transmission with graft-transmitted host ranges.

Ultrastructure and Immunolabeling of Plum Pox Virus in Herbaceous and Woody Hosts

K. Maraden and F.E. Gildow.
Dept. of Plant Pathology, Pennsylvania State University, University Park, PA 16802

Transmission electron microscopy and immunogold labeling (IGL) of PPV coat protein (CP) was used to identify PPV replicating in host cells and to study the cytopathology of infected herbaceous and woody plants. Cytoplasmic inclusions, indicative of PPV replication, were observed in the cytoplasm of epidermal cells and parenchyma cells of PPV-infected leaf tissue in both Colmo pea and GF305 peach inoculated by aphid transmission. No inclusions were observed in the vascular tissue of either host. The inclusions consisted of pinwheels and laminated aggregates characteristic of *Potyvirus* infection. Inclusions were frequently seen to be associated with the plasmalemma adjacent to plasmadesmata. Inclusions were also observed in a cortical cell of a young colmo pea root. No inclusion bodies were observed in healthy controls. Inclusion bodies were not observed in all cells of infected plants and identification of PPV virions was not always possible using only ultrastructural visualization. Therefore, an IGL protocol was developed to identify associations of virions with specific cytoplasmic sites and organelles. A protocol was developed to maintain cellular ultrastructure while retaining optimal antigenicity for labeling virion CP. Pretreatment of L.R. White plastic sections with 9% isobutanol prior to labeling aided with absorption of the polyclonal anti-PPV conjugate (AGDIA 30380 Elkhart, IN) and the10 nm gold conjugated antibody (Aurion 6702 AA Wageningen, The Netherlands). Observations indicated several associations between PPV inclusions, virions, and cell organelles. Virions appeared to “stick” to each other and labeled most strongly at these sites. Virions were frequently found attached to the plasmalemma of cells as individual particles or as strands of particles connected end-to-end. Virions were often associated with endoplasmic reticulum in cytoplasmic dense material. Virions did not appear to be physically associated with the inclusions in any way. Labeling was specific to the coat protein of the virions and did not label inclusion bodies. Immunolocalization will be used for identifying sites of replication and for studies of virus acquisition and transmission.

Impacts of Host Shifting and Mode of Transmissions on PPV Microevolution

C.Wallis[1], F. Gildow[1], and W. Schneider[2]
[1]Dept. of Plant Pathology, Penn State University, Buckhout Laboratory, University Park, PA 16802
E-mail: cwallis@psu.edu
[2]USDA-ARS Foreign Disease-Weed Science Research Unit, 1301 Ditto Ave, Ft. Detrick, MD 21702-5023

Due to an error-prone replication strategy, RNA viruses such as PPV may evolve quickly to adapt to a new hosts. In this study we examined the microevolution of PPV following a host shift from peach (*Prunus persicae*) to pea (*Pisum sativa)* during serial transmissions using either aphid-vectored or mechanical inoculation. Herbaceous host adaptation of PPV was characterized by a reduction in time to symptom development from 30 to only 10 days from serial passage 1 to 10. Transmission efficiency from peach to pea improved from 10% at passage 1 to 80% by serial passage 3 on peas using mechanical inoculation. Inoculation using aphid vectors required 10 serial passages in pea to reach a peak of 50% transmission efficiency, which was evidence of a vector bottleneck that reduced the PPV viral populations' host selection capability. Furthermore, the aphid transmission results suggested that only highly susceptible stone fruit species should be utilized in surveillance plant monitoring programs because of low transmission efficiency (5-10%) to herbaceous hosts in passages 1 to 3. Sequence analyses of specific passages from this study reveal pea host-specific mutations and mode of transmission associated mutations. Pea-adapted strains of PPV at every passage were also tested for their ability to infect the original host, peach. Regardless of the number of previous passages, all pea-adapted PPV strains consistently infected peach at 50% efficiency or greater using aphid inoculation. This indicated that herbaceous-adapted PPV strains remain very capable of infecting peach, which could undermine eradication efforts if an herbaceous PPV reservoir became established.

Agdia Inc. Summary of Activities Performed During the 2003 USDA-APHIS Plum Pox Virus National Survey

Tiffany, M. G.
Agdia Inc.

