2011

An epidemiological comparison of the US and Canadian Plum pox virus eradication programs

Andrew Gougherty
Iowa State University

An epidemiological comparison of the US and Canadian *Plum pox virus* eradication programs

by

Andrew Vincent Gougherty

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Ecology and Evolutionary Biology

Program of Study Committee:
Forrest W. Nutter, Jr., Major Professor
Mark Gleason
John Hill
Mark Kaiser

Iowa State University

Ames, Iowa

2011

TABLE OF CONTENTS

ABSTRACT

Plum pox virus (PPV) is one of the most damaging viral diseases of *Prunus spp.* worldwide. The virus was first detected in North America in Pennsylvania in 1999, and in Ontario, Canada in 2000. Following the detection of PPV in Pennsylvania and Ontario, both countries implemented PPV survey and eradication programs. The eradication program was successful in Pennsylvania, as PPV was officially declared eradicated in 2009. However, PPV remains present in Ontario. The US and Canadian eradication programs had differing effects on the epidemiology of PPV in their respective countries. The objectives of this research, therefore, were to: (i) quantify the temporal and spatial dynamics of PPV at different spatial scales, and (ii) assess the sampling and testing systems utilized by the two survey/eradication programs. Using Ripley's L function (a measure of spatial dependence), it was found that PPV-positive *Prunus* blocks in Pennsylvania in 2000 were spatially dependent (clustered) for distances of 0.7 to 4.3 km. In Ontario, spatial dependence (clustering) of PPV-positive blocks was detected for distances of 1 to 25 km. When applied to consecutive years of PPV-positive blocks in Ontario, PPV-positive *Prunus* blocks were found to be spatially dependent on the location of PPV-positive blocks that were PPV-positive the previous year. This indicates that PPV-positive *Prunus* blocks are having an impact on the health status of other *Prunus* blocks, even in subsequent years after PPV-positive trees/blocks have been removed. Distance to 50% (D_{50}) of new PPV-positive blocks in Pennsylvania from the previous years' PPV-positive blocks increased in an exponential manner until 2006, when there was a sharp decrease. Distance to 95% (D_{95}) of new PPV-positive blocks increased in 2001, but then decreased in 2002. From 2002 to 2006, the distance to 95% of new

PPV-positive *Prunus* blocks (D_{95}) was consistently between 10 and 20 km. This may suggest that when relatively few PPV-positive blocks are thought to exist, it is best to search for new PPV-positive blocks from distances of 10 to 20 km from PPV-positive *Prunus* blocks that were detected the previous year. In Ontario, D_{50} and D_{95} tended to increase over time. The distance to 95% of positive blocks was consistently between 0.5 and 1.0 km from the previous years' PPV-positive blocks, indicating that new PPV-positive blocks are very near one another. This may support the idea of Ontario implementing a PPV eradication protocol based upon distance, because a large percentage of newly detected PPV-positive blocks are so close in proximity to the previous years' PPV-positive blocks. The spatial pattern of PPV-positive trees within *Prunus* blocks was found to be random in nine of 12 blocks. In the remaining three *Prunus* blocks, PPV-positive trees were significantly clustered ($P \leq 0.05$).

A simulation model was developed to determine the relative ability of the US and Canadian sampling and testing systems to detect PPV-positive *Prunus* trees. It was found that the US system had a PPV detection efficiency of approximately 72%, whereas the Canadian system had a PPV detection efficiency of approximately 41%. This indicates that the US sampling and testing system detects approximately 30% more PPV-positive trees than does the Canadian system. The simulation model was also used to determine how the two sampling and testing protocols affected PPV detection efficiency. The ELISA test kit used in the US (Agdia) was found to have a consistently higher PPV detection efficiency compared to the ELISA test kit used in Canada (Durviz). Detection efficiency tended to increase with increasing sample size, however there were diminishing returns in detection efficiency as sample size increased. Detection efficiency

did not appreciably differ using a stratified (by scaffold) random sampling design compared to using a simple random sampling design. Finally, as the number of PPV-positive leaves required for a bulked leaf sample to test positive increased, PPV detection efficiency decreased. From these simulations, it can be concluded that PPV detection efficiency can be optimized by utilizing a 10 to 12 leaf/tree sample size that requires only one PPV-positive leaf for a bulk sample to test positive for PPV.

CHAPTER 1.

GENERAL INTRODUCTION

Thesis Organization

This thesis is divided into four chapters. The first chapter, General Introduction, provides a literature review of the history, host range, transmission, pathogen diversity, management strategies, and the impact of *Plum pox virus* (PPV) on fruit yield and quality. This chapter also provides a justification for this research. The second chapter quantifies the impact of the US and Canadian PPV eradication programs on the spatial and temporal dynamics of PPV at different spatial scales. The third chapter quantifies and compares the PPV detection efficiencies of the US and Canadian *Plum pox virus* eradication programs. The last chapter is a general summary of the conclusions reached as a part of this study.

Literature Review

History and Occurrence

Plum pox virus (PPV) is one of the the most damaging viral diseases of *Prunus* worldwide, causing significant reductions in both fruit yield and quality (8, 25). *Plum pox virus* was first described in Bulgaria in 1915 by Atanassov (4). Although PPV was first characterized on plum (*Prunus domestica*), the host range of PPV was later found to also include other stone fruit, such as peaches (*Prunus persica*), apricots (*Prunus armeniaca*), and sweet and tart cherries (*Prunus avium* and *Prunus cerasus*, respectively) (25). In the ensuing decades, PPV spread from Central Europe in all directions,

eventually reaching Southwest Asia (Georgia, Turkey, Syria, Jordan, Iran) in the late 1960s (7, 18, 49). By the 1970s, PPV had reached Western Europe (France, Switzerland, UK, Germany) (20, 28, 40, 43), and could be found as far east as India (1994) and China (2001)(32). In the Americas, PPV was first detected in Chile in 1992 (29) and was first reported in the United States in a Pennsylvania peach orchard in 1999 (22). The following year (2000), PPV was detected in Ontario and Nova Scotia, Canada (46). Currently, PPV has been detected in at least 33 countries (23).

Although the initial detection of PPV in the US occurred in 1999 in a peach orchard (cv. Encore) in Adams County, Pennsylvania (22), PPV was likely introduced into Pennsylvania in the early 1990s (47). This hypothesis is based on reports that Adams County peach growers first observed symptoms typical of PPV infection on the fruit for several years prior to the year that PPV was correctly diagnosed as the causal agent. After unsuccessfully attempting to determine the cause of the strange symptoms on the peaches, the correct diagnosis was finally made when an orchardist sent symptomatic fruit to a New Jersey County Extension Officer, who immediately suspected PPV. *Plum pox virus* was officially confirmed as the causal agent by scientists from the Pennsylvania Department of Agriculture and the United States Department of Agriculture. In subsequent PPV surveys, PPV was also detected in three neighboring counties (Franklin, Cumberland, and York) (17). These three counties border Adams County to the east, north, and west, respectively.

