Expelling a Plant Pest Invader:

The Pennsylvania Plum Pox Eradication Program, A Case Study in Regulatory Cooperation

by

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Abstract

Plum Pox Virus (Sharka), the most serious viral disease of stone fruits globally, was first identified from North America during October 1999 from infected peaches in Adams County, Pennsylvania. A Plum Pox Virus survey, eradication and outreach program was conducted in Pennsylvania from 1999-2009 by the Pennsylvania Department of Agriculture, US Department of Agriculture, Pennsylvania State University, and numerous cooperators and supporting agencies. This paper chronicles the project's evolution from detection of the disease to declaration of eradication, including types of surveys conducted and lab tests used. To determine the distribution of the virus in Pennsylvania, State and Federal personnel sampled all commercial stone fruit trees in the state for a minimum of three years, eventually concentrating most of their activities in the four south central counties (Adams, Cumberland, Franklin, and York) where the virus was detected. Staff issued numerous treatment orders leading to the removal and destruction of nearly 1,800 commercial acres of stone fruit trees. In addition, 10 acres of abandoned stone fruit trees, 54 acres of stone fruit tree nursery stock, 1,174 stone fruit trees or shrubs on residential properties, and 1,420 ornamental stone fruit trees in landscape nurseries and garden centers were ordered removed and destroyed. Wild stone fruit trees, root suckers, and seedlings in peach cull piles were tested (all negative for the virus) and then destroyed. The cost of removal and destruction of stone fruit trees, along with associated payments to growers for other control operations, totaled $30 million ($22 million – USDA, and $8 million – PDA) over a ten year period. The cost of the PPV program operations cost an additional $29 million. The eradication program succeeded because of the cooperation between stakeholders, an adequate level of funding, and the continuous reevaluation and update of policies and procedures based upon both scientific findings and the political environment.

Keywords

"Plant virus", "Plum Pox Virus", Sharka, eradication, regulatory, "federal and state cooperation", peach, *Prunus*.

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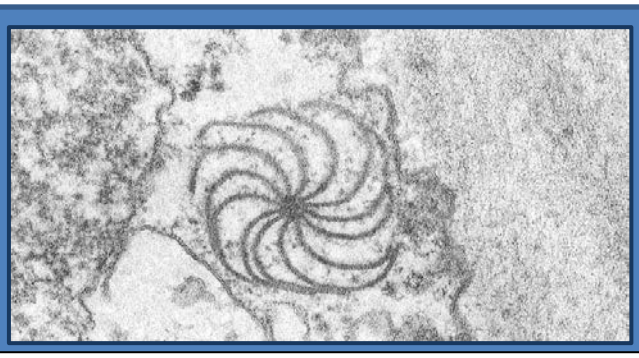
Introduction to Plum Pox Virus

Plum Pox Virus (PPV), also known as "Sharka" (Bulgarian, *sarka* = pattern, pox, stripe), globally is considered the most economically devastating viral disease of stone fruit. Plum Pox infects species of *Prunus* (including plum, peach, nectarine, cherry, apricot, and many ornamental plants). Trees infected with PPV produce fruit that may not be marketable due to noticeable blemishes, reduced taste quality and premature fruit drop. The first evidence of this disease was recorded in southwest Bulgaria during 1918. Subsequently, the disease spread throughout Europe via aphid vectors and human movement of infected propagative materials. To minimize the risk of introduction of PPV into the USA, propagative material and cut flowers of its primary *Prunus* hosts are prohibited or restricted entry into the country by Title 7, Part 319.37 of the Code of Federal Regulations. In October of 1999, the USDA Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ), and the Pennsylvania Department of Agriculture (PDA) announced the detection and confirmation of PPV in Adams County, Pennsylvania. This was the first recorded incident of PPV in the continental USA.

Causal Agent. PPV is a species in the genus *Potyvirus* (Family Potyviridae). PPV is characterized by its 750 nm flexuous, filamentous particles, containing a single-stranded positive-sense RNA of 9.8 kilobases. The virus is vectored by various aphid species in a non-persistent manner, requiring only very brief acquisition and inoculation periods for successful transmission to susceptible hosts. No latent period exists between acquisition and inoculation



Electron micrograph of PPV-PA pinwheel inclusion in peach leaf cell. Image courtesy of Fred Gildow, Penn State. *Aphis spiraecola* image by Brendan Wray, Bugwood.org. This aphid species, a vector of PPV (Gildow et al, 2004), was the most prevalent species in vacuum samples taken from PA *Prunus* trees in June and July (Greg Krawczyk, personal communication).

feeding. Aphid inoculativity declines rapidly with feeding; successful transmission is therefore dependent on time, the availability of a susceptible host and the aphid's ability to probe and feed adequately to transmit the virus.

Worldwide (as of 2009), PPV has been classified into at least seven distinct strains, identified as PPV-C, PPV-D, PPV-EA, PPV-M, PPV-Rec, PPV-T and PPV-W (Serçe *et al.* 2009). All Pennsylvania PPV isolates characterized since the initial detection in 1999 have been assigned to the PPV-D strain group. PPV-D is known not to be seed transmitted, which allows an effective eradication strategy without restricting movement of fruit.

The virus may remain latent within its plant host depending on temperature, nutrition, cultivar and type of stone fruit infected. PPV titers drop significantly in infected hosts as day/night temperatures rise during summer months to the point that detection becomes difficult. In addition, PPV may be unevenly distributed in a host, which makes it difficult to consistently detect through leaf and/or fruit samples collected for lab analysis.

PPV Symptoms. Symptoms of PPV infection may manifest on the leaves, flowers and/or fruit of susceptible hosts. Depending upon the cultivar and type of stone fruit infected, symptoms range from non-existent to severe. Globally, plum and apricot varieties appear most susceptible and express the disease most strongly. On leaves, diagnostic symptoms include chlorotic spots or rings or vein clearing and banding. Flowers may display color break and distortion. Fruit may show superficial spots and rings, but can also be distorted or misshapen. Spots and rings on fruit may become sunken or pitted, resulting in "pox-like" marks, hence the common name, Plum Pox. Some varieties show rings on the seeds after the fruit's flesh is removed. Severely susceptible plum varieties may show bark splitting on the trunk and limbs. As PPV continues to spread within an infected host, the quality and quantity of fruit is affected. Fruit production is

PPV-Infected Encore Peach grown in Adams County Pennsylvania, 1999. Courtesy of PA Dept. of Agriculture

compromised due to premature fruit drop, resulting in less produce reaching maturity. Any remaining mature fruit may be blemished with reduced sugar content, affecting taste and marketability. Infected host trees continue to decline from year to year to the point that the amount of fruit produced becomes negligible.

Disease Cycle. PPV is introduced into new areas through the movement of diseased plant material, such as cuttings, bud sticks and rootstocks. PPV is very persistent and can move from orchard to orchard by natural vectors in addition to continued movement of infected plant material. Long-distance spread of the virus is influenced by human movement of infected trees and plant material. Aphid transmission is much slower, based on the movement of the vector and its exposure to PPV-infected hosts. Because symptoms of the virus can remain mild or non-existent for a period of time, movement of the virus from its original infection point in an orchard can be significant before it is noticed. Several aphid vectors successfully transmit the virus to *Prunus* species. Peak time for transmission is during the spring season when trees produce tender, flush growth. Aphids are attracted to the succulent new plant material, and can readily probe and feed, which increases the intake of PPV and its transmission to surrounding hosts. Movement of aphid vectors is highly variable within a host, an orchard, or an area and can depend on the species of aphid, life stage (winged or wingless), weather conditions and available host material.

Some reports in Europe show that PPV occurs naturally in sweet and sour cherry, but to date no infected cherry has been found in the field in the USA. Weed species have been recorded as harboring PPV in Europe, but no movement of the virus from these species through aphid vectors has been recorded in the USA. Aphid transmission wanes during the dormant season until spring flush arrives. The virus remains within infected hosts during winter dormancy and titers rise during spring, increasing the ability of aphids to acquire the virus and transmit to surrounding susceptible host plants. As aphid activity increases with warm weather, the probability of vector transmission of PPV to these hosts becomes high. Aphids transmitting this disease are typically not host-specific feeders and colonizers; their movement to suitable hosts involves sampling any plant they contact during flight migration. During this probing process, the aphids suck plant epidermal (surface) cell contents into their stylets. If PPV is present in

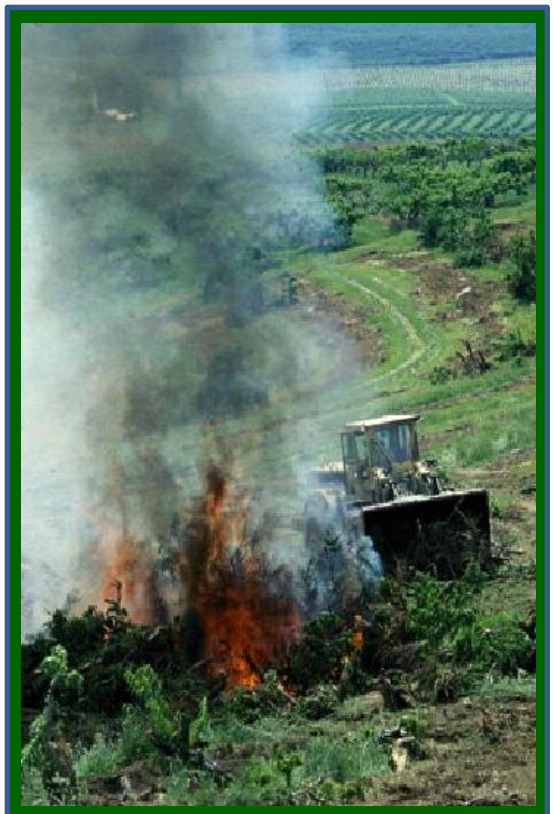
those epidermal cells, then it may become associated with the aphid stylet until the aphid probes again, setting the stage for movement of the virus to a non-infected host. When an aphid carrying PPV locates a preferred host, it may establish a colony on that host, resulting in greater movement of the disease within the host as more virus is vectored during the feeding and probing processes.

Survey and Detection. To understand the scope of PPV infection, regular surveys are necessary to determine the presence of the disease. Visual inspection of trees is not a reliable detection method because of the variability in the expression of symptoms and many years may pass before an infected host manifests symptoms. Detection surveys should locate one infected host tree in an orchard block with a minimum of samples taken. Because of the uneven distribution of the virus within a host, leaves should be taken from each major branch of the tree. At the time of the Pennsylvania eradication program, Enzyme Linked Immuno-Sorbent Assay (ELISA) and Reverse Transcription Polymerase Chain Reaction (RT-PCR) were the preferred lab tests to screen for and confirm the presence of PPV in collected leaf tissue. These tests can detect low concentrations of the virus from infected hosts that are asymptomatic. Surveys must be conducted for at least three years without PPV being detected in order for an orchard to be considered disease free.