Agdia sold 112 Durviz PPV DAS ELISA 5,000 testwells kits to nine state, one university, and one federal government laboratories that performed their own PPV ELISA testing. Three states submitted approximately 66,000 leaf samples for PPV ELISA testing and all tested negative for PPV. This testing required 14 Durviz PPV DAS ELISA 5,000 testwells kits. A total of 126 Durviz PPV DAS ELISA 5,000 testwells kits were purchased from Durviz in 2003, 32 more kits than in 2002. The Agdia stick format for the delivery of the Durviz PPV coat protein positive control replaced the lyophilized Durviz PPV control. This format is quite stable and reliably produces a high O.D. value. Agdia tested 810 prunus fruit samples by PPV PCR for the state of Virginia. There were 161 fruit samples that produced a suspect PPV PCR result. The suspect samples were sent to Beltsville for re-testing. Beltsville confirmed all of the suspect PPV PCR results negative for PPV.

The 2004 prices for the Durviz PPV DAS ELISA kits are $700.00 for the 1,000 testwell kit and $3,000.00 for the 5,000 testwell kit.

2003 Plum Pox Virus Survey Activities in South Carolina

Simon W. Scott
Dept of Entomology, Soils, and Plant Sciences
Clemson University
118 Long Hall
Box 340315
Clemson, SC 29634--0315

Phone (864) 656-5745; FAX (864) 656-0274
e-mail sscott@clemson.edu

---

49 acres of orchards identified as the potential source of suspicious fruit originating from South Carolina were surveyed. The orchards consisted of the varieties Redglobe, Contender, Nesstar #2, and PF17. These had originated from three nurseries in Tennessee and one nursery in Michigan (PF17). The trees were planted at densities of 200 or 400 trees per acre. Leaves and fruit were collected using the hierarchical system to identify trees from which the samples were to be collected. 1,008 leaf samples were collected and tested using the Durvis ELISA kit. *Plum pox virus* (PPV) was not detected in any of these samples. 3,856 fruit samples were processed by immunocapture PCR using the protocol supplied by USDA/APHIS. PPV was not detected in any of these samples.

A block of 108 plum trees was located within the last 4-6 rows of the orchard of PF17. Leaf samples were collected from individual trees and tested using the Durvis ELISA kit. PPV was not detected in any of these samples. It was not possible to collect fruit from these plum trees. Budwood was cut from the trees in October and placed in a cold room to meet dormancy requirements. The budsticks will be forced in January 2004 and tested for PPV using both ELISA and PCR. Another planting of 4 - 500 plums approx. ½ a mile from the four survey sites will be assayed for PPV during February 2004 using the same procedure.

2003 California Plum Pox Virus Survey Activities

Kathleen L. Kosta, California Department of Food and Agriculture

---

Statewide survey completed by:

- Kathleen Kosta – Northern District
- Dr. Thomas Watson – Central District
- Magally Luque-Williams – Southern District
- 5 Seasonal Employees (additional help for the Los Angeles Co. survey was provided)

Survey:

- The 2003 Plum Pox Virus survey began on April 1 and was terminated on June 26, 2003. A rainy spring caused a few problems with access to orchards and illnesses plagued the crew members throughout the season, which hindered our ability to achieve our goal of 25,000 samples
- The goal for this year's survey was to continue the sampling of commercial production stonefruit orchards in as many counties as possible throughout California. Stonefruit orchards were sampled in twenty counties. See chart (page 2).
- Trees in the Foundation Block at the University of California were tested for the second time. Additionally we tested the trees located at the Wolfskill Experimental Orchard, also on University property, which are used by plant breeders for their development of new varieties.

Results:

- A total of 1218.95 acres were surveyed; 23,750 samples were collected. (A sample is composed of two trees, resulting in two samples per quadrat.)
- All tests were negative.

Sampling Levels:

- Commercial orchards and orchards used to produce common stock are sampled according to the 25 percent hierarchical survey plan.
- Trees in the University of California Foundation Plant Material Foundation Block, which are the source of budwood or seed used to produce registered nursery stock mother trees, were sampled at the 100 percent level.
- *Prunus* spp. located at the University of California Wolfskill Experimental Orchard in Winters, CA were included in the survey and sampled at the 100 percent level.