In response to the official confirmation of PPV in Pennsylvania, a PPV Survey and Eradication Program was developed and several quarantine zones were immediately put into place, in an attempt to limit further spread of the virus. The Pennsylvania Survey

and Eradication Program eventually proved successful, as there were no new PPV detections reported over a three-year period from 2007 through 2009 (1, 2). After the third consecutive year of no new PPV positives found in Pennsylvania (2009), PPV was officially declared eradicated per the North American Plant Protection Organization (NAPPO) guidelines (31). As a result of PPV being officially eradication in Pennsylvania, less intensive systematic surveying has been conducted since 2009 (38).

Following the initial detection of PPV in Pennsylvania in 1999, a survey of *Prunus* planting material imported from Pennsylvania was conducted in the Niagara Region of Ontario (46). A less intensive survey of planting material not originating from Pennsylvania or of heterogeneous origin was similarly conducted. This initial survey revealed that three nectarine trees (cv. Fantasia) were infected with PPV in Ontario. Subsequent nationwide surveys in Canada in 2000 also revealed that PPV was present in Nova Scotia (17), however by 2006, PPV was officially declared eradicated in Nova Scotia. *Plum pox virus* has yet to be successfully eradicated in Ontario, Canada.

In 2000, 32 states took part in the United States *Plum Pox Virus* National Surveillance Program. After obtaining negative results from all US states (except Pennsylvania) for four consecutive years, the PPV National Surveillance Program was scaled back to include only the most important and at-risk *Prunus* growing states with the highest risk of economic harm (California, Maryland, Michigan, New Jersey, New York, and South Carolina). Negative test results for PPV continued to be reported for all states, except Pennsylvania in 2004 and 2005.

In 2006, the National PPV Surveillance Program personnel detected PPV outside of Pennsylvania for the first time, in both Michigan and New York (17, 41). In

Michigan, a single peach tree tested positive for PPV at the Southwest Michigan Research and Extension Center, located in Berrien County near Benton Harbor, MI. The affected tree was removed and subsequent intensive surveys and tests for PPV revealed no new PPV infections in Michigan since the initial find. In New York, PPV was detected in 2006 in two plum trees in Niagara County, located within five miles of the Canadian PPV eradication zone in 2006 (41). In subsequent PPV surveys, PPV-positive *Prunus* trees have been detected in New York in every survey year since 2007 (3). Both the Michigan and New York PPV isolates were found to belong to the same PPV strain as those found in Pennsylvania (strain PPV-D), though neither of these outbreaks is suspected of being directly related to the Pennsylvania outbreak (17, 47). It has, however, been suggested that the New York detections may have resulted from the dissemination of PPV from Canada via viruliferous aphids vectors originating from Canada, and/or the movement of PPV-infected propagative material. This hypothesis is plausible because the Ontario and New York PPV-positive trees are in such close proximity (17).

Taxonomic Classification and Description

Plum pox virus is a member of the *Potyvirus* genus in the Potyviridae family. *Plum pox virus* consists of non-enveloped, flexuous rods that contain a single molecule of positive-sense RNA that is about 10 kb in length. The rod-shaped virions are approximately 764 x 20 nm (23).

***Plum pox virus* symptoms.** *Prunus* trees infected with PPV may display a number of symptoms. Time to symptom appearance and the severity of symptoms, however, vary with environmental conditions (e.g. temperature), virus strain, host species

and cultivar and time since infection (25). Visible symptoms often do not appear until three or more years after infection, if they appear at all (14). Visible symptoms may first occur on flower blossoms, with infected blossoms exhibiting streaking or color breaking (47). These symptoms, however, can be difficult to distinguish from some *Prunus* species that produce showy blossoms, and because of the short blossoming period, streaking is often not observed by surveyors. Furthermore, blossom streaking has only been observed once in the United States (47). Leaves of infected trees may exhibit a number of symptoms, including yellow spots or markings and leaf veins that become chlorotic. Leaves may also become deformed and wrinkled. Perhaps the most distinguishing symptom of PPV is the development of chlorotic rings or 'halos' that appear on the epidermis of infected fruit. Seeds of some hosts, particularly apricot, may also display rings. The fruit of some *Prunus* species, e.g., plums and apricots, may also become mildly-to-severely deformed (25), resulting in premature fruit drop and potentially tree death (25).

Host range of PPV. *Plum pox virus* has a wide host range within the genus *Prunus*. All fruit-producing *Prunus* species are susceptible to PPV, including plums, peaches, apricots, nectarines, almonds, and sweet and tart cherries (25). A host study of three Pennsylvanian isolates revealed that nearly all *Prunus* species tested could be successfully infected by aphid and/or graft transmission (12). The isolates, however could not be successfully transmitted by viruliferous aphids to two cherry species, *P. cerasus* and *P. x* '*Snofozam*'. These negative results, however, may be due to the PPV strain (PPV-D) that was used in the study, which does not naturally infect cherry.

Alternative hosts. A number of woody and herbaceous non-*Prunus* species have been shown to be mechanically- and/or naturally-infected by PPV (24). The presence of alternative hosts in the landscape can be problematic, as alternative hosts may provide epidemiologically-important sources of initial PPV inoculum that must be accounted for, if PPV eradication programs are to be successful. Several studies in Europe have revealed the presence of a number of weed genera infected with PPV in *Prunus* orchards that had a high incidence of PPV, including *Sonchus spp.*, *Clematis spp.*, *Trifolium spp.*, and *Cichorium spp.* (24). Similar studies in the United States and Canada, however, have not detected PPV in any weed species present within the orchard landscape or in home gardens (44). It has been suggested that the discrepancy between the presence of PPV alternative weed hosts in Europe and the lack of alternative weed hosts in the US may be due to the occurrence of a variety of PPV strains being present in Europe (PPV-D, M, and Rec), while there is a single predominant strain in North America (PPV-D) (24). Even so, it has been argued that alternative PPV hosts do not play an epidemiologically-important role in the dissemination of PPV, as transmission from PPV alternative hosts to *Prunus* has not been adequately documented (24). Thus, the importance of alternative weed hosts in the dissemination of PPV within orchards remains unclear.