Control/Eradication. When PPV is detected, the most effective control method involves the destruction of host trees. If infection is detected early, then eradication of the disease may be attainable providing host material is destroyed rapidly. In Pennsylvania, infected orchard blocks were quickly destroyed by pushing and burning the trees. Buffer zones of adjacent trees, potentially inoculated recently by infectious aphids, were also destroyed to contend with the natural aphid spread of the disease. A PPV Quarantine was established which regulated any movement of *Prunus* spp. into and out of the area. The quarantine also prohibited replanting *Prunus* within the affected area until a 3-year disease-free period could be achieved. Planting of other non-*Prunus* species (apples and field crops) was permitted within the quarantine zone. Such quarantines may need to involve entire states or counties, but in the case of Pennsylvania, small areas within a county were placed under regulation. PPV was not widespread in the USA, so an area-wide management strategy was not necessary. In Europe the distribution of PPV

includes many countries, so a regional management approach is used. Eradication is not realistic in Europe. The management approach in France, for example, involves regular visual surveys followed by prompt removal of symptomatic trees. New disease-free trees are replanted and the cycle continues each year wherever disease presence is 10% or more.

Insecticidal control of aphid vectors is generally not effective for control of PPV because many aphid species move in and out of orchards each season and could still transmit the virus before succumbing to the insecticide. In addition, insecticidal sprays targeted for aphid control do not match established pest management programs in stone fruit and may only increase resistance and cause secondary pest outbreaks.



Destruction of condemned orchards, Adams County Pennsylvania, 2000. Image courtesy of Penn State.

Resistance. The introduction of resistant *Prunus* varieties is a long-term solution to the devastation caused by PPV. To date, varieties with absolute natural resistance to PPV are not commercially available. A resistant *P. domestica* variety, Honey Sweet plum, has been developed through genetic modification in work conducted by USDA, Agricultural Research Service (ARS) (Scorza & Ravelonandro 2006); no similar resistance has been introduced into peach. Even with extensive work in Europe and North America, more time is needed before genetically resistant varieties are released in numbers sufficiently large to reach mainstream production nurseries globally. The use of certified virus-free planting stock is essential to prevent further spread of PPV. This, coupled with a program of survey, testing and tree removal, will prevent PPV from gaining hold in areas where the disease is not known to occur or is present at low levels.

Plum Pox Disease History. A conference on PPV hosted by the European Plant Protection Organization (EPPO) in 1994 summarized the history and status of Plum Pox on that continent (Roy & Smith 1994). PPV was first described in Bulgaria in plums in the early 1900s and had spread through much of Europe and to several Mediterranean countries by the time of the conference. The first report of PPV in the Western hemisphere, in Chile (1992), was also presented at the 1994 EPPO conference.

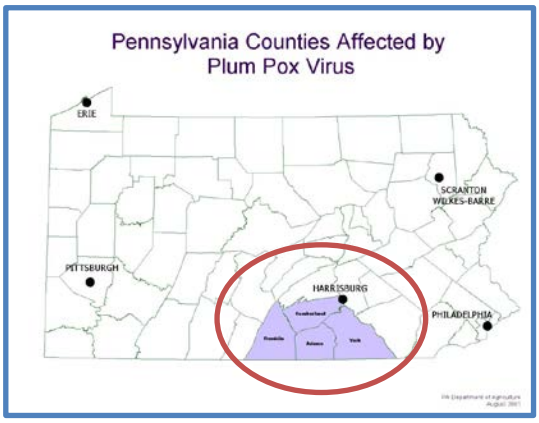
The first report of PPV in North America came in 1999 with Pennsylvania's detection (Levy *et al.* 2000). This was followed very quickly by reports of a Canadian infestation (Thompson *et al.* 2001). In 2006, PPV was detected in Michigan and New York. In 2006, all North American isolates were classified as PPV D-strain; subsequently, Canada reported single-tree detections of PPV-W and PPV-Rec. By 2006, the disease had been reported in many Middle Eastern countries, China, and Argentina (Capote *et al.* 2006), and in 2009 PPV was reported in Japan (Maejima *et al.* 2009). Virtually everywhere *Prunus* has been planted, the sharka disease has also been found. Notable exceptions to date are New Zealand and Australia where border surveillance and rigorous biosecurity still hold PPV at bay. Some more northerly countries, such as Finland, where *Prunus* is a relatively minor crop, have also remained PPV-free (Capote *et al.* 2006).

A complete understanding of the chronology of spread is impossible, owing to the lack of specific detection tools available until more recent years. However, several key influences on virus distribution patterns are apparent. The center-of-origin of PPV was likely in Eastern Europe near the first description of the disease. Local virus spread was fairly slow in Eastern Europe. Rapid spread of the virus occurs with movement of infected stock when biosecurity and selectivity of stock are given low priority. An example would be in reconstruction efforts after World War II, when the desperate demand for planting stock allowed nursery stock, along with any graft-transmissible agents it might harbor, to move freely across international lines. By the time more care could be taken in the importation of nursery stock, the virus had already become established in orchards and in natural areas, making it impossible to eliminate (Maria Kolber, pers. comm.).

Another escalation in virus distribution is seen when a more aggressive strain, such as PPV-M, is introduced into a new area, or when an introduced isolate is particularly well adapted to economically important *Prunus* hosts in a new region. Economic damage caused by PPV in France and Spain give evidence of this (Capote *et al.* 2006).

Program History in Pennsylvania

Pennsylvania Detection History. A fruit grower in Adams County, Pennsylvania, noticed yellow markings on mature Encore peach over a period of several years. As the numbers of fruit with markings grew more common, and began to affect the marketability of the fruit, the grower shared the description of the fruit with various experts in the area who attempted to explain the symptoms based on environmental or pest conditions known to occur in the area. In 1999, Jerry Frecon of Rutgers University, after seeing the fruit, suggested the markings might be caused by Plum Pox Virus, a virus not known to occur in North America. A consultant had already invited a Pennsylvania state regulatory plant pathologist to visit the growing location. Since the specter of Plum Pox had been raised, the regulatory community addressed that hypothesis. Because Plum Pox was not present in North America, a complete and specific PPV test kit was not commercially available in the USA. At the time, an ELISA reagent set was available for the general potyvirus group and a marginal Plum Pox reagent set was available, but cross-reacted with other potyviruses, had no true positive control, and gave weak reactions with sampled fruit. Suspect plant material was sent to the USDA lab in Beltsville, MD for direct testing via both ELISA and immunocapture RT-PCR. There, the Encore peaches were indeed confirmed positive for PPV.



Program Establishment and Decision Processes. Once PPV was confirmed from Adams County, the PDA and USDA conducted a delimiting survey to determine the extent of the infestation. Using the block of Encore peaches as the geographic center of the survey, the USDA and PDA teams attempted to survey all blocks of PPV-susceptible *Prunus* within a 10-mile

radius. The delimiting survey began October 12, 1999, with the last collection made on November 4, when nearly all the leaves had yellowed or dropped from the trees.

The survey method was informal, not systematic, as time was of the essence. Two team members were assigned to each block of trees, each person starting at an adjacent corner of the block. Each person then walked a diagonal line to the opposite corner and collected 2-4 leaves on randomly selected trees. No attempts were made to search for PPV symptoms. The leaves from each block were pooled and then tested at the PDA lab using the superior ELISA reagents secured through USDA. USDA and PDA personnel surveyed other counties in a similar manner to determine whether the PPV infestation was confined to south central Pennsylvania.

This informal survey revealed a PPV infestation spanning two neighboring townships (Huntingdon and Latimore) in northern Adams County. Four growers were involved, and nearly 220 acres (18 blocks) of their trees were infected with PPV to some degree. An epidemiological study was also begun that fall, directed by Tim Gottwald (USDA-ARS), to understand the distribution and level of infection within orchard blocks. Fortunately, more than 200 sites in Adams County and 32 other counties in the Commonwealth tested negative for PPV. The surveys gave an early indication that the PPV situation most likely resulted from a single introduction followed by localized spread.

The rolling hills of Adams County PA intermingle stone fruit orchards with apple orchards, wooded areas, and residential properties. Image courtesy of PA Dept. of Agriculture.

The results of the preliminary survey led to the decision to eradicate the virus. The decision was based on the following information: 1) Surveys conducted in Fall 1999 indicated the virus distribution was localized; all 18 positive blocks were within a 1.5 mile radius of the block of Encore peach where the initial detection was made. 2) The scattered distribution of stone fruit blocks within the infested

townships and the presence of woodlands, numerous blocks of commercial non-stone fruit trees and presence of field crops, all served to slow the spread of the virus. 3) Investigations and trace-backs of trees in infected blocks and testing of trees in stone fruit nurseries gave a preliminary indication that nursery stock was not the source of the infection. 4) Trace-back investigations indicated that the virus had been in Adams County a short time, possibly less than eight (8) years. 5) All samples of PA nursery stock and nursery source trees (which PA stone fruit nurseries used as sources of budwood) tested negative for PPV. 6) A sophisticated method of lab testing to determine whether trees were infected with PPV was in place. This was especially valuable because only two of the 18 infected blocks of trees displayed any clear symptoms of PPV. 7) There was commitment from federal, state, university, and grower communities on a unified effort to eradicate the virus.

The lateness of the detection allowed the use of the fall and winter months to organize the survey and eradication program for calendar year 2000. Major challenges confronted were: 1) funding for the program; 2) developing rules for removal and destruction of infected blocks; 3) deciding on and obtaining commitments to reimburse growers for their losses; 4) setting up and equipping a testing lab; and 5) all the logistics associated with a large program.

- On October 18, 1999, all state regulatory agencies, Canada and Mexico were informed of the detection of PPV in Adams County. On October 21, PDA established a PPV quarantine in Huntington and Latimore Townships, prohibiting the movement of stone fruit trees and stone fruit budwood both within and out of the regulated townships.
- On December 14-16, 1999, a PPV Technical Workshop was held at the Pennsylvania State University (Penn State) Fruit Research and Extension Center in Biglerville, Adams County. Thanks to funding from USDA, specialists on PPV from France, Hungary and Spain presented information on PPV to more than 100 attendees from 16 states and Canada.
- On December 20, 1999, PA Governor Tom Ridge signed Act 57, the Drought, Orchard and Nursery Indemnity and Flood Relief Act, part of which made grants available to indemnify owners of commercial orchards and fruit tree nurseries for the following activities related to Plum Pox Virus: 1) removal and destruction of commercial trees and

nursery stock; 2) treatment with herbicides to control suspected weed hosts of PPV; 3) vector (aphid) control; and 4) other activities necessary to eradicate PPV. This statute gave PDA legal authority to develop the necessary guidelines by which grant applications would be approved.