COUNTY	VARIETIES	# OF SAMPLES	# OF ACRES	RESULTS
Butte	Peach, Prune	2024	82.25	Negative
Colusa	Almond, Prune	1030	65.20	Negative
Fresno	Peach, Nectarine	1226	54.30	Negative
Glenn	Prune	1412	83.60	Negative
Kings	Nectarine, Peach, Cherry	809	42.30	Negative
Los Angeles	Apricot, Nectarines, Peach, Various Prunus	1215	62.5	Negative
Madera	Peach	1080	61.40	Negative
Merced	Pluot, Peach	770	43.60	Negative
Placer	Peach, Plum	249	7.85	Negative
Sacramento	Various Prunus	143	7.24	Negative
San Benito	Apricot	768	57.81	Negative
San Joaquin	Peach	2183	60.70	Negative
Santa Clara	Apricot, Cherries	1195	81.40	Negative
Shasta	Prune	810	58.40	Negative

Solano	Apricot, Cherry, Peach, Plum, Plumcot, Prune	996	17.50	Negative
Stanislaus	Apricot, Peach, Plum Nectarine	2458	103.00	Negative
Sutter	Peach, Prune	1349	97.90	Negative
Tehama	Almond, Peach, Prune, Various Prunus	1035	90.45	Negative
Tulare	Plum	1579	90.60	Negative
Yolo	Various Prunus, Prune	1419	50.95	Negative

Risk Assessment of Imported Stone Fruits as Sources of Plum Pox Virus

F.E. Gildow[1] and W. Schneider[2]

[1]Dept. of Plant Pathology , Penn State University, University Park, PA 16801
[2]USDA-ARS foreign Disease-weed Science Research Unit, Ft. Detrick, MD 21702

We have identified six indigenous aphid species (of 13 tested) that are capable of transmitting Pennsylvania strains of PPV from infected trees to healthy trees. Recent research has verified the ability of two species of aphids to transmit plum pox virus (PPV) to peach seedlings after first feeding on peach fruit harvested from PPV-infected trees. When given a 3-day probing access period simultaneously on PPV-infected peach fruit and on healthy peach seedlings, the vectors *Myzus persicae* and *Aphis spiraecola* transmitted PPV to 50% and 35% of the healthy seedlings. Under controlled conditions *M. persicae* and *A. spiraecola* fed and survived on harvested peach fruit for 3 and 7 days, respectively. Trapping experiments over the past two years have identified large numbers of A. *spiraecola* landing on peach fruit in trees in orchards and in cull piles of discarded fruit. Results support the hypothesis that infected fruit has the potential to function in long distance spread of PPV. Prior to the discovery of PPV in Pennsylvania in 1999, PPV was detected infecting stone fruits in Chile. To test for the probability of PPV-infected stone fruits being imported into the U.S., assorted stone fruits imported from Chile were collected weekly at the Port of Philadelphia by APHIS personnel during January to March, 2003 and tested by ELISA and PCR for PPV infection. A total of 1400 fruit (apricot, nectarine, peach, and plum) were collected from 13 shipments representing 100-200 fruit per shipment from 34 growers or packers. In 2003, none of 1400 fruit tested by ELISA were positive for PPV, and none of 180 randomly selected fruit were positive by PCR. In addition, aphids failed to transmit PPV from any of 120 randomly selected peach fruit to healthy peach seedlings. At present there is no evidence to support the idea that PPV is readily imported on Chilean stone fruits. Although the risk is probably low, there exists the potential for PPV to spread locally or interstate on peaches harvested from PPV-infected trees of unknown origin. Often infected trees and fruit are symptomless. Disposal of stone fruits in buried landfill is recommended.

Host Range and Transmission of the Canadian Isolate of PPV

D. Thomas Lowery
AAFC-PARC, Box 4200, Hwy 97, Summerland, B.C. V0H 1Z0

Collaborative research conducted over the past year and a half relating to the epidemiology of the Canadian isolate of PPV will be presented. In addition to studies of aphid vector efficiencies and alternate hosts, we are also monitoring aphid flight activity in the Niagara Region and investigating other possible means of virus spread.

Numbers of migrant aphids captured in yellow pan traps and suction traps in Niagara peach orchards were variable between years with respect to trap counts and species composition, but there was little variation among sites. Several of the more common species, such as the spirea aphid, *Aphis spiraecola*, are known to be important vectors of PPV in Europe. Detection of virus from individual aphids by means of PCR has proven to be problematic. With refinement of the technique, we hope to be able to relate detection rates to transmission efficiencies.

To date, none of the common weeds encountered in or around peach orchards have proven susceptible following mechanical inoculations; aphid inoculations are ongoing. *Nicotiana benthamiana* has proven to be the most suitable herbaceous host, and it is being used in parallel with peach in laboratory studies. Five of the six ornamental *Prunus* species tested are susceptible to the most common isolate of the 'D' strain occurring in Ontario. The virus appears to be well adapted to peach, *P. persicae*. Using green peach aphid, *Myzus persicae*, as the vector, infection of peach seedlings from dwarf flowering almond, *P. glandulosa*, or from peach averaged more than 20%. Symptoms were visible on peach seedlings inoculated in the laboratory, and plants tested positive by ELISA after only four to six weeks.

