An Epidemiological Comparison of the US and Canadian Plum pox virus Eradication Programs

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Abstract
Plum pox virus (PPV) was first detected in North America in 1999 in Pennsylvania, and the following year in Ontario, Canada. In response to these outbreaks, both countries implemented eradication programs in an effort to eradicate the virus before it could have a significant effect on the Prunus industry in their respective countries. The objectives of this study were to: (i) quantify the impact of the US and Canadian PPV eradication programs on the spatial and temporal dynamics of PPV in Pennsylvania and Ontario; and (ii) compare the detection efficiencies of the US and Canadian PPV sampling systems. Ripley’s K function revealed PPV-positive Prunus blocks in Pennsylvania to be clustered between distances of 0.7 and 4.3 km in 2000, while in Ontario, PPV-positive blocks were clustered between distances of 1 and 25 km over the period 2006-2009. A simulation model was developed to determine the relative detection efficiencies of the US and Canadian PPV eradication programs. The US eradication program was found to have a detection efficiency of 71.7%, whereas the Canadian eradication program had a detection efficiency of 40.5%. The data generated in this study should help to improve the PPV eradication programs currently used in the US and Canada, as well as provide a scientific basis to evaluate future eradication programs.

Introduction
Plum pox virus (PPV), also known as Sharka disease, is one of the most damaging diseases of stone fruit worldwide (5). The virus was first characterized in Bulgaria in 1915 (1), and has since been detected in much of Europe as well as in Asia and South America (5). Plum pox virus was first detected in North America in Pennsylvania in 1999 (4), and in Ontario, Canada the following year (8). In response to these outbreaks, the US and Canadian governments implemented PPV survey and eradication programs with the goal of eradicating PPV before it could have a significant negative impact on the stone fruit industry in either country. The US and Canadian PPV eradication programs differed in a number of ways including sample size (leaves collected per tree), testing method, and tree removal policy (eradication).

In the United States, Prunus blocks in areas where PPV has not been previously detected Prunus trees for testing are sampled using a hierarchical sampling protocol whereby approximately 25% of trees within Prunus blocks are sampled and tested for PPV by ELISA (3). In areas where the virus has been previously detected (quarantine areas) all trees within Prunus blocks are sampled. In Pennsylvania, 8 leaves are collected per tree sample, and these are bulk-tested using a commercially available double antibody sandwich (DAS) enzyme linked immunosorbent assay (ELISA) test kit (Agdia Inc., Elkhart, IN). Detection efficiency is a critical element of the Pennsylvania eradication program, as all Prunus trees within 500 m of a PPV-positive tree are removed, regardless of their health status.

Sampling and testing protocols in Ontario are similar to the Pennsylvania protocols, in that a hierarchical sampling design is also used in non-quarantine areas (sampling approximately 25% of trees) to sample and test Prunus trees, and a census is used in PPV-quarantine areas. However, in Ontario, 20 leaves are collected per tree sample and these 20 leaves are bulk tested using a
different double antibody sandwich indirect (DASI) ELISA test kit (Durviz Inc., Valencia, Spain). When a PPV-positive tree is detected in Ontario, the entire Prunus block is removed if the PPV incidence exceeds a given threshold. Otherwise, only individual PPV-positive trees are removed. The PPV-incidence threshold for block removal has decreased over the years since the implementation of Canada’s eradication program, i.e., from 10% in 2000 to 0.5% in 2010. Prunus blocks may also be removed if a block is positive for PPV for three consecutive years.

The Pennsylvania and Canadian eradication programs have had good-to-excellent success in eradicating Plum pox virus. In Pennsylvania, PPV was officially declared eradicated within 10 years of implementing its PPV eradication program. The Canadian program has also been met with some success (i.e., fewer PPV-positive trees were detected each year following the implementation of their eradication program) PPV has yet to be eradicated in Ontario, and there was an increase in the number of PPV-positive trees detected in 2010.

The effect of the US and Canadian Plum pox virus eradication programs on the temporal and spatial dynamics of PPV epidemics has not been well studied. Therefore, the goal of this research was to investigate the epidemiological impacts of the US and Canadian Plum pox virus eradication programs. Specifically, the objectives were to: (i) quantify the impacts of the Pennsylvania and Canadian Plum pox virus eradication programs on the spatial and temporal dynamics of the PPV epidemics in Pennsylvania and Ontario; and (ii) quantify and compare the detection efficiencies of the US and Canadian eradication programs sampling testing protocols.

Spatial and Temporal Dynamics of PPV
Plum pox virus survey data were obtained from the Pennsylvania Department of Agriculture and the Canadian Food Inspection Agency. Survey data included the GPS coordinates for all Prunus blocks surveyed each year and the status of PPV within the block. Pennsylvania survey data consisted of all Prunus blocks surveyed from 1999 through 2009. In Ontario, GPS survey data was not collected until 2006, hence spatial analyses were only performed for the period 2006-2009.