Transmission of *Plum Pox Virus* and Sources of Inoculum

Plum pox virus can be successfully transmitted in two ways: the grafting of infected plant material onto a healthy tree or rootstock, or by viruliferous aphids. For decades, PPV was also thought to be seed transmitted (37), but subsequent studies have shown that, although the seed coat and cotyledons may contain the virus, the embryo

does not become infected. Therefore, no seed-to-seedling transmission is possible and, at present, this is considered true for all PPV strains (37). Still, a number of recent studies suggest that seed transmission of PPV may be possible in some *Prunus* cultivars with some strains of the virus (often the Marcus strain). Thus, more research is required for a definitive answer to the question of seed-to-seedling transmissibility of PPV.

Graft transmission of PPV. *Plum pox virus* can be disseminated over long distances by the transport of infected plant (grafting) material, including rootstocks, budwood, and scions (12). Because grafting is the most common method of propagating *Prunus*, care must be taken to ensure that all propagative material is 'clean' (i.e. virus-free) before introducing propagative plant material into an orchard. It is highly probable that this was the mechanism by which PPV was initially introduced into the US and Canada (17).

Aphid Transmission of PPV. *Plum pox virus* can be disseminated over short-to-meso-distances by several aphid species (12, 13). However, the acquisition and transmission efficiencies can vary by aphid species, geographic location, and PPV strain (13). In the US, four aphid species have been associated with PPV acquisition and transmission (13). Two of these, *Myzus persicae* and *Aphis spiraecola*, are considered the most efficient as well as the most abundant PPV vectors (47). A number of other aphid species in the US have been identified as potential vectors, but these are less efficient and/or less abundant than *M. persicae* and *A. spiraecola* (13).

Plum pox virus transmission by aphids is nonpersistent, meaning that the virus does not have a latent period within the insect vector. Consequently, the virus can be immediately acquired and transmitted by the aphid to other host plants during aphid

feeding (13). The virus adheres to the proboscis and/or inside of the aphid food canal when aphids probe PPV-infected leaves or fruit. Transmission then occurs when viruliferous alate aphids fly to another tree *Prunus* and probe a leaf or fruit, and inoculate a healthy plant cell with the virus (14). Aphid transmission is likely responsible for the dissemination of the virus from tree-to-tree, block-to-block, and orchard-to-orchard. Because aphids are poor fliers, their movement is often determined by wind currents (47). Consequently, prevailing winds and physical barriers that may impede aphid movement are important factors in aphid-vectored transmission (13). New infections of PPV are reported to often occur downwind from previously infected trees (13).

Aphids may also facilitate the dissemination of the virus within an individual tree, by transmitting the virus from scaffold-to-scaffold, and leaf-to-leaf within the same tree (i.e., making an infected tree a larger lesion "epidemiologically" from which aphids can acquire and transmit PPV).

Diversity of PPV Strains.

There are currently six known PPV strains, which vary geographically and by host range. These are the Dideron, Marcus, El Amar, Cherry, Recombinant, and Winona PPV strains.

The PPV-Dideron strain. The Dideron strain was originally isolated from an apricot tree in southern France (23). This is the most prevalent strain in Western Europe and in the Americas (Chile, United States, Canada, Argentina). In North America, only in Canada have other strains been reported (19, 45). The predominant hosts for this strain include apricots, peaches, and plums. Epidemiologically, this strain is less efficiently

acquired and transmitted by aphids than other PPV strains, so it is thought to be more easily contained and eradicated than other PPV strains (23).

The PPV-Marcus strain. The Marcus strain was originally isolated from a peach tree in Greece (23). The Marcus strain is prevalent throughout Southern, Eastern, and Central Europe and is generally limited to peaches (23). Because the Marcus strain is efficiently transmitted by a number of aphid species, it is considered the most aggressive strain of the virus. Orchards infected with this strain are reported to have very high PPV incidence due to a rapid increase in PPV incidence, and this strain is reported to cause more severe symptoms than other strains (9).

The PPV-El Amar strain. Relatively little is known about the El Amar strain, which is found exclusively in North Africa (Egypt), and is known to infect both peach and apricot (23). Unlike the PPV strains found in other geographic locations, infected stone fruit in Egypt are often devoid of obvious symptoms, with the infrequent exception of necrotic/chlorotic rings on leaves (48). The absence of visible symptoms may be the consequence of the high temperatures in the region, as PPV symptoms are often diminished or entirely masked at high temperatures (25).

The PPV-Cherry strain. The cherry strain of the virus infects both sweet and tart cherries, and is found in Eastern and Central Europe, as well as Italy (23). The cherry strain has the widest experimental host range of all PPV strains, and is the only known strain to infect cherries (25). Despite its wide host range, the cherry strain has only been found (naturally) in sweet and tart cherry orchards.

The PPV-Recombinant strain. A recombinant of the Marcus and Dideron strains was initially collected in Eastern Europe from a PPV-infected apricot tree in

Slovakia in 1996 (15). However, it was not until 2001 that this isolate was determined to be a recombinant of the two strains. The genome of the recombinant strain consists of the coat protein region of the Marcus strain genome and the remaining genome of the Dideron strain. Because PPV strain typing often targets only the coat protein region of the PPV genome, it is thought that some of the isolates in Europe originally typed as Marcus, may in fact, be recombinant PPV strains (15). A follow-up study of PPV isolates collected during a 2001 survey in Slovakia revealed that 16 of 23 isolates were indeed the recombinant strain. However, the testing of selected of isolates collected in France, where the Marcus strain is prevalent, between 1985 and 2001 revealed no recombinant strains (16). Despite this, the authors suggest that the PPV recombinant strain may be more prevalent than currently thought. During the 2008 Home Owner Survey in Ontario, PPV-Rec was detected for the first time in North America. Three trees were found with the recombinant strain on peach/plum, peach/apricot, and plum grafted onto a plum rootstock. The trees were removed following detection, and no new detection of the recombinant strain in Canada have since been reported (45).

The PPV-Winona strain. A sixth unique strain, named Winona, was isolated in 2004 from two plum trees sampled from a homeowner's property in Stoney Creek, Ontario (19). Phylogenetic analyses, as well as serological and nucleic acid-based typing revealed that these isolates are distinct from other PPV isolates (19). However, since this report, the trees from which this strain was isolated have been removed, and the Winona strain is thought to be eradicated in Canada (19). More recently, however, an isolate from infected germplasm illegally imported into the United States from the Ukraine was intercepted and identified as belonging to the Winona strain. This has led some

regulators to believe that the Winona strain may have originated in Eastern Europe, where it continues to exist (10). The isolation of both the Winona and recombinant strains on homeowner's properties in Ontario illustrates the substantial risk that homeowner's potentially play in the long distance dissemination of PPV strains to new geographic areas.