Regional Spatial Pattern Analyses of Plum Pox Virus.

T.R. Gottwald[1] S. Dallot[1], and R. Favin[2]
[1]USDA, ARS, USHRL, Ft. Pierce, FL and [2]CFIA, Guelph, Ontario, Canada

---

Regional data for GPS locations of all and PPV+ orchards were collected for both Adams Co. Pennsylvania and Niagara Peninsula, of Ontario Canada. Preliminary analysis using a modified Ripley's K function for both epidemics indicated that the range of spatial dependency (RSD) for the epidemics ranged from 3 to 35 km. Nearest neighbor distributional analysis was then conducted to examine the demographics of the disease versus total population of orchards in an attempt to determine differences in the regional planting structure. Inverse correlations were found for Pennsylvania at distance ranges from 10. to 6.6 km and for Ontario from 0.7 to 4.3 km, indicating that the distribution of PPV-diseased orchards differed from the total population of orchards within these ranges. This was interpreted as possible evidence that aphid transmission and/or plant movement heavily influenced the regional disease pattern over these ranges, respectively. For Ontario, the distribution of individual cultivars was also examined and five processing peach cultivars were identified that occur in greater than expected frequency near PPV+ orchards, indicating some influence on the epidemic. Survival analysis via a modified Cox model was also conducted for 19 orchards infected with PPV-m in southern France that were subjected to roguing. Roguing maintained these orchards at a low incidence or only allowed a slight increase but did not eradicate the disease. Estimates indicated that more intense roguing of infected and asymptomatic trees within a 12-18 m radius surrounding PPV+ trees would be a more effective approach to eradicating the disease. In addition, risk analysis further indicated that, 1) an increase of 100 m from the edge of a block sharing a boundary with another PPV infected orchard results in 58% less risk of infection, 2) an increase in orchard area of 1 ha reduces the risk of infection by 10%, 3) an increase in planting density by the addition of 500 trees/ha reduces the risk by 43%, and 4) an increase in distance of only 10 m from an infected tree corresponds to 2 to 5 trees in the direction of the rows and 1 or 2 trees in the across row direction reduced the risk of infection by 40.3%.

Survey of Potential Aphid Vectors of Plum Pox Virus (PPV) in Pennsylvania Stone Fruit Orchards and Ornamental Prunus Trees

Grzegorz (Greg) Krawczyk, Ph. D
Penn State University
Department of Entomology
Fruit Research and Extension Center
Biglerville, PA 17307

Project Objectives:

1. Survey possible alternative PPV fruit tree (cherries and plums) and ornamental hosts in southern regions of Pennsylvania (Adams County and surrounding counties) for potential aphid vectors of PPV during the growing season.
2. Identify the aphid species present in various sites and determine their temporal and spatial distribution.

Materials and Methods:

During the 2003 season aphid surveys started in mid-May and continued until the end of October. Seven commercial stone fruit orchards listed in Table 3 were used for collecting vacuum samples of aphid species. Sucking samples of aphids were collected weekly from nine trees from each site. Aphids collected from sampling trees were placed in plastic containers and then in coolers with blue ice. Immobilized specimen was then transferred into glass vials with 70 percent ethanol. To monitor relative abundance of aphids throughout the season, the yellow sticky traps (IPM Tech, Inc, Portland OR) were also placed at each evaluated site. Traps were exchanged weekly and the number of collected aphids and the location was monitored. Additionally, starting from late May seven sites with ornamental stone fruit trees located on non-commercial properties were also identified and vacuum aphid samples were collected from various hosts: purple leaf plum, weeping cherry, ornamental cherry, tulip poplar and peach. All collected aphids were stored in 70% ethanol and an aphid taxonomist conducted the species identification during the winter.