A modified form of Ripley’s K function, known as the L function, was used to characterize the distribution of PPV-positive blocks (2,7). The K function is given as:

\[ K(d) = \lambda^{-1} E[\text{number of extra marked points within distance d of a randomly chosen marked point}] \]

where \( \lambda \) is the density of all marked points (2,7). The L function is given as:

\[ L(d) = \sqrt{\frac{K(d)}{\pi}} - d \]

where d is distance (2,6,7). Because the underlying pattern of all Prunus blocks was clustered, a random labeling null hypothesis was used to generate the L function. The L function was calculated using the software program Programita (9).

To determine how the spatial distribution of PPV changed over time in Pennsylvania and Ontario, 50% (D\(_{50}\)) and 95% (D\(_{95}\)) quantiles were calculated for nearest neighbor distances between successive years. This analysis allows for the determination of the distance for newly detected PPV-positive blocks relative to the GPS locations of PPV-positive blocks the previous year.

PPV Sampling and Testing Protocols
To address the question of how the US and Canadian sampling and testing schemes in the US and Canada affected PPV detection efficiency, 100-leaf samples per tree were collected from 19 known PPV-positive trees. Each leaf was
cut in half, and each half was tested using either the US ELISA test kit protocol (Agdia), or the ELISA test kit used by Canada (Durviz). Using the database of ELISA results for each leaf, a simulation model was developed to simulate repeated sampling (without replacement) of known PPV-positive trees. The simulation included the following variable inputs: ELISA result (US kit and Canadian kit), sample design (random, stratified random), sample size (4 to 40 leaves per tree, by 4’s), and the number of PPV-positive leaves required for a bulk leaf sample to test positive for PPV (1 to 5).

Two sampling designs were simulated: (i) random; and (ii) stratified random. When utilizing the simple random sampling design, the simulation selected leaves at random among the 100 leaves that were sampled and tested individually by ELISA, for any given tree. The stratified random design randomly selected an equal number of leaves, from each of the four main scaffolds per tree. Simulated sample sizes increased in increments of four from 4 to 40. Increments of four were utilized so that an equal number of leaves would be selected from each of the four tree scaffolds when the stratified random sampling design was simulated. Finally, the number of PPV-positive leaves required for a bulk sample to test positive for PPV was varied from 1 and 5.

For each of the 200 possible variable combinations of ELISA results, sampling design, sample size, and the PPV-positive leaves required for a bulk sample to test positive, 500,000 simulations were run. Detection efficiency (%) was then calculated as the number of PPV-positive iterations/total number of iterations performed × 100.

Impacts of Pennsylvania and Canadian Plum pox virus Eradication Programs

Spatial dependence of PPV-positive Prunus blocks was observed in both Pennsylvania and Ontario PPV spatial data. Prunus block incidence over the time period analyzed were similar for Pennsylvania (3.65%) and Ontario (3.22%), allowing for a direct comparison of the spatial dependence of PPV. In Pennsylvania, clustering of PPV-positive Prunus blocks was observed for distances between 0.7 and 4.3 km, indicating that PPV-positive Prunus blocks were having an effect on the health status of other Prunus blocks for these distances (Fig. 1). In Ontario, PPV-positive blocks were found to be clustered for distances of 1 to 25 km for each year between 2006 and 2009 (Fig. 2).
Distance to 50% of newly detected PPV-positive blocks from the previous years PPV-positive blocks ($D_{50}$) in Pennsylvania was 1.3 km in 2000, and increase each year up to 2005 (17.1 km) (Fig. 3). In 2006, however, $D_{50}$ decreased to 6.3 km. Distance to 95% of new PPV-positive blocks in Pennsylvania initially increased to 34.6 km in 2001, followed by a decrease to 11.2 km in 2004. The $D_{50}$ then again increased in 2005 and 2006. The average nearest neighbor distance between all Prunus blocks in Pennsylvania was 546.1 m (data not shown), indicating that all Prunus blocks, regardless of health status, were nearer to one another than PPV-positive blocks were to one another. In Ontario, the $D_{50}$ increased each year from 20007 to 2009; however, $D_{95}$ values decreased between 2007 and 2008, and then increased in 2009 (Fig. 4). Like in Pennsylvania, all Prunus blocks were nearer to one another than PPV-positive blocks. The average nearest neighbor distance was 51.25 m.
Ripley’s L function analysis, a measure of spatial dependence, for PPV-positive Prunus blocks detected in Ontario in (A) 2006, (B) 2007, (C) 2008, and (D) 2009. A random labeling null hypothesis was used to account for the underlying pattern of all Prunus blocks.
Fig. 3. The distance to 50% ($D_{50}$) and 95% ($D_{95}$) of new Plum pox virus-positive Prunus blocks in Pennsylvania plotted over time. Values represent the 50% and 95% quantiles for the minimum distances between PPV-positive Prunus blocks and the nearest PPV-positive Prunus block from the previous year.
Fig. 4. The distance to 50% ($D_{50}$) and 95% ($D_{95}$) of new Plum pox virus-positive Prunus blocks in Ontario plotted over time. Values represent the 50% and 95% quantiles for the minimum distances between PPV-positive Prunus blocks and the nearest PPV-positive Prunus block from the previous year.