- On December 21, 1999, PA Agriculture Secretary Samuel E. Hayes, Jr. signed the Commercial Orchard and Fruit Tree Nursery and Indemnity Program, which provided commercial growers and stone fruit nurseries with the information necessary to apply for indemnities associated with the PPV eradication program pertaining to the four activities in the above paragraph.

Size and complexity of federal systems meant that publication of information at the federal level lagged behind state-level action. However, the close working relationship between staff at the state and federal levels meant that field operations could proceed smoothly, and state and federal actions stayed consistent and clear. The first federal public notice related to Pennsylvania's PPV detection occurred March 2, 2000, when a "Declaration of Emergency" and a "Declaration of Extraordinary Emergency Because of Plum Pox" were published in the Federal Register. Publication of a federal quarantine paralleling the state quarantine followed on June 2, 2000, and a Plum Pox Compensation Rule was published September 14, 2000.

Penn State and USDA Agricultural Economists met with the affected growers, who provided information on fruit yields and costs. Using this information, the Agricultural Economists developed an indemnification table that detailed values of fruit production for trees aged from 1 to 25 years old. In addition, PDA staff worked with growers of stone fruit nurseries to determine indemnification for any *Prunus* nursery stock that would have to be removed and destroyed. Both these programs were detailed and then published in the Pennsylvania Bulletin and Federal Register in order for PDA and USDA to reimburse growers and stone fruit nurseries for their losses. By the end of January, 2000, PDA's web site had posted a copy of the Notice Secretary Hayes had signed on December 21, 1999, and a copy of the grant application that PDA had developed and approved by its Legal Office and the PDA Comptroller.

Both PDA and USDA worked with their agencies' administrators and legislators and each other to determine levels of compensation for lost fruit production, costs associated with removing and destroying trees, and cost of treatments for the PPV-vectoring aphids. In March 2000, PDA and USDA administrators met in Washington, DC, and agreed to share costs associated with reimbursing growers and stone fruit nurseries for lost fruit production and loss of stone fruit nursery stock, respectively. These two items comprised a significant portion of the payments that were to be dispersed. In addition several other funding programs were developed to reimburse growers and homeowners; all of these were the responsibility of PDA.

On March 4, 2000, at the National Association of State Department of Agriculture's annual meeting in Washington, DC, PDA staff detailed the USDA/PDA plan to eradicate PPV from PA and asked the Association members to support a resolution that: 1) Requested USDA to issue a federal quarantine of the infected area in PA, declare an extraordinary emergency which was to include language stating that compensation to the growers would be provided once rates were established; 2) requested the federal Office of Management and Budget to make funds available to compensate growers whose trees had to be destroyed in order to eradicate PPV from the USA; and 3) requested that USDA develop and distribute a standardized survey protocol and fund a 3-year national survey to determine whether PPV was present in other states with commercial stone fruit production.

Much of the compensation program had been resolved by April, 2000, allowing time for growers to remove and destroy the trees that were confirmed positive in late 1999. The growers responded quickly, removing the PPV-infected blocks before leaf development, thus denying aphids the opportunity to feed on the leaves and spread the disease to healthy trees.

PDA and USDA worked cooperatively in all aspects of the PPV program. PDA had the added responsibility for conducting the statewide survey of commercial orchards, eventually including 52 of the state's 67 counties. USDA provided funding to PDA during the 10 years leading to the eradication of PPV from the Commonwealth. PDA received federal dollars under a series of annual Cooperative Agreements to hire up to 100 temporary employees per season to sample commercial orchards, conduct the necessary lab testing of leaf samples, and assist with

the homeowner survey that USDA supervised. Federal dollars also allowed PDA to purchase and/or lease vehicles for survey work, pay operating costs and purchase survey and testing supplies.

From the initial detection of PPV, it became apparent that communication with growers and the general public in the affected areas would be of paramount importance. PA Agriculture Secretary Samuel E. Hayes, Jr. held a public meeting in Adams County and reaffirmed the state and federal goals to eradicate the virus from the Commonwealth. A dynamic speaker, Hayes was the perfect spokesperson for the program, having served as a Pennsylvania legislator (House of Representatives) during an outbreak of Avian Influenza in the Commonwealth in 1983-84.

Hayes, along with Lyle B. Forer, Director, PA Bureau of Plant Industry, maintained communications with local legislators whose districts were impacted by the virus, worked with federal officials to establish rates of indemnities for growers and met with the impacted growers to secure their cooperation. In addition, Hayes and Forer held periodic press conferences to keep the public informed about the eradication program. After the first year of intensive communication, they began annual meetings with the growers to update them on progress made on the program; USDA staff also participated in these meetings. Hayes' successors (Dennis C Wolff and Russell E. Redding) continued these policies. Forer's successor, Earl M. Haas, a long-time Bureau employee, supported the eradication program, providing the continuity needed to maintain it at the Bureau level.

Penn State Cooperative Extension staff helped immensely in the early years of the program. Master Gardeners from Adams County assisted initially with a day-long homeowner survey that helped develop the logistics associated with this important part of the program. Two Adams County Cooperative Extension staff members assisted USDA and PDA with survey work, introduced growers to eradication staff and assisted with delivering treatment or tree destruction orders to the growers.

Staff at Penn State's Fruit Research and Extension Center in Adams County published a booklet that detailed the detection of PPV in the county, provided historical information on the

virus and had color photos of damage symptoms on both leaves and fruit. The Penn State staff also produced a video on the PPV situation in Adams County and established a web page on PPV. The site was maintained even after eradication was declared. Penn State Cooperative Extension, Adams County, continues to provide outreach on PPV and on replanting of stone fruit trees.

Several Penn State University researchers received support from USDA for additional studies on PPV. Their work provided information critical to eradication success. Chilean stone fruits were sampled at the Port of Philadelphia to determine whether this was a pathway of introduction of the virus. These studies found no PPV-infected imported fruit, indicating that it is not a frequent or a likely pathway (Fred Gildow, pers. comm.). The researcher also worked with USDA staff at their national quarantine facility at Ft. Detrick, MD to study aphid transmission of PPV. A second researcher tested hundreds of plant species (weeds, trees, shrubs, *etc.*) growing in and around the PPV-infected blocks as potential hosts of the virus. His work found no non-stone fruit hosts of PPV in Pennsylvania environs (John Halbrendt, pers. comm.). His team also monitored trap plants that were placed near PPV-positive sites to determine whether a virus reservoir still existed in these areas. A third line of research, performed jointly by Penn State and PDA staff, documented aphid species and their seasonal abundance in Pennsylvania orchards. Before PPV was detected in the state, only aphid species causing direct damage to stone fruit had been studied. The presence of PPV demanded that we document presence of non-colonizing aphids that had potential as PPV vectors.

A trap plant site established to detect aphid transmission from PPV reservoirs. Image courtesy of John Halbrendt, Penn State.

From the beginning of the project, we were fortunate to receive assistance from local, national and international specialists. A Scientific Issues Working Group of specialists was formed by USDA to provide advice and counsel to the eradication program staff. The working

group was composed of state and federal agencies, industry and university personnel. It met sporadically throughout the life of the eradication program whenever an operational question was raised that could be addressed scientifically. In addition, a USDA-supported working group was formed, originally given the name NE1006, "Eradication, Containment, and/or Management of Plum Pox Disease (Sharka)". The group held its first meeting in Gettysburg, PA during the fall of 2000. While the name changed over the years, this group continued to meet annually through the life of the eradication program, with the meeting evolving into a joint USA/Canadian Plum Pox Conference that attracted participants from all around the world, and provided a forum for eradication and management program updates as well as research reports. The interaction these meetings facilitated among various state and provincial operational programs was invaluable in allowing those programs to work effectively.

An early example of the benefits of interaction between the PPV eradication program operational group and the Scientific Issues Working Group involved PPV control recommendations. In 2000, when a positive tree was identified, treatment orders were issued to remove and destroy all trees in the block of trees to which that positive tree belonged. In a residential setting, that meant that perhaps a single tree was removed. In a commercial orchard, thousands of trees might need to be removed. But in either case, sometimes trees were left standing that were situated very close to the positive tree, by virtue of their being in a different block. If the positive tree happened to be on the very edge of a large orchard block, it might have neighboring trees in a second block that were closer to it than were trees in its own block that we required removed. This control strategy made biological sense if the planting stock was the only source of PPV, but did not make sense in the case of aphid spread of the virus. Local operational staff questioned whether aphids could be spreading virus ahead of the detection and removal program. In a conference call in December 2000, the Scientific Issues Working Group considered the question. After thorough evaluation of available literature and robust discussion, the panel members agreed that the most scientifically sound data on aphid movement and vectoring capability, along with considerations of specific PA terrain and land use patterns, consistently supported the removal of trees in a zone of 500 meters around a PPV-infected block. The operations group then incorporated that 500-meter buffer zone into all new treatment orders. While this policy was re-examined on a regular basis throughout the life of the eradication

program in PA, it withstood challenge and remained in place through the life of the program. There is no doubt that this policy contributed to the success of the eradication program in Pennsylvania.

Research Conducted. Europe's long-running battle with PPV meant that many tools had already been developed and were available for the USA to use in shaping its PPV detection and control policies. The international workshop in December 1999 provided a well-informed start for Pennsylvania that would have been impossible if dealing with a new, unstudied disease. However, questions existed that European colleagues could not adequately answer. In part, this was because the virus had already become fairly widely distributed in Europe before the availability of highly sensitive detection tools now accessible. In other words, Europe did not have the same opportunity to investigate a new introduction with the tools available in this century. In addition, some questions arose because we could not directly extrapolate information from Europe to Pennsylvania. To develop a strategy for in-depth understanding of the PPV situation in Pennsylvania, a list of topics arising from the international workshop was developed, and assignments given to develop focus papers in those key areas. From these focus papers, research questions were developed and various organizations or scientists began to address them. Collaboration among the research and regulatory arms of USDA, PDA, and PSU research and extension communities were particularly important in responding rapidly to answer research questions that would shape an effective control program.

Primary areas of investigation included:

- Most appropriate detection tools and protocols (USDA-APHIS-PPQ-CPHST 2010; Schneider *et al.* 2004)
- Aphid species and population densities (Gildow *et al.* 2004; Wallis *et al.* 2005)
- Characteristics of disease spread (Dallot *et al.* 2004; Hughes *et al.* 2002; Labonne & Quiot, 2001; Quiot *et al.* 2006; Schneider *et al.* 2005; Stobbs, 2005)
- Host plants (*Prunus*, other virus reservoirs) (3 Damsteegt *et al.* 2001, 2004, 2007)
- Molecular characteristics of the Pennsylvania virus isolates (Levy *et al.* 2000; Wallis *et al.* 2007; Schneider *et al.* 2011).