Results:

During the 2003 sampling season 21 aphid species were collected from monitored hosts. The highest species diversity was observed on plum trees (n=9 aphid species), followed by peach trees, cherry and ornamental trees with the identical number of collected species (n=6). Four aphid species were identified for the first time from stone fruit hosts in Adams County: *Aphis coreopsidis* (Thomas), cowpea aphid *Aphis craccivora* Koch, tuliptree aphid *Illinoia liriodendri* (Monell), chrysanthemum aphid *Macrosiphoniella sanborni* (Gillette) and *Nearctaphis crataegifoliae* (Fitch). The quantitative study with the yellow sticky straps revealed that the highest numbers of migratory aphids in commercial orchards were collected during late June and July reaching over a thousand aphids per trap per week. The identification of aphids collected during the same time period (June 20- July 30) in vacuum samples showed that over 90 percent of aphids belonged to a single species: *Aphis spiraecola* Patch. Other, most abundant species collected during the season included: tuliptree aphid *Illinoia liriodendri* (Monell), corn leaf aphid *Rhaphalopsiphum maidis* (Fitch), pea aphid *Acyrthosiphum pisum* (Harris) and green artichoke aphid *Capithophorus eleagni* (del Guercio) Other species although present in the system, were collected sporadically, often being represented by a single specimen in a sample (8 species). In contrast to previous years of collection, during the 2003 season the green peach aphid, *Myzus persicae* was not collected in the samples. Also, the total number of collected aphids during this past season was much lower than during the previous seasons. The above normal precipitation and below normal temperatures during most of the season may have effect on the number of aphids collected during the 2003 season. Work on potential vectors for the PPV virus transmission conducted by other scientists will hopefully determine the importance of identified species in potential spread of the PPV virus in PA orchards and ornamental stone fruit hosts.

Overview of Plum Pox Virus in Europe

Michel Ravelonandro
Institut de Biologie Végétale et Moléculaire, IBVM, INRA-Bordeaux, Villenave d'Ornon, France.

Europe is the continent that represents the source of plum pox virus (PPV). Whether *Prunus* is originated from Asia and recognized as the natural host of PPV, no evidence of PPV detection has been presently reported from this continent. In reference to PPV, Europe is the main area affected however the chronological spread of the disease lets us to distinguish between the programmed works developed in European Union countries and the rest. Leadership in the knowledge about the biological and the immunological properties of PPV can be found in the European Union countries however the rest and notably the eastern and central countries play a major role with the high diversity of PPV. Cooperative research activities conducted by a few representative laboratories showed practically the scientific progress achieved in virus control. But around this theme as well as the ultimate goals, every country has built its respective strategy that exhibited the lack of harmony in virus resistance protocols. So the speediness of cultivar spread that satisfied breeders and fruit-tree growers did not cover the strict safeguards on the possible outbreak of PPV that emerges from the tolerant cultivars. Obviously that requires more knowledge about virus, plant-virus and virus-vector interactions to fulfil the strict survey and to perform an efficient eradication system. Resistance is the hardiest topic to target the control of PPV, therefore no any restrictive means must be chosen, and genetic engineering approach must be valued in parallel with the conventional breeding program.

Analysis of Methylation and Post-Transcriptional Gene Silencing in Embryos and Seedlings of C5, A Transgenic PPV-Resistant Plum [Prunus Domestica (L.)]

R. Scorza[1], K. Webb[1], and M. Ravelonandro[2]
[1]USDA-ARS Appalachian Fruit Research Station, 45 Wiltshire Rd., Kearneysville, WV 25443
e-mail: rscorza@afrs.ars.usda.gov, fax: ++1 304 7282340
[2] Institut de Biologie Végétale et Moléculaire, INRA, BP 81 33883 Villenave d'Ornon, France
e-mail: ravelona@bordeaux.inra.fr, fax: ++33 5 57122381

We have previously shown that in the PPV resistant transgenic plum clone C5 the PPV-CP insert is post-transcriptionally silenced (PTGS) and methylated (Scorza et al., Transgenic Res.1021-1029, 2001). We have also demonstrated that the transgene insert and PPV resistance are mutually inherited in progeny of C5 (Ravelonandro et al., Acta Hort. 478:67-71, 1998; Scorza et al., Acta Hort. 472: 421-427, 1998). In herbaceous systems, PTGS has been shown to be "re-set" following seed germination. To our knowledge, this phenomenon has not been studied in woody perennials. *P. domestica* is a woody perennial that normally requires stratification (moist chilling) for germination. Seeds of open-pollinated C5 carrying the PPV-CP insert were germinated with and without stratification, as were seeds of C3, a non-PTGS PPV-CP transgenic clone that is susceptible to PPV. DNA analyses showed that at one month following germination the PPV-CP gene in C5 was methylated in seedlings from both stratified and non-stratified treatments at levels comparable to the levels in leaves of the C5 parent, whereas the NPTII and *uid* transgenes were unmethylated as expected. No transgene methylation was found in C3 seedlings. Based on these results, methylation and RNA expression were evaluated in embryos prior to germination, and in one-month-old seedlings. These evaluations indicated that the PPV-CP transgene in embryos of ungerminated C5 seeds displayed reduced levels of methylation and higher levels of RNA expression than in leaves of either the one-month-old C5 seedlings or the C5 parent. These results suggest a rapid and concurrent increase in PPV-CP transgene methylation and RNA silencing that occurs within one month of germination of C5 seedlings.