Detection efficiency for the US PPV eradication program, which employs the Agdia test kit, a stratified random sampling design, and requires one PPV-positive leaf per 8 leaf bulk sample, was 71.8%. This indicates that the US sampling scheme would detect 71.8% of PPV-positive trees that are sampled and tested (Fig 5A). This indicates that the US sampling scheme would detect 71.8% of PPV-positive trees that are sampled and tested. Detection efficiency for the Canadian eradication program was 40.5% (Fig 5B). The Canadian program uses a Durviz ELISA test kit, a stratified random sampling design, and a 20 leaf sample size. Of the twenty leaves in a bulk sample, two of the leaves must be PPV-positive in a 20 leaf bulk sample for a bulk sample to test positive for PPV. These results indicate that the US PPV sampling and testing protocols detected approximately 30% more of the actual PPV-positive trees tested than the Canadian PPV sampling and testing protocol.

When all other input variables kept constant, sample size was found to have a positive impact on detection efficiency. As sample size (leaves sampled and bulk tested per tree) increased, detection efficiency also increased (Fig. 5A and B). However, as sample size increased, the gain in detection efficiency diminished. This was observed for both the US and Canadian ELISA tests and both sample designs.
Fig. 5. Simulation results using a stratified random design and (A) the US Plum pox virus ELISA kit and (B) the Canadian PPV ELISA kit for repeated sampling (500,000 iterations) of 13 known PPV-positive Prunus trees in Ontario, Canada.
Sampling design was found to have minimal effect on detection efficiency for either country. Using the US system, detection efficiency for a random sampling design was approximately 70.4%, while a stratified random design resulted in a detection efficiency of 71.8%. Using the Canadian system, a random sampling design had a detection efficiency of 40.6%, while a stratified random sampling design had a detection efficiency of approximately 40.5%. Since both the US and Canadian sampling and testing protocols already approximate a stratified sampling design, and there were no appreciable differences compared to using a simple random sampling design, no changes are recommended for this part of either countries protocols.

Sample size was found to have a positive impact on detection efficiency. As sample size (leaves sampled and bulk tested per tree) increased, detection efficiency also increased (Fig. 5). However, as sample size increased, the gain in detection efficiency diminished. This was observed for both the US and Canadian ELISA tests and both sample designs.

Increasing the number of PPV-positive leaves required for a bulk sample to test positive decreased detection efficiency. Detection efficiency was highest when only a single PPV-positive leaf was required for a bulk sample to test positive for PPV.

**Implications**

The results of simulation analyses (based upon actual PPV-test results) presented in this study provide important new information concerning the detection efficiencies of the US and Canadian Plum Pox eradication programs. Spatial analyses revealed that PPV-positive Prunus blocks are clustered in space, reinforcing the concept of sampling most intensively in areas where PPV has already been detected. Results also suggests that a tree removal policy (eradication of PPV-positive trees) based upon distance may be the most appropriate strategy to eradicate PPV. In Ontario, the distance to 50% (D_50) and 95% (D_95) of newly detected PPV-positive Prunus blocks from the previous years PPV-positive blocks, revealed that PPV-positive Prunus blocks tended to be fairly close (less than 1 km). If the block removal policy in Ontario were replaced by a removal policy based on the proximity to PPV-positive blocks, many of the ensuing seasons PPV-positive blocks would have been removed before being detected positive, thus greatly shortening the period of time that PPV-positive blocks are infectious before being removed. Thus, not only would this decrease the number of PPV-positive trees and blocks detected in future years, but it would also prevent the undetected PPV-positive blocks from acting as sources of inoculum contributing to new future positive blocks in the future.

Results from the simulation model similarly provided important information on the most efficient sampling and testing methods to detect PPV. Though neither of the two countries PPV sampling and testing programs had perfect detection efficiencies, detection efficiency was significantly higher using the US sampling and testing program compared to Canada’s program. Our results also strongly suggest that PPV detection efficiency could be further optimized by utilizing 8-12 leaf bulk sample sizes that require only one PPV-positive leaf for a bulk sample to test positive for PPV. Furthermore, it was found that the Agdia ELISA test kit had a higher detection efficiency than the Durviz ELISA test kit, when the same leaves were sampled and tested. This suggests that detection efficiency in Canada could be greatly improved by switching to an Agdia PPV ELISA kit.

**Literature Cited**