As we gained a better understanding of the epidemiology of our situation in Pennsylvania, through investigation of these topics as well as through an increasing body of survey data, we found it essential to re-examine our program regularly to make certain that eradication was still an achievable goal, and that we were using the best available means to reach our goal. This was completed on the scientific level by the USDA Scientific Issues Working Group, as well as at the operational level at PDA and USDA. At the same time, continuous discussion was essential with industry to ensure that the program was designed to be least burdensome to them, and considered the practical consequences of program policy.

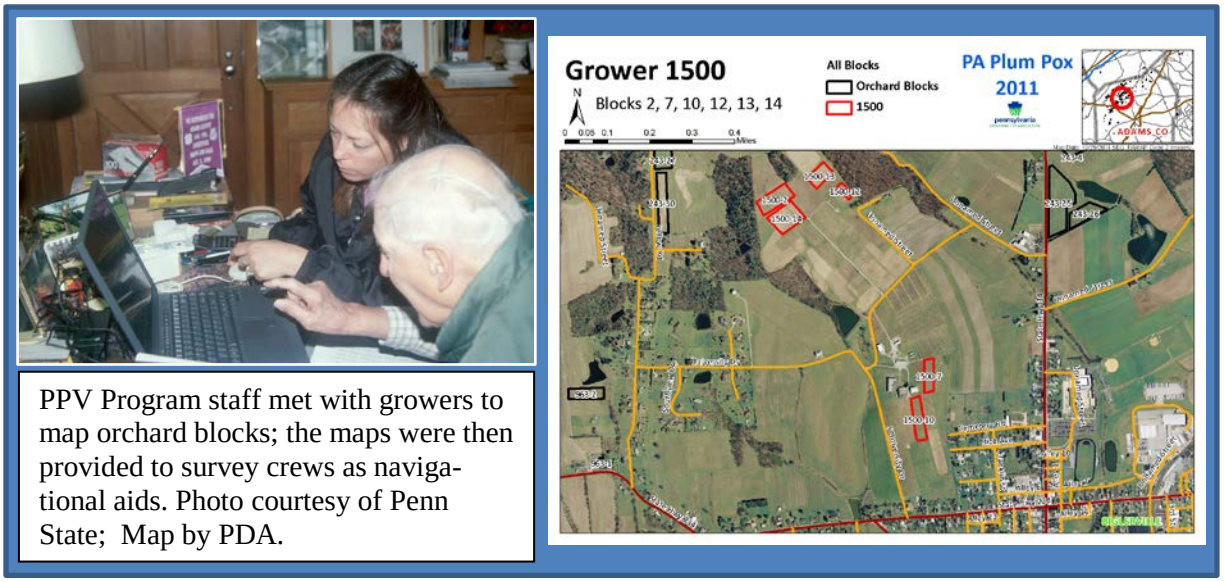
Surveys Conducted

Commercial Orchard Survey. In Pennsylvania, a commercial orchard was defined as any stone fruit tree(s) whose fruit was sold. By this definition a commercial orchard might consist of any number of trees, from a single tree to many acres of trees. Each commercial orchard was further divided into a survey unit termed "block" which was defined in terms of practicality for survey, either as a management unit designated by the owner, or as an obvious survey unit by the survey team. A single block could include more than one species or variety of crop and trees of differing age. The location of each orchard block was recorded with global positioning system (GPS) receivers (Corvallis Microtech, Inc, Corvallis, OR) and incorporated into a geographical information system (GIS), initially using Arcview 3.x or ArcMap software (ESRI, Redlands, CA).

From the year 2000 onward, orchards were sampled using a hierarchical survey method specifically adapted for the Pennsylvania Plum Pox situation (Hughes *et al.* 2002). In any orchard block larger than 200 trees, a total of 25 % of all trees in the block were sampled in groups of four spatially-related trees. Because of the uneven distribution of virus in trees, multiple leaves were taken from each sampled tree, taking those leaves from as many different major scaffolds of the tree as possible. Earlier studies from France (Quiot *et al.* 2006), confirmed informally in Pennsylvania and formally in Canada (Stobbs 2005), indicated that surveyors should avoid leaves at the tips of branches in favor of leaves on older wood, or towards the center of the tree or limb. In Pennsylvania, at least four leaves were collected from a tree. In situations where less than 20 trees were present, or in areas with greater risk of virus presence,

eight leaves were taken from a tree. In very rare instances, sixteen leaves were selected from a single tree. Finally, in cases where a suspect tree was identified, an eight-leaf sample was collected from each major scaffold of the tree. All trees and their associated samples were tagged with a barcode that was specific to the county, grower, block, and individual tree or group of trees that was sampled. A database system adapted from a system provided to Pennsylvania by the Citrus Tristeza Agency in California allowed us to associate these barcode numbers with testing results, in a paperless lab data processing system.

The priority for all Plum Pox survey was the immediate vicinity where the virus was detected (Adams, Cumberland, Franklin, and York Counties). Also, commercial orchards were considered appropriate targets for statewide and national survey. When PPV was discovered in Pennsylvania, there was no detailed information on where all of the *Prunus* orchards were located. Federal and County agencies that worked with crop insurance, conservation, statistics, and education had acreages and some grower contact information but much of this was considered confidential and not readily shared. Project developers needed a detailed and accurate record of size and location of all orchards that could become infected by PPV. During the winter of 1999 – 2000, PDA gathered this information by initiating a mapping project using ArcView 3.0. Penn State was contracted to customize “off the shelf” software so newly hired seasonal employees could collect detailed information about each grower and their *Prunus* orchard blocks without extensive software training. Laptop computers were loaded with the simplified mapping program along with digital orthoquad imagery of areas where mappers would be working. PDA hired temporary personnel and USDA brought in emergency response personnel to meet with growers and gather targeted data. The information collected included grower contact information, pesticide treatment information and posting locations, and a digitized image of each orchard block. The block-specific information included trees per acre, year planted, crop planted, source of the stock, a unique grower ID, a site ID, any unique field names used by the grower to identify the block, who collected the data, and any special information about the block such as 'mixed planting of peach, plum, and pears'. Over six months, almost every known *Prunus* orchard in the state was mapped, with at least some commercial production mapped in 52 of its 67 counties. As new growers and blocks were discovered, they were added into the data set and surveyed as well.



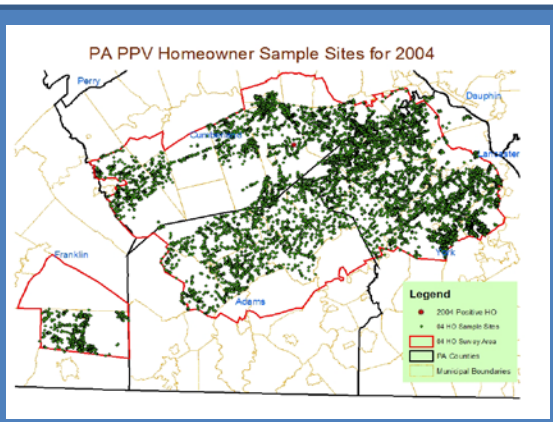
PPV Program staff met with growers to map orchard blocks; the maps were then provided to survey crews as navigational aids. Photo courtesy of Penn State; Map by PDA.

Because Pennsylvania's mountains are not hospitable for stone fruit production, growing areas generally cluster in a few distinct regions of the state, with the majority (over 60%) of all commercial production occurring in a single county (Adams). A statewide survey of commercial orchards was carried out for three years. After that period, survey areas were focused based on perceived risks and available resources.

The geospatial data, and the ability to generate detailed maps with it, was key to the project's success. It allowed for: 1) Ease of navigation to known sites each survey season; 2) efficient development of buffer zones and quarantine boundaries when a positive tree was identified; 3) calculation of acreage to predict number of samples and prepare sample labels before field survey; and 4) calculations and verification of acreage for destruction and compensation purposes. Accurate sample numbers could be projected using various protocols and comparisons of quarantine changes could be examined. The number of temporary personnel needed could be estimated, and disease detections could be traced geographically. In addition, project managers could improve accuracy of budgets, track project progress, and plan much more easily than tracking the information on paper and spreadsheets as had been done in the past.

Geospatial data is only useful if it is maintained. Each year PDA made changes to about 20% of the *Prunus* orchard-block information in the state, keeping pace with orchardist management of their farms and crops.

Homeowner Survey. When PPV was confirmed and eradication was decided upon, project personnel knew that in order to be successful, all possible host plants in and around the infested areas would need to be sampled and tested for the disease. This included the sampling of all residential properties within five miles of a positive site. The five-mile intensive-survey buffer around a positive site was established after several years of survey. Positive sites were being detected every five miles or less from previous year's positive sites. This buffer appeared to be the least distance that could be used to establish the outer limits of intensive survey that would establish the highest probability of detecting isolated infected plants while limiting survey costs.



Homeowner survey images, courtesy of USDA-APHIS-PPQ PPV Eradication Program.

During the summer of 2000, about 2000 properties adjacent to positive orchards were surveyed, generating 500 samples. No positives were detected. All sampling records were kept on paper. That fall and winter, project personnel attempted to gather GPS points of the sampled properties for mapping purposes. This was a difficult task resulting in about 80% accuracy since the person collecting the points had to rely on the address information given on the sample forms. All samples were collected from rural areas where properties are not typically identified by a house number and mailboxes are often grouped together at the end of a lane. Over 10,000 residential properties were sampled during the following year. The number of properties and mode of data collection created a monumental task when locating a property to resample for confirmation of suspect positives samples. Locations were found by tracking barcode sample numbers that were issued to teams and where they were working that date to narrow the search of the property address.

The end of the 2001 survey prompted a search for a better way to track sample collection data since the continued detections of positive orchards and residential sites would expand the survey to a projected inspection of over 40,000 residential properties in 2002. The ideal solution would be to collect all of the data while in the field, along with the GPS way-point, in an electronic format that could be transferred into a central searchable database. At that time, GPS units were relatively inexpensive and a few Personal Data Assistants on the market could be obtained for double the cost of a GPS unit. The challenge was assembling a handheld unit that was capable of collecting GPS and collection-site data that did not cost several thousands of dollars or require extensive post-processing of the collected data. Pennsylvania USDA-APHIS-PPQ personnel had been working with GPS units in the field, using laptops with the units attached via data cables. Based upon that experience, we knew that this was not an option − a person cannot simultaneously hold a GPS unit, hold a computer, and collect data. The initial unit used in 2002 was an Ipaq Personal Data Assistant with a compact flash sleeve attached that held a CF GPS unit. Within a few years, Personal Data Assistant manufacturers were marketing units with built-in GPS for a price comparable to our original stand-alone Personal Data Assistant. This improved the efficiency and reliability of homeowner survey data collection with fewer errors due to poor connections between the GPS and Personal Data Assistants.