Progress Towards Engineering Resistance to the Canadian Isolate of Plum Pox Virus in Model Plants

Sanfacon, H.[1], E. Barszcz[2], D. Brown[3], S. Sibbald[3], L. Stobbs[2], A. Svircev[2], L. Tian[3], R. Wen[1], K. Whybourne[2], S.C. Zhang[1]
[1] Pacific Agri-Food Research Centre, Summerland, B.C., Canada V0H 1Z0 [2] Southern Crop Protection and Food Research Centre, Vineland, Ontario, Canada L0R 2E0 [3] Southern Crop Protection and Food Research Centre, London, Ontario, Canada , N5V 4T3

As part of a team effort aimed at developing resistance to PPV in peaches, we have tested the intron-hairpin approach as a method to efficiently induce resistance to PPV. In an effort to engineer broad-spectrum resistance effective against various PPV isolates, two short regions of the PPV genome were selected in the P1 and HC-Pro region for their high degree of sequence identity among characterized PPV isolates. Each of these regions were cloned in a conformation allowing the formation of a hairpin containing the PPV sequence interspaced with an intron derived from the cherry genome. Several lines of *Nicotiana benthamiana* and *Arabidopsis thaliana* (two hosts for PPV) were produced for each of these constructs. Thirteen *Nicotiana* lines confirmed to contain the HC-Pro intron-hairpin construct and nine *Nicotiana* lines confirmed to contain the P1 intron-hairpin construct were tested using a Canadian isolate of PPV as an inoculum and the CFIA-approved ELISA assay to confirm virus infection. In the control lines, 80 to 95% of the inoculated plants were PPV-positive. Of the thirteen HC-Pro lines, nine displayed excellent resistance to PPV in initial resistance assays (i.e. none of the inoculated plants were PPV-positive) while three displayed good resistance (i.e. only 5 to 15 % of the inoculated plants were PPV-positive). Similarly, five of the nine P1 lines displayed excellent resistance to PPV (i.e. 0 % PPV-positive plants) while 3 lines displayed good resistance (5 to 10% PPV-positive plants). Additional resistance assays will be conducted in collaboration with Michel Ravelonandro to test the breadth of the resistance using other PPV isolates. These initial results suggest that resistance to PPV can be induced very efficiently using the intron-hairpin approach. The efficiency of this method should prove very useful when attempting peach transformation. Finally, other approaches to engineer resistance to PPV, such as the selection of specific inhibitors of PPV proteinases that could be expressed in plants, will be discussed.

Evaluation of DNA Methylation of the Plum Pox Virus Coat Protein Transgene in Mature Field-Grown Plum Pox Virus Resistant Plum Trees (Prunus domestica L.)

Hughes-Watson PL and Scorza R. 2003.

Plum pox virus (PPV), a member of the genus *Potyvirus,* is one of the most destructive viral diseases of *Prunus* species. PPV causes fruit symptoms such as light green or yellows rings, deeply engraved rings and spots, and brown gum like deposits in the flesh of plum (*Prunus domestica* L.) rendering the fruit unmarketable and resulting in economic losses for producers. Traditional plant breeding has produced few varieties of *Prunus* that are highly resistant to PPV. Through the use of genetic transformation, PPV resistant plum trees containing the PPV coat protein (CP) have been produced. PPV-CP transgenic plum clone C5 was found to be highly resistant to PPV. C5 plants displayed characteristics typical of post-transcriptional gene silencing (PTGS), including a high level of transcription in the nucleus, low levels of transgene mRNA in the cytoplasm, and methylation of the silenced PPV-CP transgene (Scorza *et al*., 2001, Transgenic Research 10: 201-209). DNA methylation has consistently been associated with PTGS in many species. The aim of the current study was to evaluate the level of methylation of the PPV-CP transgene in leaf tissue from 6-8 year old field-grown C5 trees. PPV-CP transgene DNA in the two C5 trees was stable and consistently more highly methylated at three time points (April, July, and October) during the three growing seasons as compared to other PPV-CP transgenic plums that were neither silenced nor PPV resistant. Methylation levels in C5 started out relatively low in April, increased to their highest levels in July and then decreased again in October. Even though methylation levels decreased in October these levels were at least twice that seen in April. This study demonstrates both the long term stability of PTGS-related transgene methylation and seasonal fluctuation of methylation in a perennial fruit tree.