Importance of *Plum Pox Virus*

Impact of PPV on *Prunus* yield. *Plum pox virus* can have a number of detrimental impacts on the yield and quality of stone fruit production. Infected trees often prematurely drop their fruit, even if no visible symptoms are present on the fruit or leaves (25). Infected trees will decline over time, though there are conflicting reports on whether PPV will eventually kill infected trees (8, 47). In Europe, growers can expect 5% of PPV-infected trees to die prematurely each year (8). It is estimated that about 1.5 million metric tons of European plum fruit are lost annually due to PPV infection (8). In North America, there have been no reports of PPV affecting yield, likely because PPV was initially detected relatively soon after being introduced, as well as the implementation of eradication programs immediately following detection in the US and Canada.

Impact on *Prunus* quality. Fruit from PPV-infected trees may display a variety of symptoms that negatively impact marketability, especially for the fresh fruit market (8). Though the impact on fruit quality depends on the host species, cultivar, and virus strain, there are several characteristic symptoms that are present on nearly all *Prunus* fruiting species infected with PPV (14). Fruit from infected trees often display chlorotic rings on the epidermis (25). Although these markings are only "skin deep", these

symptoms prevent the fruit from being sold on the fresh fruit market (47). Some infected stone fruit, especially plums, may have smaller, and misshapen fruit, which further decreases the likelihood of obtaining top-price on the fresh market (25). The taste of the fruit may also be adversely affected, as PPV-infected fruit tend to have decreased sugar content, and may have an acidic taste (33). It is worth noting that the impact of PPV on fruit quality may not be realized for a number of years after the initial year of infection, as it often takes several growing seasons for disease symptoms to appear (i.e. there is a long incubation period) (14).

Management of *Plum Pox Virus*

Exclusion. Several management strategies are available to limit the spread and impact of PPV within the United States and Canada. The first line of defense is exclusion, i.e., prevent the importation of PPV-infected propagation material. Done successfully, initial inoculum (Y_0) will remain at zero, and this strategy will prevent PPV from being disseminated over long distances to new geographic areas where the virus is not yet present (34). This strategy is particularly important in preventing the reintroduction of PPV into areas where it has already been eradicated (Pennsylvania) or new geographic areas where it has not been found previously.

Eradication. If the virus is detected in a new geographic area, an eradication program may be employed in an attempt to reduce sources of virus inoculum to zero, thereby preventing further virus spread over time and space (35). Because PPV-infected trees cannot be 'cured,' it is necessary to remove all PPV-infected trees to prevent future dissemination of the virus to healthy trees. This strategy, however, is laborious, and requires rigorous surveying and sampling. This strategy can be undermined by the fact

that the latent period of infected trees is shorter than the incubation period. Therefore, PPV-infected trees may serve as potential sources for virus spread for several growing seasons before PPV symptoms are expressed and infected trees are removed. Though some European PPV survey programs previously relied solely on surveying for visible symptoms (11, 21, 26), US and Canadian PPV surveys now employ virus detection kits to test leaf samples for the presence of PPV. This strategy, however, is not foolproof, as it requires survey programs to strike a balance between sample size (number of leaves per tree), the number of sampling units (leaves) required to be positive, and the number of trees sampled per *Prunus* block.

Comparison of Eradication Programs in the United States and Canada

The US and Canadian PPV Eradication Programs differ in a number of ways, including sample size, bulk testing, ELISA test kits and test protocols, and the PPV incidence threshold required for tree and block removal (eradication).

The Pennsylvania PPV eradication program utilizes a hierarchical sampling method of surveying *Prunus* outside its quarantine zone. Quarantine zones in Pennsylvania are established on easily defined geographic areas, often by townships borders. Within the Pennsylvania quarantine zones, all *Prunus* trees are sampled for PPV. The Pennsylvania removal protocol differs greatly from the Canadian protocol. Rather than relying on a threshold, under the Pennsylvania program, *Prunus* trees are removed based on their proximity to a positive tree. The removal radius is 500 meters, and has remained constant in the 10 years since the virus was first detected. The eradication program required the removal of all *Prunus* trees within 500 m of a PPV-positive tree. Because Pennsylvania makes up only a small portion of the *Prunus*

industry in the United States, combined with the limited distribution of PPV in Pennsylvania, this more aggressive removal program has been implemented without a dramatic effect on the national *Prunus* industry.

The Ontario quarantine area is divided into three sub-areas, each of which has a different survey protocol. The division of the quarantine area into Sub-Areas was meant to allow for more intensive surveying in areas where PPV incidence was highest. In Sub-Area A, all the trees growing within previously positive or suspect *Prunus* blocks are sampled individually (census). Trees in blocks within 200 meters of PPV-positive/suspect blocks are similarly sampled individually. In the remaining blocks in Sub-Area A, trees are sampled in pairs. In Sub-Areas B and C, trees within all previously positive and suspect blocks are sampled individually. The trees in the remaining blocks are sampled in groups of four (25% of trees in each block).

Because of the widespread distribution of the virus when initially detected in Canada, and the density of Canadian *Prunus* blocks in the Niagara Region of Canada, a "grower friendly" tree removal protocol was established. The protocol is based on block incidence, rather than proximity to a PPV- infected tree. Thresholds have similarly been used in a number of European countries (e.g. France, Italy, UK), where virus incidence is high (27, 30, 39, 42). In Canada, if a block is found to have an incidence above a set threshold, the entire block is removed. If a block is below the threshold, only the individual PPV-positive trees are removed. If a block is found to have positive trees for three or more years, the entire block is removed regardless of whether the incidence is above the threshold. The initial threshold was set at 10% in 2000, and has decreased annually since that time. Currently the threshold is 0.5%. Eventually, the threshold will

be decreased to 0%, whereby a single positive tree will require the removal of an entire block.

As of 2006, in Pennsylvania, approximately 190,000 trees have been removed, while in Canada 264,000 trees have been removed (8). It is anticipated that the eventual success of both Country's Eradication Programs will compensate *Prunus* growers for damages incurred to the *Prunus* industries (8). Eradication programs have had a mixed history of success, though several countries (Switzerland, Belgium, Netherlands) have effectively eliminated PPV within their borders because of aggressive eradication programs (23).

Protection. Because the virus is vectored by aphids, insecticide applications might appear to be an attractive management tactic. However, it has been reported that insecticide applications do not effectively limit aphid acquisition and transmission of plant viruses, as aphids can acquire and transmit the virus before the insecticide can act (6, 47). Furthermore, it is well known that insecticides can act as an irritant, resulting in increased vector movement (6), facilitating even faster temporal and spatial spread of the virus.