In the first years of Homeowner Survey, four leaves were taken from each sampled tree. When the project had determined the extent of the infected areas and removals of positive and exposed trees reduced the incidence level of the disease, sampling intensity was increased to one 8-leaf sample per tree. Every tree was sampled since there was no statistically significant sampling level identified between the hierarchical survey level of 25% sampling and 100% sampling. Each tree within five miles of a positive site was sampled.

Residential survey continued around all positive sites until the regulated areas were removed from quarantine. For an area to be deregulated, three consecutive years of negative survey had to be achieved, followed by three additional years of monitoring survey in a one-mile radius around the positive site.

As scientific knowledge of the Pennsylvania PPV isolates accumulated, we realized that, although we could experimentally infect several cherry species with the isolates (Damsteegt *et al.* 2007), the cherry types were not optimal hosts for the virus and the virus was not easily detected in these species. Therefore (in the eighth year of survey) cherry species were dropped from homeowner survey except on properties where more susceptible *Prunus* species were also present. This change significantly decreased the complexity and cost of the homeowner survey. (Cherry represented two-thirds of all homeowner properties and trees sampled.) The homeowner survey teams then covered more territory when confining survey to most susceptible species. Also, the PPV testing lab was able to handle more samples of those species for which our detection system was most appropriate.

Sentinel Trees. As the eradication program unfolded, surveillance activities identified a hole in the monitoring system: A large core area where all commercial orchard blocks and susceptible homeowner trees had been removed. Nothing was left here to easily monitor, yet virus reservoirs could be present undetected in the area − in a fence row, in a wild area or in an unanticipated host. To fill this monitoring void, a sentinel program was established, placing highly susceptible plant material back into the area. Regular testing of these plants might uncover aphid transmission from unknown reservoirs of the virus located nearby. One sentinel site was established for each five acres of orchard removed. Two or three trees were planted at each sentinel site, including at least one peach and one plum. The earliest sites used Loring Peach and Brompton Plum varieties. As they became available, GF-305 peach seedlings replaced or were added in addition to the other sentinels. All of these varieties were known to be highly susceptible to PA PPV isolates.

Sentinel trees were planted along the edges of the former orchards if the site was planted with row or forage crops. If the site was replanted with a non-*Prunus* orchard crop (such as apple or pear), then the sentinel sites were established within the orchard rows. This helped protect the trees from mowing damage, minimized the need for watering the trees during drought periods and gave the sentinels the benefit of pest and disease control treatments applied within the orchard. Trees were maintained by project personnel who pruned, sprayed, and watered the

sentinel trees as needed. Sentinel trees were sampled and tested for PPV during the spring and fall each year, using both ELISA and real time RT-PCR.

At the peak of the sentinel program, 300 sites were planted with over 500 trees. Sentinel trees were removed and destroyed when quarantine restrictions were lifted from the area in which they were planted. At that point, growers were free to again plant *Prunus*, thus providing a commercial host to monitor. Sentinel removal assured us that these highly PPV-susceptible trees would not be a reservoir for virus should PPV be re-introduced into the area.

The GIS portion of the sentinel survey project was a very basic data set that was comprised of a waypoint for each site established, with each assigned a unique identification number. This information was maintained by USDA-APHIS-PPQ personnel throughout the project. The information was simple but vital, because each year new personnel would occupy temporary positions to sample and maintain the trees at the three hundred sites distributed throughout the core infected area across four counties. Many sites located along field edges and wood lots would quickly become overgrown during the growing season and would have been difficult to locate without a GPS. The site data enabled crews to easily locate the sites at each farm. The data was also used by permanent personnel to track work progress and to identify sites that needed trees replaced or removed as areas were deregulated.

Seedling/Sucker. Field crews assigned to maintain the sentinel sites also inspected previously-positive orchard sites for three years after tree removal, searching for any growth of seedlings and root suckers. All host plants found were sampled and then destroyed. Positive orchard blocks found in the first years of the project generated a lot of positive suckering root shoots, requiring extra work to clean the fields. As the project progressed and the disease incidence levels decreased, less positive plant material was detected. All positive plants found were suckering roots; no uncultivated seedlings found ever tested positive for PPV. Plum and cherry rootstocks were prolific producers of root suckers; peach trees typically did not produce root suckers under Pennsylvania conditions, although the active search during the Plum Pox program noted that peach orchards that were removed during hot dry drought conditions did indeed generate root suckers.

Wild Bloom / Feral Trees. Locating orchards and homeowner trees was relatively straight-forward, and these two programs produced nearly all the leaf samples collected for testing. However, a major concern was the presence of wild trees and cull piles where *Prunus* seedlings, saplings, or even mature trees could be present. PDA and USDA cooperated to carry out a Spring Bloom survey. Stone fruit trees are among the first to flower in late winter-early spring (in some years, apricots began to blossom in February). Teams of two people each would drive through the quarantine areas, with emphasis on the 500-meter buffer zones around known positive sites, searching for blooming wild stone fruit trees. When such trees were detected, they were sampled and tested, the property owners were contacted, and arrangements were made to remove and destroy the trees. In addition, several growers reported wild trees on their properties or ones they had seen while driving through the quarantine areas. This project was successful and resulted in the detection and removal of hundreds of wild trees. Fortunately, no wild trees were found to be infected with PPV.

PA Dept. of Conservation and Natural Resources staff assisted in identification of *Prunus* and other plants in woodland settings. Image courtesy of Penn State.

Abandoned / Untended Orchards. Another potential reservoir for PPV was abandoned or untended orchards. During the first 10 years of the program, a few abandoned *Prunus* orchards were found and mapped by PPV program staff. Realizing the danger these trees would pose as reservoirs for the virus, a policy for their removal was developed. Property owners were reimbursed for tree removal and destruction, but no indemnities were provided for lost fruit production.

Uninfected Orchards in Quarantine Areas. The removal of hundreds of acres of stone fruit trees and the moratorium on planting in quarantine areas created an unexpected problem for growers who had stone fruit trees remaining in quarantine areas. Many of these trees were older

and past their prime fruit-bearing years. Since these growers were under a quarantine order prohibiting planting of new PPV-susceptible trees in the area, they could not replace dead trees or plant new blocks. If growers wanted to sell any stone fruit, then they were forced to retain trees they would have otherwise removed in a normal cycle of orchard rejuvenation. The PPV program was concerned that, if virus infected these much older trees, it would be more difficult to detect. A program was developed that offered growers; 1) an opportunity to remove these older trees; 2) reimbursement for removal and destruction costs; and 3) reimbursement for 70% of the lost fruit production costs. Sixteen growers took advantage of the program and removed 37 blocks, totaling 184 acres.

Statewide Landscape-Nursery Survey. PDA took advantage of a statewide staff of 18 people responsible for plant inspection in garden centers, production nurseries, greenhouses, and Christmas tree plantations. Plant Inspectors randomly sampled *Prunus* from ornamental nurseries and retail garden centers annually. This action was a proactive measure intended to survey these outlets and ensure that landscape *Prunus* were not infected with PPV. No retail landscape *Prunus* were found infected with PPV.

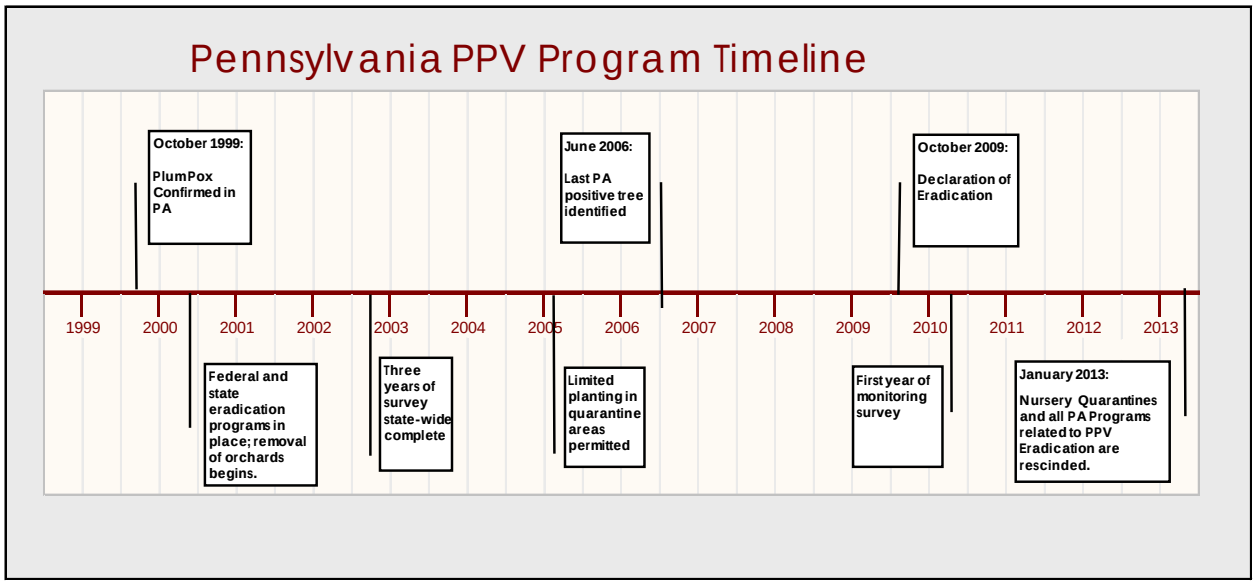
Statewide Production-Nursery Survey. With the establishment of the initial Plum Pox quarantine in October 1999, no PPV-susceptible nursery stock production was permitted in a quarantined area. By 2002, Pennsylvania regulation prohibited propagation of PPV-susceptible source material (either budwood or rootstock) within the PPV quarantine zones or within one mile of any quarantine zone boundary. In addition, any propagator of susceptible *Prunus* within the entire Commonwealth was required to have all budwood sources tested for PPV. Two nursery establishments entered into compliance agreements with the PDA that allowed them to store dormant *Prunus* in cold storage/shipping facilities within the quarantine zone. Because of restrictions on *Prunus* propagation on land that had traditionally been nursery ground, Pennsylvania fruit tree nurseries were forced to move away from the Adams County area.

Peach seedling nursery bed.
Photo by Sarah Gettys, PDA

Special arrangements were made with neighboring states and with USDA, for inspection and testing by the PDA, so that the Pennsylvania nurseries involved could still meet specialized virus-free certification regulations.