Working with Select Agents-Specifically Plum Pox Potyvirus

Charles Divan
Agricultural Microbiologist
Biological and Technical Services
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
Riverdale, MD

The Agricultural Bioterrorism Protection of 2002 (ABPA) is codified in the APHIS Regulations 7 CFR 331 and 9 CFR 121. The intent of the ABPA is to establish and enforce safety procedures for select agents and toxins to protect, in part, animal and plant health. The effective date of the ABPA is November 12, 2003. In part, the administrative burden to the applicant is: entity registration, security risk assessment for individuals by the FBI, and record keeping. The USA Patriots Act, 2002 restricts access to select agents and toxins by nationality. The ABPA regulates the possession, use, and transfer of select agents and toxins. ABPA Regulations for select agents and toxins are in addition to the Plant Protection Act Regulations. The APHIS select agents and toxins are: PPQ, 10 select agents (5 bacteria, 4 fungi, 1 virus, and no toxins); Veterinary Services, more than 80 agents and toxins. Diagnostic labs, as described in 7 CFR or 9CFR, are exempt from the ABPA. The Plant Protection Act Regulations provide a permit system for research. The APHIS Administrator may exempt individuals or entities from the requirements of the ABPA for up to 3 years. There are 5 forms required under the ABPA: APHIS form 2040-2044. Civil and/or criminal penalties for violation of the ABPA are up to $500,000.00 and 10 years in jail. The Secretary of Agriculture is required to review and republish the list of select agents and toxins every 2 years.

How Secure Is Our Defense Infrastructure?

Bill Howell, Manager
NRSP5, National Virus-Tested Fruit Tree Program
24106 N. Bunn Road, WSU-IAREC, Prosser, WA 99350
Phone 509-786-9251; Fax 509-786-9370; Email wehowell@wsu.edu
Web site www.nrsp5.prosser.wsu.edu

With the arrival of plum pox virus to orchards in North America, the plant protection organizations in both the United States and Canada mobilized to minimize the economic effects of this pathogen. Much of this effort included surveys and tree elimination. However, it is important to remember that in the long term the most effective mechanism for limiting virus diseases in pome and stone fruit orchards is through certification of planting stock, the most likely means of spread of virus and virus-like pathogens in these crops. Certification in the United States is based on foundation grade virus-tested propagation material, which is supplied by the National Research Support Project #5 to nurseries throughout the country. State departments of agriculture monitor and certify the production of trees from this source. This scheme for protecting our orchards from virus problems has been highly effective for many years. Unfortunately, this success leads to ambivalence by many in the industry and research. Lack of interest, and thus, of funding, puts the important national and state infrastructures for these certification programs in jeopardy. It is time for all concerned with the health of our tree fruit orchards to study and devise methods for re-establishing these programs in viable, proactive, and productive ways so that the consequences of the presence of viruses such as plum pox and other serious virus diseases are greatly reduced now and for the future.

PPV in Ontario: Getting the Message Out

Michael Celetti, Plant Pathologist, Horticulture Program Lead, OMAF, Guelph, Ontario, Canada
Neil Carter, Tender Fruit and Grape IPM Specialist, OMAF, Vineland , Ontario, Canada
Hannah Fraser, Entomologist, Horticulture Program Lead, OMAF, Vineland , Ontario, Canada

---

After the discovery of Plum pox virus strain D in a few orchards in Adams County, Pennsylvania during the fall of 1999, one of the first extension activities in Ontario, Canada was to educate growers on what PPV is and what it means to the *Prunus* fruit and ornamental industries. Our strategy was to use every available form of media to provide a wide range of information on PPV to fruit growers and ornamental producers.

Information on PPV biology, symptoms and impact was developed and disseminated during the winter of 2000 through various newsletters including the "Ontario Tender Fruit Producers' Marketing Board Newsletter"(monthly) and an OMAF publication "The Tender Fruit Grape Vine"(bi-monthly) (http://www.gov.on.ca/OMAFRA/english/newsletters.html). These two publications are distributed by the OTFPMB to 1400 subscribers within the industry. In addition, feature articles were written by OMAF extension staff and published in The Grower Newspaper (11,700 subscribers) and the Fruit and Vegetable Magazine (7,000 subscribers). Information was also provided at grower conventions such as "The Niagara Peninsula Fruit and Vegetable Growers Association Convention and Trade Show" now called "Ontario Fruit and Vegetable Convention and Trade Show" which attracted 1,500 delegates in February, 2003. A segment on PPV was produced by OMAF's "Town and Country" for television which was broadcast across Ontario.