Genetic host resistance. One of the most effective management strategies is the development host resistant *Prunus* cultivars. Researchers from the United States, France, Spain, Poland, Romania, Chile, and the Czech Republic first began developing PPV-resistant cultivars in 1989. Researchers were able to confer resistance to an existing plum cultivar 'Bluebyrd' by inserting the PPV coat protein sequence into the host genome, via *Agrobacterium tumefaciens*. The new resistant cultivar was named 'HoneySweet'. Subsequent greenhouse and field studies in Spain, Poland, and Romania have shown this

new cultivar to be resistant to the local strains of PPV in these countries. Though this cultivar has been shown to be completely resistant to aphid inoculation, it is still at least somewhat susceptible to graft inoculation. Grafting virus-infected budwood onto the resistant cultivar will often cause some minor infection near the graft union, which seems to indicate that this cultivar may not be completely resistant to PPV. HoneySweet, however, remains unavailable to growers. In the United States, deregulation proceedings have begun with the USDA, FDA, and EPA, with the hopes of eventually making the cultivar available to growers soon. Though incidence levels are likely too low in the United States for HoneySweet to have a dramatic effect on PPV eradication in the U.S., it does provide an option, should the virus ever become widespread or should the incidence level dramatically increase. In Europe, however, where the infection level is much higher, HoneySweet could be a valuable tool in eradicating PPV, while minimizing unnecessary crop loss.

Therapy. Although whole trees cannot be 'cured' of the virus, small tissues of a plant can be treated. One treatment method is cryopreservation. In a study conducted in the late 1990s, researchers infected a *Prunus* rootstock, via chip budding, with the PPV-M strain of the virus (5). After rapid cooling of the rootstock and immersion into liquid nitrogen, tests for PPV the following day revealed 50% of the infected rootstocks were now virus-free, while only 20% of the control rootstocks were virus-free. The researchers suggest that the higher percentage of virus-free rootstocks after cryopreservation is a result of larger cells, which were hypothesized to be more likely infected with PPV than smaller cells, being destroyed during cryopreservation as a result

of having a higher water content than smaller cells. Ultimately, the researchers suggest, resulting in a larger percentage of small, healthy (virus-free) cells after cryopreservation.

Another potential therapy treatment involves heat. The objective of heat therapy is to heat PPV-infected plant tissue over a period of time to denature or inactivate the virus. Little research has been done using heat treatment to "cure" plant tissue infected with PPV. A review in 1969, however, reported PPV to be "difficult to inactivate" requiring heating at 38 ^{o}C for 39 days (36).

Justification

The potential negative impact of PPV on the *Prunus* industry in North America is great. Were the virus to become established within a *Prunus* producing area, yield losses could be similar to those in Europe, where losses approached 100% in some areas (23). For this reason it is important to assess the current US and Canadian Eradication Programs to determine if the most efficient sampling designs, testing methods, and removal (eradication) protocols are being utilized to eradicate PPV. Little has been done to attempt to quantify the effect the sampling, testing, and eradication programs in the US and Canada have had on the progress of the PPV epidemics in their respective countries. The only study investigating the epidemiology of PPV in North America to date, was undertaken at the beginning stages of the PPV epidemics in the US and Canada. In 2000, Gottwald investigated the spatial dynamics of PPV in North America, including the distribution of PPV within and among *Prunus* blocks (17). Within *Prunus* blocks, it was found that PPV-infected trees within rows were randomly distributed in 80% of blocks, while accross rows trees were randomly distributed in only 55% of *Prunus* blocks.

Gottwald further investigated the spatial distribution of PPV-infected blocks in Ontario in 2000. It was found that the spatial dependence (clustering) of PPV-positive blocks ranged up to 35.9 km; however, when the underlying pattern of all blocks was accounted for, spatial dependence ranged from 0.7 to 4.3 km. This is the only study that has been conducted on the epidemiology of PPV in North America. Since this initial study in 2000, both countries have developed and implemented large scale eradication programs that have had differing effects on the epidemiology of PPV in their respective countries.

The US and Canadian Eradication Programs differ in a number of ways. Among these are: (i) the number of leaves sampled from individual trees, and (ii) the test kit used to test leaf samples for PPV. In the US leaf samples consist of 8 leaves per tree, while in Canada samples consist of 20 leaves per tree. To our knowledge, the relative efficiency of the Canadian sampling scheme has never been compared to the efficiency of the US sampling scheme in their ability to detect PPV-positive trees. Therefore the objectives of this study are to:

(i) Quantify the impacts of the US and Canadian *Plum pox virus* eradication programs on the spatial/temporal spread of PPV at different spatial scales.

(ii) Quantify and compare the detection efficiencies of the US and Canadian *Plum pox virus* sampling and testing protocols.

Literature Cited

1. Anon. 2007. 2007 Plum Pox Survey Summary. Pennsylvania Department of Agriculture, Harrisburg, PA.

2. Anon. 2008. 2008 Plum Pox Survey Summary. Pennsylvania Department of Agriculture.

3. Anon. 2010. Annual *Plum pox virus* Survey. Cornell University http://web.pppmb.cals.cornell.edu/fuchs/ppv/.

4. Atanassov, D. 1932. Plum Pox: A new virus disease. Annals of the University Sofia, Faculty of Agriculture and Silviculture 11:49-69.

5. Brison, M. 1997. Effect of cryopreservation on the sanitary state of a cv Prunus rootstock experimentally contaminated with Plum Pox Potyvirus. Plant Science 123:189.

6. Broadbent, L. 1957. Insecticidal control of the spread of plant viruses. Annual Review of Entomology 2:339-354.

7. Caglayan, K. 2006. *Plum pox virus* (PPV) in Turkey. EPPO Bull. 36:216-217.

8. Cambra, M., Capote, N., Myrta, A., and Llacer, G. 2006. *Plum pox virus* and the estimated costs associated with sharka disease. EPPO Bull. 36:202-204.

9. Candresse, T., Cambra, M. 2006. Causal agent of sharka disease: historical perspective and current status of *Plum pox virus* strains. EPPO Bull. 36:239-246.

10. Croft, H., Malinowski, T., Krizbai, L., Mikec, I., Kajic, V., Reed, C., Varga, A., and James, D. 2008. Use of Luminex xMAP-derived Bio-Plex bead-based suspension array for specific detection of PPV W and characterization of epitopes on the coat protein of the virus. J. Virological Methods 153:203-213.

11. Dallot, S. 2004. Factors affecting the spread of *Plum pox virus* strain M in peach orchards subjected to roguing in France. Phytopathology 94:1390.