Because of a PPV-positive nursery find in 2003, roughly five miles from any quarantine boundary, an effort to expand the nursery quarantine restrictions in an area around the primary PPV quarantine zones was initiated in 2004, completed January 2005. The expanded nursery quarantine zone covered all primary quarantine areas for three years after the primary quarantine has been rescinded. It also covered any additional area within 11.5 km (7.15miles) of a PPV-positive found in the previous three years. This expanded area did not prohibit the sale of *Prunus* from retail sites that simply resold material bought from elsewhere, but it did prohibit propagation or long-term grow-out of *Prunus* near quarantine areas. This lessened potential exposure of nursery stock to aphids that may carry PPV, and subsequent movement to areas where it could be the source of a new PPV infection focus.

Although nursery sampling was a small PA survey component in terms of time and sample numbers, this portion of the survey was absolutely critical because of the risk of long-distance movement of virus within or outside Pennsylvania. After adjustments to nursery quarantine restrictions were made in 2003, all nursery and budwood source tree samples tested negative for PPV for the remainder of the life of the program.



Diagnostics. The Plum Pox eradication program lab functions benefited from a foundation of experience and experimentation already in place within USDA, both at APHIS and ARS. For years, a USDA PPV study group had been working pro-actively to develop means of detecting PPV if it arrived in the USA. Dr. Laurene Levy was part of a team that assisted in diagnosis of PPV when it was first detected in Chile during the 1990s; subsequently she headed an APHIS lab at the U.S. National Quarantine facilities in Beltsville, MD that was fully equipped for PPV diagnostics. Dr. Levy quickly developed a national ELISA testing standard, and also provided extensive confirmatory testing and strain typing services for the program. The USDA-ARS team in Fort Detrick, MD, was also positioned to answer research questions related to Plum Pox introduction and establishment in the USA.

Because of their experience with available ELISA reagents, USDA was able to name a national testing standard based on a commercially available ELISA kit that incorporated the universal PPV monoclonal antibody 5B-IVIA (Cambra *et al.* 1995). When difficulties arose with the international distribution of ELISA kits, USDA quickly considered other options. A new ELISA kit from Agdia Inc, based on a Canadian polyclonal antibody source, was validated and accepted as a national standard for use in the 2006 survey season (USDA-APHIS-PPQ-CPHST, 2008).

Molecular detection tools have changed over time. In 1999, PDA relied completely on USDA for all molecular testing of suspect or positive samples. In 2003, USDA-ARS provided a new real-time RT-PCR test to Pennsylvania that has become a powerful confirmatory tool for borderline ELISA test results (Schneider *et al.* 2004). This allowed a certain volume of work to shift from USDA to a state lab. With the real time RT-PCR in place at the state level, only ELISA-positive samples from outside established quarantine zones were sent to USDA for confirmation, along with any samples yielding particularly strange results that occasionally arose and generated concern at the state level. Throughout the life of the PPV eradication program, USDA continued to run an extensive range of molecular tests, including immunocapture RT-PCR, conventional RT-PCR, and real-time RT-PCR, and took the lead in developing and evaluating new procedures and diagnostic platforms as they became available (CPHST, personal communication).

Public Relations. Good communications are prerequisite for any program to be successful. From the beginning, the Pennsylvania PPV program partners tried to make program information readily available to all stakeholders. Public meetings and press conferences were successful in providing information on the program to large numbers of people. However, equally important were all the communications necessary to make the program run smoothly. Periodic meetings of program staff members were held throughout the year, especially during the survey season, and these were attended by state, federal, cooperative extension, and university personnel. Updates were given on progress made and situations encountered. In addition, the USDA National Plum Pox Virus Program Coordinator typically provided a periodic report on samples collected, tests performed, homeowner survey results, *etc.* PDA issued press releases when the virus was detected and when quarantines were put in place or rescinded in a regulated municipality.

At the end of each calendar year, USDA and PDA prepared an annual report with input from all other agencies and individuals involved. The reports included information of any detections of PPV, numbers of samples taken and tested, homeowner samples, maps of the areas quarantined for PPV, and any program notices published in the Pennsylvania Bulletin.

Good communications were especially important when dealing with people who were ordered to remove and destroy trees, including growers and homeowners. When a commercial stone fruit tree tested positive for PPV, the grower was contacted and informed of the test results. An appointment was made for the USDA and PDA staff to present the grower with treatment orders, explaining them and answering any questions. Growers also were provided with the necessary paperwork and reimbursement forms. When requested, state and federal staff assisted growers with completing the paperwork.

Homeowners whose trees were either infected with PPV or were within a 500 meter buffer zone were given treatment orders along with information on how to proceed with tree removal. Typically, homeowners were given the option of removing a condemned tree or hiring a vendor to do the work. Homeowners received $25 for each tree in the lawn or managed portion of their property plus the cost of removal and destruction. PDA also reimbursed homeowners for

removal and destruction costs associated with any wild trees on their properties but did not provide any indemnity for these trees. Whether processing claims of growers or homeowners, the goal was to have the application approved within two days in either agency so it could be forwarded for final approval and payment.

Changes to Compensation and Planting Restriction Policies. Over the course of the eradication effort, a number of restrictions on planting of *Prunus* were put in place that changed the baseline information used in developing appropriate compensation levels for affected commercial growers. As concerns were identified, several changes in indemnity payment programs were adopted.

In the first Pennsylvania Plum Pox Virus Quarantine established in October 1999, movement of regulated commodities was prohibited both *out* of the quarantine area and *within* that area. Restricting movement *within* a quarantine area was unusual, but the rationale was that the quarantine area was large enough that positive trees from an already-infested area could be moved to a new area, establishing a new focus of infection within the quarantine zone which might take several years to identify. In a subsequent order in August 2001, the language was strengthened to say "This quarantine order also prohibits the planting of stone fruit trees (apricot, nectarine, peach and plum) in the quarantined area. This prohibition applies to both fruit-bearing and ornamental varieties of stone fruit trees." Creating a moratorium on planting essentially locked the host universe in quarantine areas; once host material had been identified for survey, no additional individuals or groups of trees should be found in subsequent survey years. This made survey more manageable, especially the very time-consuming and difficult homeowner survey; allowed confirmation that material was not moving within the quarantine area; and prevented new bridges of host material from being built for aphids to move PPV ahead of survey and detection capabilities. The moratorium was an attempt to prevent an increase in virus reservoirs during the eradication phase of the program.

The planting moratorium was most difficult to communicate to homeowners. A campaign to educate homeowners, municipalities, and landscape contractors was initiated to get the message out. USDA included information about the moratorium in mailings sent out ahead of

homeowner survey. Homeowner survey crews distributed information about the moratorium to all surveyed properties. All PA-licensed nurseries and nursery dealers in zip codes within quarantine zones and bordering quarantine zones received annual mailings, and many were visited by PDA Inspectors. Many local nurseries and garden centers just outside quarantine zones posted maps and information so that homeowners shopping at their facility would be aware of the moratorium.

While the moratorium was not as difficult to communicate to commercial growers, they were the ones that felt its impact most strongly. The initial compensation payments made to growers for lost fruit production were calculated based on a 3-year ban on planting. When eradication was not accomplished within that time, the budget was reviewed and deemed sufficiently healthy that growers were offered an additional two years of payments.

The five years of indemnity payments for the growers initially impacted by the virus ended in 2004. A new program for replanting trees was offered to growers who had exhausted their indemnity payments and were still in a quarantine area subject to the planting moratorium. The following replanting options were made available to these growers beginning in 2005. *1) Filling in missing or dead trees in an existing stone fruit orchard.* Growers were permitted to replace missing or dead trees in existing orchards; however, limits were imposed on the rate of replanting. In the first year of the replanting process, a grower could replant 20% of the original number of trees in the block. In the second year, no more than 10% of the original number of trees could be added, and the third year, 5% of the original number. Adding new trees to existing blocks was permitted as long as the trees were planted within the boundaries of the original block. If these blocks subsequently tested positive for PPV, the growers would be eligible for grants for removal and destruction of the trees, replanting costs and lost fruit production costs. *2) Planting new blocks of stone fruit trees in an existing quarantine area.* Eligible growers were permitted to plant a percentage of the stone fruit trees they had to remove and destroy as a result of PPV. Planting would be approved only on specific ground that fell at least 1.5 km from any positive found in the previous two years and 600 meters from any other existing commercial *Prunus* block (other than tart cherry). These trees would not be eligible for

any compensation if PPV were detected in the newly planted blocks or if the newly planted blocks fell within a 500 meter buffer zone from a positive tree or block.

Results of PPV Program Activities. More than two million samples (2,012,053) were collected and tested in Pennsylvania from 2000 through 2009. Commercial orchard survey was conducted across all stone fruit growing areas of the state. While samples were taken from 52 PA counties, PPV-positive samples were identified from only four; sample results for those four counties are shown in Table 1. The vast majority of the 459 positive samples were from peach or nectarine trees (*P. persica*), which are also the dominant commercial crops in the area. Fruit-bearing plum trees were second in frequency of positive detections, followed by very infrequent finds in flowering almond (*P. glandulosa*) and apricot.

Table 1. Four-County Sample Numbers by Year for Adams, Cumberland, Franklin, and York Counties, 2000 - 2010

Year	Orchard Samples	Homeowner Samples	Other Samples	Total Samples	Total Positives	% Positive
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	155,970	36,530	6,845	199,345	11	0.006
2004	166,306	42,730	2,059	211,095	4	0.002
2005	213,005	51,158	3,280	267,443	5	0.002
2006	166,568	45,702	4,418	216,688	6	0.002
2007	173,180	44,295	2,689	220,164	0	0
2008	218,198	10,230	2,634	231,062	0	0
2009	204,251	10,843	264	215,358	0	0

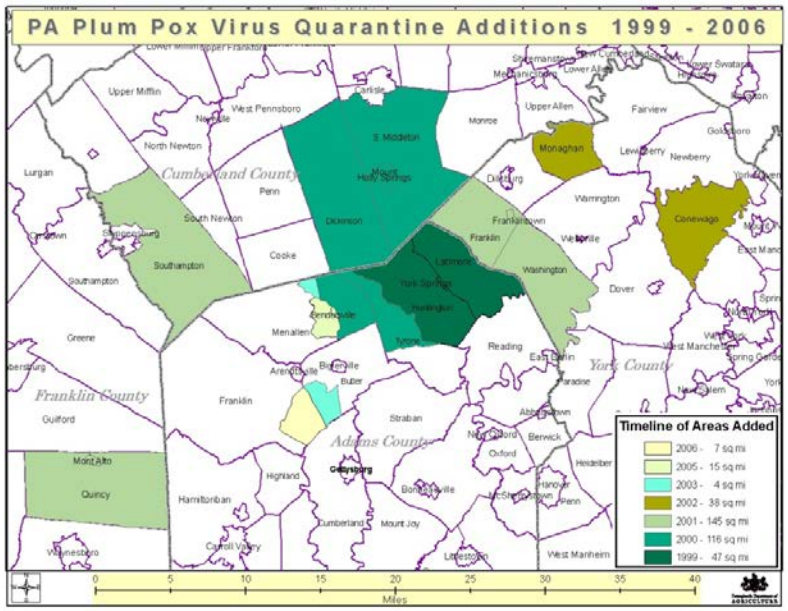
Over the course of the program, some sampling occurred in every month of the year from March (dormant budsticks) through early November (many leaves already yellow and falling from trees). The majority of sampling took place from mid-May through August when abundant

seasonal labor was available. An overwhelming majority of PPV-positive samples were collected in May and June, although some positive samples were collected in every month sampling took place. Although weather patterns influenced suitability of survey in an unpredictable way, survey of this size needed to be planned ahead and could not easily be switched on and off without jeopardizing ability to retain sufficient trained labor to accomplish the work. Positive detections during suboptimal months indicated that survey was not wholly unproductive then. An effort was made to sample highest risk geographic areas in the optimal window for virus detection, and then to cycle other sampling so that different orchards were sampled in the optimal sampling window each year.