On June 23, 2000, the Canadian Food Inspection Agency (CFIA) announced the discovery of PPV in Niagara-on-the-Lake during an extensive survey conducted to determine if PPV was present in Canada. To determine what should be done about PPV in Ontario, OMAF, together with OTFPMB, coordinated a PPV Workshop for growers in the fall of 2000. International experts working on PPV including Christine Colas from France, Dr. Mariano Cambra from Spain and Rayanne Lehman from the US where brought in to present the latest information on PPV biology, regulatory status, management and eradication efforts in other countries. Growers had the opportunity to ask questions and obtain information about PPV and the experts were able to witness first hand the PPV situation in Ontario. Recommendations for action were written up based on the information obtained at the workshop along with the survey results from 2000 and submitted to Federal and Provincial Ministers of Agriculture.

Around the time PPV was discovered in Ontario, the internet was already recognized as an effective communication tool and Pennsylvania State University Extension developed an extensive PPV website to transfer up to date information to their local industry. Because of the pace of new information on PPV from research and surveys, OMAF also utilized the internet and developed a PPV page on the government website. This webpage provided growers with up to date information on biology, distribution, survey updates and PPV workshops. Eventually the information was developed into a Factsheet "*Sharka (Plum Pox Virus) of Stone Fruit and Ornamental Prunus species*". The factsheet was rolled out at Plum Pox workshops "Lessons Learned from 2000 and Beyond" conducted across southern Ontario during 2002 to help train growers on the identification, biology, impact on IPM programs and strategies for eradication of PPV. At the same time, OMAF staff were developing an on-line newsletter called Hortmatters (www.gov.on.ca/OMAFRA/english/crops/hort/news/hortmatt/2003/02hrt03.htm) that is used to update and transfer information to Agribusiness and Agriculture consultants in Ontario (during September 2003 Hortmatters was visited 1658 times). Plum pox information and updates were also provided to the industry through radio reports broadcast through a station in southern Ontario. To reach a wider geographic audience, the radio reports can now be downloaded as sound bites from the OMAF website (http://www.gov.on.ca/OMAFRA/english/crops/radio/radiodaily.htm).

The CFIA has also been transferring information through a series of posters developed to educate growers using photos of PPV from other regions. CFIA and OMAF have recently developed a 19.5x28 inch poster that includes photos taken of PPV symptoms observed in Ontario. Grower groups had requested a poster that included photos of symptoms of the disease as observed in Ontario. The poster was distributed to all tender fruit producers in Ontario through OTFPMB.

The strategy of providing information through many different media required repackaging and updating of ideas on a continual basis. It also required rethinking how to best present messages about PPV for different formats. Although time consuming, this strategy ensured that all affected growers were regularly informed about this important issue.

The latest information obtained from attending and participation at meetings such as the Canadian/American Plum Pox Virus Update and NE 1006 will be transferred to growers through blanketing the media with a series of PPV informational materials during 2004. This will be particularly important to foster the continuing grower support and understanding for the eradication program needed to make the program a success.

Stone Fruit Certification in the US: Excellence in Patches

Ruth A. Welliver, Pennsylvania Department of Agriculture

National and state virus certification programs in the United States were established when virus problems threatened to destroy nursery and fruit industries. Industry demand for quality stock drove the development of quality certification programs. Unfortunately, those programs have suffered from their success, now struggling to maintain their integrity without adequate research, development, or even maintenance support. This breakdown in certification programs may have contributed to the ability of plum pox to sneak into the country.

Viruses continue to cause economic damage to nurseries, fruit growers, and consumers. Without good clean stock sources, losses from virus diseases will increase. It is crucial that researchers, regulators, and industry representatives work together to evaluate and renovate certification programs in the U.S. A committee under the auspices of the National Plant Board has been working this year to study the issue. They are ready to ask the National Plant Board to recommend that USDA-APHIS-PPQ establish standards for a national certification program. Such a standard could begin the process toward a program in the United States that would provide defense against any international challenge to the current fruit tree importation system; provide enhanced surveillance for quarantine and regulated pests; focus limited resources to critical areas; allow adjustments to bring certification in line with industry practice; and protect the U.S. industry against production losses due to virus infection.

Certification is an inexpensive means of complementing national pest detection and pest exclusion programs, and is critical to the health of green industries and the environment. It will only be a useful and vital system, however, if we engage in ongoing dialogue among research, extension, regulatory, and industry interests.