12. Damsteegt, V. D., Scorza, R., Stone, A. L., Schneider, W. L., Webb, K., Demuth, M., and Gildow, F. E. 2007. Prunus host range of *Plum pox virus* (PPV) in the United States by aphid and graft inoculation. Plant Dis. 91:18-23.

13. Gildow, F., Damsteegt, V., Stone, A., Schneider, W., Luster, D., Levy, L. 2004. Plum pox in North America: Identification of aphid vectors and a potential role for fruit in virus spread. Phytopathology 94:868-874.

14. Gildow, F., Travis, J., and Halbrendt, J. 2000. *Plum Pox Virus* of Stone Fruit.

15. Glasa, M., Kudela, O., Marie-Jeanne, V., and Quiot, J. B. 2001. Evidence of a naturally occuring recombinant isolate of *Plum pox virus* from Slovakia. Plant Dis. 85:920.

16. Glasa, M., Marie-Jeanne, V., Labonne, G., Subr, Z, Kudela, O., Quiot, J. 2002. A natural population of recombinant *Plum pox virus* is viable and competitive under field conditions. Eur. J. Plant Pathol. 108:843-853.

17. Gottwald, T. R. 2006. Epidemiology of sharka disease in North America. EPPO Bull. 36:279-286.

18. Ismaeil, F. 2006. *Plum pox virus* (PPV) in Syria. EPPO Bull. 36:216-216.

19. James, D., and Varga, A. 2005. Nucleotide sequence analysis of *Plum pox virus* isolate W3174: Evidence of a new strain. Virus Research 110:143-150.

20. Jarausch, W. 2006. *Plum pox virus* (PPV) in Germany. EPPO Bull. 36:209-209.

21. Labonne, G. and Dallot, S. 2006. Epidemiology of sharka disease in France. EPPO Bull. 36:267-270.

22. Levy, L., Damsteegt, V., and Welliver, R. 2000. First report of *Plum pox virus* (Sharka disease) in *Prunus persica* in the United States. Plant Dis. 84:202.

23. Levy, L., Damsteegt, V., Scorza, R., and Kolber, M. 2000. Plum plox potyvirus disease of stone fruits. http://www.apsnet.org/online/feature/plumpox/top.html.

24. Llacer, G. 2006. Hosts and symptoms of *Plum pox virus*: Herbaceous hosts. EPPO Bull. 36:227-228.

25. Llacer, G. and Cambra, M. 2006. Hosts and symptoms of *Plum pox virus*: fruiting *Prunus* species. EPPO Bull. 36:219-221.

26. Mikec, I., Kajić, V., Krajačić, M. and Škorić, D. 2008. Occurrence and distribution of *Plum pox virus* in Croatia. Acta Hort. 781:193 - 197.

27. Mumford, R. A. 2006. Control and monitoring: control of *Plum pox virus* in the United Kingdom. EPPO Bull. 36:315-318.

28. Mumford, R. A. 2006. *Plum pox virus* (PPV) in the United Kingdom. EPPO Bull. 36:217-217.

29. Muñoz, M. and Collao, M. 2006. *Plum pox virus* (PPV) in Chile. EPPO Bull. 36:207-207.

30. Myrta, A., Di Terlizzi, B., Savino, V., and Martelli, G. P. 2006. Control and monitoring: monitoring and eradication of sharka in south-east Italy over 15 years. EPPO Bull. 36:309-311.

31. NAPPO 2004. Guidelines for phytosanitary action following detection of *Plum pox virus*. NAPPO Regional Standards for Phytosanitary Measures http://www.nappo.org/Standards/Standards%28all%29/RSPM18-18-10-04-e.pdf.

32. Navratil, M., Safarova, D., Karesova, R., and Petrzik, K. 2006. *Plum pox virus* (PPV) in China. EPPO Bull. 36:207-207.

33. Nemeth, M. 1994. History and importance of plum pox in stone fruit production. EPPO Bull. 24:525-536.

34. Nutter, F. W., Jr. 1997. Quantifying the temporal dynamics of plant virus epidemics: a review. Crop Protection 16:603-618.

35. Nutter Jr, F. W., Jr. 2007. The role of plant disease epidemiology in developing successful integrated disease managment programs. Pages 43-77 in: General Concepts in Integrated Pest and Disease Management. A. Ciancio and K. G. Mukerji, eds. Springer, Dordrecht, The Netherlands.

36. Nyland, G. and Goheen, A. C. 1969. Heat Therapy of Virus Diseases of Perennial Plants. Annual Review of Phytopathology 7:331-354.

37. Pasquini, G., and Barba, M. 2006. The question of seed transmissibility of *Plum pox virus*. EPPO Bull. 36:287-292.

38. PDA 2009. Revision of Quarantine and Program Orders Relating to *Plum pox virus*. Pennsylvania Bull. 39:7242

39. Penvern, S., Bellon, S., Fauriel, J., and Sauphanor, B. Peach orchard protection strategies and aphid communities: Towards an integrated agroecosystem approach. Crop Protection 29:1148-1156.

40. Ramel, M. E., Gugerli, P., and Bünter, M. 2006. *Plum pox virus* (PPV) in Switzerland. EPPO Bull. 36:215-216.

41. Snover-Clift, K. L., Clement, P. A., Jablonski, R., Mungari, R. J., Mavrodieva, V. A., Negi, S., and Levy, L. 2007. First report of *Plum pox virus* on plum in New York State. Plant Dis. 91:1512.

42. Speich, P. 2006. Control and monitoring: *Plum pox virus* quarantine situation in France. EPPO Bull. 36:307-308.

43. Speich, P. 2006. *Plum pox virus* (PPV) in France. EPPO Bull. 36:208-209.

44. Stobbs, L. W., Van Driel, L., Whybourne, K., Carlson, C., Tulloch, M., and Van Lier, J. 2005. Distribution of *Plum pox virus* in residential sites, commercial nurseries, and native plant species in the Niagara Region, Ontario, Canada. Plant Dis. 89:822-827.

45. Thompson, D. 2009. First Report of *Plum pox virus* Recombinant Strain on Prunus spp. in Canada. Plant Dis. 93:674.

46. Thompson, D., McCann, M., MacLeod, M., Lye, D., Green, M., and James, D. 2001. First report of Plum pox potyvirus in Ontario, Canada. Plant Dis. 85:97.

47. USDA 2007. Recovery plan for *Plum pox virus* (Sharka) of stone fruit. National Plant Disease Recovery System. http://www.ars.usda.gov/SP2UserFiles/Place/00000000/opmp/Plum%20Pox%20PPV%2070222.pdf.