Pennsylvania sampling confirmed that virus could be detected in many parts of a plant, including fruit, flowers, and leaves. Leaf sampling made best logistical sense for a large-scale survey effort, both in terms of the timeframe when the sampling unit was available, and the storage characteristics of the samples. Symptoms were occasionally noted on blossoms, fruit, and leaves in PA, but many trees that tested positive had no obvious symptoms at the time they tested positive.

Pennsylvania sampling also confirmed the erratic distribution of detectable virus in a tree. When a positive tree was identified, it was resampled scaffold-by-scaffold. Trees were found that were uniformly positive throughout the tree, but more frequently only a portion of the tree tested positive – sometimes only a small portion of one branch would test positive. Since positive trees were removed the season they were identified, it was not possible to record change over time in virus distribution within the tree.

At its peak, the PA PPV quarantine area covered more than 300 square miles. Approximately 1,600 acres of commercial stone fruit orchards, roughly 20% of all stone fruit orchards in the Commonwealth, were removed and destroyed as PPV-positive and exposed blocks. Tree removal from commercial and non-commercial settings is summarized in Table 2.



Although extensive investigation was attempted both formally through the USDA-APHIS Investigative and Enforcement Service, and informally by the PPV operational team, the original source of the Pennsylvania PPV infestation was never identified. It did appear from the distribution pattern of positive trees that primary spread of the disease once introduced into Pennsylvania was via aphid vectors, although some assisted spread through human activity could not be ruled out.

Sequence analysis of PPV isolates from Pennsylvania showed some variation, separating into two clades within the PPV-D strain group (Schneider *et al.* 2011). However, because PA clades were more closely related to each other than to PPV-D sequences from other geographic areas (either in North America or worldwide), and since individuals from both clades were present in the single geographic epicenter of infection in Pennsylvania, the possibility existed that the two clades were present in or arose from a single introduction event. Only one PPV positive find in Pennsylvania was obviously different from all others: In 2006, two PPV-positive plum trees were identified through the homeowner survey. Although also strain-D, the PPV isolate from these two trees had a significantly different sequence identity, more closely related to some European isolates than to other Pennsylvania isolates (Schneider, personal communication). This find, in the very last year that PPV positives were found in Pennsylvania, was a shock. It apparently represented a separate introduction of PPV into Pennsylvania, found

only because of the homeowner survey design to find spread from the originally-identified infestation. How many other introductions could be lying outside the homeowner survey area – in Pennsylvania and in the USA?

Cost of Eradication Program and Economic Impact. Stone fruit production is an important industry in Pennsylvania. In data summarized by the National Agricultural Statistics Service, Pennsylvania consistently ranks fourth or fifth in stone fruit production among the states. The state had 5,344 acres of peaches in 1997, with a production value of approximately $24 million. In 2008, peach acreage had dropped to 3,860 acres, but growers anticipated increasing acreage significantly over the next several years (USDA, NASS-PA Field Office. 2010).

As with any functioning economic system, direct impacts of a single change in the system are relatively simple to document, while the "ripple effects" to the economy require careful study to unravel. The easily measurable costs of the Plum Pox eradication program in Pennsylvania include trees removed and destroyed (Table 2), survey program expenses (Table 3), and money paid out for control actions and indemnification (Table 4). With over 59 million dollars paid out by USDA and PDA together to cover survey, control costs, and compensation to affected parties, it is clear that eradication does not come cheaply. But those are simply the costs that are easiest for us to measure. Additional costs of the PPV eradication program that USDA and PDA did not measure include: 1) Lost market share of Pennsylvania stone fruit nurseries and orchards; 2) loss of income due to prohibition on planting in quarantine areas; 3) loss of income for laborers traditionally employed in the stone fruit orchards that were destroyed; 4) shifts in labor availability as those laborers were forced to look elsewhere for sufficient work; 5) losses of income to packing houses and other post-harvest operations relying on stone fruit production; and 6) general loss to the local economy associated with the disruption in the stone fruit industry. Expenses that are not catalogued here also include production of educational materials and programs, largely done by Penn State; cost of research programs addressing operational needs of the program, many supported by USDA funds but some by PDA and other sources as well; cost of survey conducted in other states to assure trading partners that fruit and nursery stock were uninfected; and cost of PA monitoring surveys that continued beyond 2009.

Table 2. PPV ERADICATION PROGRAM, 1999 - 2009

ACREAGE REMOVED & DESTROYED/PLANTS REMOVED & DESTROYED

Category	Acreage or #Plants Destroyed
Commercial trees infected with PPV or within 500 meters of infected trees	**1,598 acres**
Voluntary removal and destruction of uninfected commercial trees within PPV quarantine areas	**184 acres**
Stone fruit nursery stock	**54 acres**
Untended (abandoned) orchards	**10 acres**
Total acreage destroyed	**1,846 acres**
Landscape nurseries, garden centers	**1,420 plants**
Homeowners	**1,174 plants**
Total plants destroyed	**2,594 plants**

Table 3. Operating Costs for PA PPV Eradication Program, 2000-2009*	
Federal	$ 23.7 million
State	$ 5.2 million
TOTAL	$ 28.9 million

* Figures based on USDA's Operational Budget for the PA PPV Eradication Program, including USDA/PDA Cooperative Agreement financial reporting.

Table 4. PPV ERADICATION PROGRAM, 1999 - 2009

PAYMENTS	PDA COSTS	USDA COSTS
TO COMMERCIAL FRUIT GROWERS		
Removal & Destruction	**$1,578,841**	**0**
Indemnities for lost fruit production	**$5,627,094**	**$22,067,223**
Replanting	**$ 200,099**	**0**
Vector Control	**$ 81,673**	**0**
Herbicides	**$ 79,917**	**0**
Lost Inputs	**$ 393,732**	**0**
Sub-Total, Commercial	**$7,961,356**	**$22,067,223**
TO OTHERS		
Homeowners	**$ 74,000**	
Landscape Nurseries and Garden Centers	**$ 191,821**	
Sub-Total, Others	**$ 265,821**	
TOTAL	**$8,227,177**	**$22,067,223**

Keys to Success

Detecting recently introduced invasive species is a challenging task and eradicating these unwelcome invaders is an even greater challenge. The successful eradication of PPV from Pennsylvania resulted from a combination of timely events, key decisions and some good fortune.

Strong Industry. Our first good fortune came when a conscientious grower sought answers for his blemished peaches from a number of specialists until one of them suggested that PPV

could be the cause of the problem. Without the grower's persistence, PPV would not have been detected as early as it was.

This circumstance highlights the importance of vigilance on the part of growers. They are in the orchards daily, and are in the best position to notice when there is a problem. State and federal regulatory agencies will never have sufficient personnel and funds to carry out all the surveillance necessary to detect invasive species. Clearly, the viability of American agriculture depends, in part, on proactive pest detection programs designed with a strong industry outreach and participation component.

Funding. Adequate funding paved the way for our success. Most importantly, funds allowed compensation for stone fruit growers and stone fruit nurseries for their lost fruit production and lost nursery stock. Funds allowed compensation for removing and destroying trees, replanting efforts, and spraying for aphids and weeds. The funding was critical to growers surviving this epidemic.

Funding provided by USDA allowed PDA to purchase the necessary supplies and equipment for field sampling and lab testing of the leaf samples collected from stone fruit orchards, homeowner trees, stone fruit nurseries, and landscape/garden centers. Federal dollars supported PDA's costs for hiring seasonal staff and purchasing/leasing vehicles and their operating expenses as well as research/testing by PSU staff that helped immensely in the eradication program.

Fortunately, sufficient state dollars were committed to the program in its early stages. PDA agreed to share costs with USDA in compensation to growers and stone fruit nursery owners for stone fruit trees that were condemned because of the presence of PPV. Although USDA paid 85% of lost fruit production costs, PDA provided 100% of the monies required to remove and destroy trees, replant stone fruit blocks with non-PPV host material, spray for aphids and weeds, and recover cost of lost inputs (money invested in stone fruit trees prior to removal and destruction). In addition, PDA paid 100% of the costs for the residential tree removal program.

The timing of the need for funding was fortunate for both PDA and USDA. In the early years of the eradication program, during a booming economy, sufficient state funding was committed to the indemnification program to sustain it through 2009, the year eradication was declared. The most costly years of the program were 2000-2004 when most PPV-infected stone fruit trees were detected, removed and destroyed. From 2005-2009, fewer positive trees were found, which meant fewer funds were needed for compensation. From state and federal revenue surpluses in 1999, the country moved into a period of unusually low revenues and budget deficits. Had PPV first been detected in Adams County in 2009 instead of 1999, the necessary amount of state and federal funds may not have been available to us, undoubtedly having an enormous impact on the eradication program. At the very least, it would have prolonged the work, but may have prevented us from pursuing eradication at all.