48. Youssef, S. A., Shalaby, A. 2006. *Plum pox virus* (PPV) in Egypt. EPPO Bull. 36:208.

49. Zamharir, M. G., Bashir, N. S., and Khakvar, R. 2006. *Plum pox virus* (PPV) in Iran. EPPO Bull. 36:210-210.

CHAPTER 2.

SPATIAL AND TEMPORAL ANALYSES OF *PLUM POX VIRUS* IN PENNSYLVANIA AND ONTARIO

Manuscript prepared for submission to *Phytopathology*

Abstract

Plum pox virus (PPV) was first detected in Pennsylvania in 1999, and then in Ontario the following year (2000). Both countries implemented PPV eradication programs the same year that PPV was first detected; PPV was officially declared eradicated in Pennsylvania in 2009, but remained present in Ontario. Differences in how the US and Canadian National Eradication Programs were implemented had varying effects on the spatial and temporal dynamics of the PPV epidemics. The frequency of PPV-positive trees detected over time (year) decreased in both countries. *Plum pox virus* incidence (number of PPV positive trees/total number of trees sampled and tested x 100) decreased in Pennsylvania approximately 1.5 times faster than in Ontario. Marked point pattern analysis revealed that PPV-positive *Prunus* blocks were clustered in Pennsylvania for distances of 0.7 to 4.3 km, whereas in Ontario, PPV-positive blocks were clustered for distances of 1.0 to 25.0 km. Multi-year spatiotemporal analyses revealed that PPV-positive blocks in Ontario were clustered for distances of 0.1 to 3.0 km in 2007, whereas clustering was observed for distances of approximately 0.1 to 17.0 km in 2008 and 2009, indicating that the location of PPV-positive blocks detected in one year were spatially dependent upon the locations of PPV-positive blocks detected the previous year.

Distance to 50% and 95% of new PPV-positive blocks from one year to the next in Pennsylvania revealed that 95% of new PPV-positive blocks were between 10 and 20 km from PPV-positive blocks detected the previous year. This suggests that PPV surveys should be more intensive at these distances as new PPV-positive blocks become rare events. In Ontario, 95% of PPV-positive blocks were between 500 and 900 m from PPV-positive blocks detected the previous year. Thus, because PPV-positive blocks are close to previously positive blocks, a tree removal policy based on the distance from PPV-positive trees may be better suited to eradicate PPV from Ontario, as opposed to a removal protocol based on block incidence. Spatial analyses of PPV-positive trees within *Prunus* blocks revealed that PPV-positive trees were clustered in 25% of 12 PPV-infected blocks. This finding suggests that a systematic sampling design should be continued to be utilized when surveying for PPV-positive trees in blocks in which the status of PPV is unknown. This study provides new information concerning the spatial and temporal dynamics that may prove to be important for present and future PPV eradication programs.

Introduction

Plum pox virus (PPV) is the one of the most damaging viruses of *Prunus* worldwide (4) causing severe losses in yield and fruit quality (19). In countries where PPV is well established, losses attributed to PPV can reach 100% (18). Since PPV was first characterized in Bulgaria in 1915 (1), the virus has spread throughout much of Europe (5, 7, 15, 23, 30), the Mediterranean (2, 3, 14, 21, 31, 37), the Middle East (39, 41), Asia (26), South America (24, 28), and North America (16, 34). *Plum pox virus* was

first detected in North America in Pennsylvania in 1999 (17) and the following year (2000) in both Ontario and Nova Scotia, Canada (35).

Following the detection of PPV in the US and Canada, both countries established PPV survey and eradication programs (33, 36). However, the eradication programs implemented differed in sampling designs, number of leaves sampled per tree, ELISA protocol and test kit used to test leaf samples, and how each nation's eradication program was implemented once a PPV-positive *Prunus* tree was detected. Plum pox was officially declared eradicated from Pennsylvania in 2009, according to North American Plant Protection Organization (NAPPO) guidelines (25). However, PPV has not yet been eradicated in Ontario.

Few studies have investigated the temporal and spatial dynamics of PPV at different spatial scales (leaf, scaffold, tree, block, and orchard). Only one study has analyzed the spatial dependence of PPV in North America, and this analysis was only performed for a single year (10). In 2000, PPV-positive blocks in Pennsylvania and Ontario were found to be significantly clustered over long distances. After the underlying pattern on all *Prunus* blocks were accounted for, PPV-positive blocks in Pennsylvania were reported to be clustered over distance of 1.0 to 6.6 km., while in Ontario *Prunus* blocks were reported to be clustered for distances of 0.7 to 4.3 km (10, 11).

It has been previously reported that the spatial pattern of PPV-positive trees within *Prunus* blocks in Pennsylvania were mostly random (10). The within-block spatial pattern of PPV-positive trees has been analyzed in Europe (6, 9); however, the

within-block spatial pattern of PPV-positive trees in Canada has not been determined, nor have subsequent studies been conducted in North America since the initial study in 2000.

Thus, the impact of the US and Canadian *Plum pox virus* Eradication Programs on the temporal and spatial dynamics of PPV epidemics has not been investigated. Therefore, the objectives of this study, were to: (i) quantify the temporal dynamics of PPV epidemics in Pennsylvania and Ontario since the implementation of each country's PPV eradication program, (ii) determine the spatial pattern of PPV-positive blocks for each individual PPV survey year (and over combined years), and (iii) determine the spatial pattern of PPV-positive trees within *Prunus* blocks in Canada.

Material and Methods

Temporal progress of PPV epidemics. The number of PPV-positive *Prunus* trees detected in Pennsylvania and Ontario were plotted over time (2000 – 2009). Four models were evaluated to determine which model best explained the relationship between the number of PPV-positive trees detected (y) versus years of detection (x). The models evaluated were: the linear model, the exponential model ($ln\ y$), the logistic model ($ln\ (y/1 - y)$), and the Gompertz model ($-ln\ (-ln\ y)$). Models were evaluated based on the following criteria: a significant F statistic ($P \leq 0.05$), the coefficient of determination (R^2), and the standard error for the estimate of y (SEy) (27).

The temporal progress of PPV-positive sample incidence (number of PPV-positive samples/total number of samples collected) was also plotted over time. Models were fit to PPV incidence in the same manner as described using the same evaluation criteria.