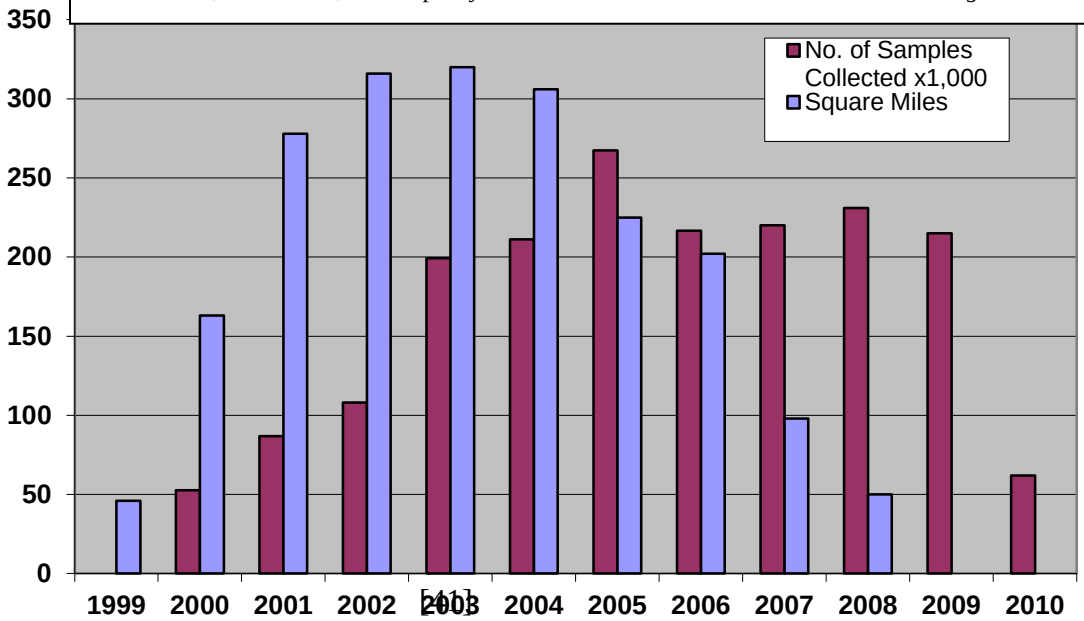
Collaboration between State and Federal Agencies. Long before detection of PPV, PDA and USDA staff in Pennsylvania had worked cooperatively on several pest mitigation programs, including Cereal Leaf Beetle, exotic pest surveys, invasive weeds, Asian Longhorned Beetle, and Gypsy Moth. The staff knew one another and had the benefit of a long-term positive working relationship. Neither group of workers had a political agenda, and PPV eradication became the focus for the next ten years. Having several agencies cooperating allowed the optimization of program operational efficiencies. Sometimes, one agency could easily perform a program function when another agency was blocked by procedural dictates outside program control.

Part of the reason for the good relationships was the close proximity of offices in Harrisburg, only ¼ mile apart. All staff met frequently, and typically attended common training sessions. Shortly after the detection of PPV, USDA established a satellite office for the PPV program in Carlisle PA, about 20 miles from Harrisburg. This site and location actually increased the efficiency of the eradication program because it was larger and allowed USDA to house more program staff. This office was also closer to the PPV quarantine area. The close proximity of offices meant that leaf samples collected by USDA crews could easily be driven, rather than shipped, to the PDA lab for testing.

Cooperation of Affected Growers and Homeowners. The Program was extremely fortunate that the affected growers cooperated. While the indemnification funding made a huge difference, there was clearly more than that behind their sacrifices. Generally, growers had never heard of PPV before the Pennsylvania detection. They learned very quickly, and immediately mobilized to proactively shape the program, supporting eradication while ensuring that grower concerns were addressed. They totally supported the efforts, even assisting where possible, such as reporting locations of wild stone fruit trees, or providing navigational or on-farm support to PPV survey crews. Fortunately, all the impacted growers remain in business today, although not all grow peaches. The program also dealt with hundreds of homeowners who were required to remove and destroy stone fruit trees on their properties, and these people were good cooperators. Only on one occasion did we require a police escort in order to deal with an issue involving condemned trees.

The initial PPV detection can be attributed to a grower, and the first crucial months of program development were guided by grower input. But a program spanning a decade requires active, evolving support and cooperation from the industry throughout its program life. To be effective, survey had to be repeated on the same farms across many years, and survey actually had to intensify as virus levels decreased. A smart and cohesive industry was essential to maintain cooperation of its members, keep pressure on funding sources, and allow the work to be completed.

Pennsylvania Primary Quarantine Area Size and Survey Size Over Time. While quarantine area (blue bars) reached a maximum size in 2003, declining to zero in 2009, sampling (red bars) plateaued between 200,000 and 250,000 samples/year in 2003 and was maintained at that level *through* 2009.



Assistance from Penn State. PSU Cooperative Extension, entomology and plant pathology faculty provided much assistance during the project. Production of outreach materials (video, written material and a PPV web site) all helped educate the public and growers on the virus. Research on transmission of PPV and surveillance/testing of imported stone fruits at the Port-of-Philadelphia all contributed to increase our knowledge of PPV. Work by a PSU Ag Economist in the early stages of the program was critical in developing an indemnification table that was used as the basis for compensating growers for lost stone fruit production. Penn State also took the lead in producing a recovery plan for plum pox virus under the auspices of the National Plant Disease Recovery System (USDA-ARS 2007).

Support from State and Federal Administrators / Legislators. From the beginning of the program, local legislators supported it by appropriating the funds that PDA needed to pay indemnities to the affected parties. Pennsylvania's congressional staff also assisted. USDA staff cooperators assisted by preparing a PPV emergency declaration which set the stage for making federal funding available to Pennsylvania's stone fruit growers. Support at all levels of government paved the way to a successful program outcome.

October 29, 2009. Declaration of PPV Eradication in Pennsylvania included a gathering of partners from USDA, PDA, local government, and the fruit industry. A ceremonial tree planting marked the end of all planting restrictions in Pennsylvania. Photo courtesy of PA Department of Agriculture.

Reviewing/Revising the Program. The program that began in late 1999 underwent several changes during the 10 years leading to the eradication of PPV. The PPV program was the largest and most intensive plant protection program in Pennsylvania that the current staffs of USDA or PDA had ever encountered. The biggest challenges were initiating the program with little knowledge of the virus and the absence of any previous situations that we could reference involving successful eradication of PPV. Knowledge gained from communications with many virologists, statisticians, and people engaged in virus survey work and lab testing provided the initial information that helped move the program forward.

However, after the program was begun, change became the rule rather than the exception, driven by the knowledge gained about the virus and the various situations encountered during field survey, lab testing, and related research work. As our scientific knowledge increased, change was both necessary and inevitable. Some examples of changes made include:

- As the size of the quarantine area expanded, so did the numbers of growers affected by PPV – whether a positive tree was detected in their own orchard, or their orchard fell within a buffer zone from a positive find on a neighboring property, or they were simply trapped in a quarantine zone. Growers had to continually learn and adapt to the changing situation, often becoming trainers and advisors for other newly impacted growers.
- The size of the seasonal crew hired by USDA and PDA to perform survey and laboratory work nearly doubled in the first few years, eventually topping 100. More employees were needed to collect and process more leaf samples in order to detect the virus more quickly. The full-time seasonal lab staff was expanded to include a part-time crew that worked from 3:30 – 8 PM Monday – Thursday, helping us to decrease the backlog of samples.
- Likewise, sampling intensity, especially in the five mile radius around infected blocks of trees, was increased to speed up detection of the virus.
- After 2003, Real Time RT-PCR was routinely employed by PDA as a supplemental testing tool whenever elevated readings were encountered during routine ELISA testing.

- Logistics to accommodate an expanding survey were constantly modified. For example, a mobile home provided by USDA, deployed as a field office, facilitated stationing a sampling crew in Adams County thus eliminating the need for these workers to drive to Harrisburg, pick up their state vehicles and return to Adams County to begin sampling. Travel time saved meant more sampling time each day! Another example of logistical change involved procurement protocols. Federal requirements to purchase "sole source" items such as PPV test kits were streamlined as compared to PDA's guidelines. Consequently, USDA purchased these items, thus saving PDA staff up to a 6-week waiting period for permission to purchase them. USDA's approval process required only two days.
- Training programs were fine-tuned and improved. Field and lab workers were cross-trained and melded into a single team, thereby allowing all workers more opportunity to vary their experience, a practice that definitely boosted morale. Requiring all seasonal employees to receive defensive driver training paid big dividends in terms of avoiding a rash of minor accidents following the first season of survey work. No serious injuries were incurred, but vehicle downtime presented a problem.
- Establishing a 500 meter buffer zone around each infected block of trees and ordering removal and destruction of all stone fruit trees within the buffer zone also helped shorten the eradication program.
- When PPV was first detected in each of the counties (Adams, Cumberland, Franklin, and York), quarantines were established on a township basis as a conservative measure. As less virus was being detected in the program, and more testing history was accumulated, the size of any new quarantine areas were made as small as possible to reduce impact on the industry yet not create any undue risk for stone fruit producers.
- Use of hand-held data recorders in the residential survey program allowed for electronic storage of data, a major improvement over the paper records kept during the first two years of the program.
- An industry request to revisit crippling planting moratorium policies led to several permit-based programs that allowed limited planting in low-risk situations in PPV quarantine zones.

- Research in France and in Pennsylvania highlighted the risk associated with aphid transmission from infected fruit to trees, sparking an initiative to incorporate proper disposal of off-farm fruit into industry best management practices.

When PPV was found in Pennsylvania, there was no precedent and no guarantee that eradication would be achievable. Basing regulatory policy on existing scientific knowledge, while developing a robust system for continuing input from industry representatives, survey operations, and research scientists, allowed the evolution of policy and practice that kept eradication strategy viable.

Acknowledgements

The dedication, insight, and hard work of so many are reflected in the Pennsylvania plum pox story; we cannot provide a complete list of participants. We acknowledge our admiration for the stone fruit growers of Pennsylvania, and particularly those who were directly affected by PPV, whose patience and forward-thinking throughout this program were key to the success achieved. The Pennsylvania State University research and extension were always at the ready to assist growers. On that team, Jayson Harper in Agricultural Economics, James Travis, John Halbrendt, Frederick Gildow, and Greg Krawczyk, among others, provided critical needs throughout the life of the program. The Cooperative extension staff in fruit trees (Lynn Kime, Tara Baugher, Bill Kleiner) kept outreach and communication strong. Finally, we acknowledge the assistance of:

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- Administrators, Scientists, and Officers in USDA, including:
  - administrative, budget, IES
  - USDA-APHIS-PPQ
  - PPV Program Managers and Staff

- Eastern Regional Office
- Headquarters
- Pennsylvania SPHD's office and domestic staff
- CPHST, including the Scientific Issues Working Group participants
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List of Acronyms

APHIS – USDA's Animal and Plant Health Inspection Service
ARS – USDA's Agricultural Research Service
ELISA – Enzyme-Linked ImmunoSorbent Assay
GPS – Global Positioning System
PA – Pennsylvania
PDA – The Pennsylvania Department of Agriculture
PPQ – USDA-APHIS' Plant Protection and Quarantine
PPV – Plum Pox Virus
PSU – The Pennsylvania State University, Penn State
RT-PCR – Reverse-Transcription Polymerase Chain Reaction
USA – United States of America
USDA – The United States Department of Agriculture