Spatial analyses of PPV-positive blocks. *Plum pox virus* survey data were obtained from the Pennsylvania Department of Agriculture (PDA) and the Canadian Food Inspection Agency (CFIA). Survey data included the GPS locations of all PPV-positive and all PPV-negative *Prunus* blocks. Pennsylvania survey data included the location of all *Prunus* blocks surveyed from 1999 through 2006 (the final year that PPV-positive blocks were detected). The survey data from Ontario consisted of *Prunus* blocks surveyed from 2006 through 2009, as GPS spatial data was not collected until 2006. To characterize the spatial pattern of PPV-positive *Prunus* blocks, Ripley's K function was used (8, 29). The K function is a marked point process used to characterize the point patterns of completely mapped points. Ripley's K is calculated by comparing the density of marked points within a given radius around each single marked point to the density of all marked points in the study area. 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 λ is the density of all marked points. This process is repeated for all marked points in the study area and for all distance intervals defined by the user.

Spatial dependence is determined by calculating the K function for a randomized simulation of points, which is then compared to the observed K function obtained from the known locations of points. If the observed K(d) for a given distance is greater than the simulated K(d) for that distance, then the marked points are considered spatially dependent (clustered) at that distance. Similarly, if the observed K(d) for a given distance is less than the simulated K(d), then marked points are considered regularly distributed at that distance. Finally, when K(d) is within the simulated upper and lower

confidence envelopes, marked points are considered randomly distributed for that distance.

Because the spatial pattern of all *Prunus* blocks, regardless of whether they are PPV-positive or not, is clustered in both Pennsylvania and Ontario, a random labeling null hypothesis was used to calculate the simulated confidence envelopes (8, 38). This null hypothesis allows for the determination of whether the spatial pattern of PPV-positive blocks was significantly different from the underlying pattern of all *Prunus* blocks (8, 38). The random labeling null hypothesis uses a Monte Carlo simulation to randomly label the status (in this case, PPV-positive or PPV-negative) of points (i.e., blocks). Ripley's K is calculated for the randomly labeled points to generate a confidence envelope, from which spatial dependence (PPV spatial pattern) is then inferred. Visual interpretation of the plotted K function is more intuitive using a transformed version, known as the L function (8). The L function stabilizes the variance and removes the scale dependence associated with the K function (8, 38). Plots, therefore, were generated using the L function, which is given as:

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

The univariate L function was calculated for individual years of PPV-positive blocks. The L function requires at least 30 marked points (i.e., PPV-positive blocks); hence, for Pennsylvania, the L function was calculated only for the positive blocks detected in 2000, as all subsequent survey years had fewer than 30 PPV-positive *Prunus* blocks detected. For Ontario, the L function was calculated for PPV-positive blocks in 2006, 2007, 2008, and 2009.

Using a bivariate form, the L function can also be used to assess the presence of spatial dependence over the course of multiple survey years (8). This analysis allows for the determination of whether the locations of PPV-positive blocks in one year are spatially dependent upon the locations of PPV-positive blocks the previous year. The bivariate K function is given as:

$$K_{ij}(d) = \lambda_j^{-1}E[\text{number of type j marked points within distance d of a randomly chosen type i marked point}]$$

,where λ_j is the density of j marked points. For our purposes, "i marked points" are PPV-positive blocks from year 1, and "j marked points" are PPV-positive blocks from year 2. Like with the univariate form, a random labeling null hypothesis was appropriate. As with the univariate form, the bivariate K function was transformed to the L function using the above equation. The bivariate L function was calculated for three pairs of consecutive years for the Ontario PPV survey (i.e., 2006/2007, 2007/2008, 2008/2009). The univariate and bivariate L functions were calculated for each instance using the software program PROGRAMITA (38).

Similar to the concept of time to 50% (T_{50}) or 95% (T_{95}) disease incidence, we developed a method to determine the distance to 50% and 95% of new PPV-positive blocks (D_{50} and D_{95}, respectively) relative to the previous year's PPV-positive blocks. D_{50} and D_{95} were calculated for each available year of data by determining the nearest neighbor distance from a PPV-positive block in one year to a PPV-positive block from the previous year. Nearest neighbor distances were calculated using ArcMap software (Environmental Systems Research Institute, Redlands, CA). The nearest neighbor distances (between subsequent years) were then divided into 50% and 95% quantiles, and plotted over time (year).

Spatial analyses within PPV-positive blocks. To determine the spatial pattern of PPV-positive trees within infected blocks, all trees within 12 *Prunus* blocks were mapped with regard to their PPV-status (positive or negative). *Prunus* blocks that had previously tested positive for the presence of PPV-positive trees were arbitrarily selected by CFIA personnel. Each tree in each block was individually sampled and tested by the CFIA for PPV by enzyme linked immunosorbent assay (Durviz Inc., Valencia, Spain). Ordinary runs analysis was used to determine how the PPV-positive trees were distributed within the block (13, 32). A run is defined as a sequence of like events (20). Here, a run is a sequence of healthy or PPV-infected trees either within or across rows. To determine the presence of spatial dependence (clustering) of PPV-infected trees within a block, a *z*-statistic was calculated to compare the observed number of runs to the expected number of runs that would occur if PPV-positive trees were randomly distributed within a block, for a given level of PPV incidence. The *z*-statistic is calculated as:

$$z = \frac{O - E}{s(O)}$$

,where O is the observed number of runs, E is the expected number of runs, and s(O) is the standard deviation of the observed number of runs. A *z*-statistic value of <-1.64 indicates that PPV-positive trees are spatially clustered within the *Prunus* blocks (rejection of the null hypothesis). Conversely, a *z*-statistic value >-1.64 indicates that PPV-positive trees are randomly distributed within the *Prunus* block. A *z*-statistic was calculated for each block within and across tree rows.

Results

Temporal progress of PPV epidemics in the US and Canada. In both Ontario and Pennsylvania, the number of PPV-positive trees decreased over time.

In Pennsylvania, the relationship between year and the number of PPV-positive *Prunus* trees detected decreased exponentially (0.59 ln units/year) ($F = 8.43$, $P = 0.0337$), with the independent variable (year) explaining 62.8% of the variation in the ln number of PPV-positive trees detected over time ($R^2 = 62.8\%$) (Fig. 1A and B).

There was a significant linear relationship between year and the number of PPV-positive trees detected in Ontario ($F = 81.43$, $P < 0.0001$). For 2000 through 2009, the frequency of PPV-positive *Prunus* trees detected in Ontario decreased by ~195 trees/year, with year explaining 91.1% of the variation in PPV-positive trees ($R^2 = 91.1\%$) (Fig. 2A and B).

Plum pox virus incidence (number of PPV-positive trees/total number of trees tested) in both Pennsylvania and Ontario also decreased over time. Incidence of PPV was highest in both Pennsylvania and Ontario the first year that PPV surveys were initiated (2000). The incidence of PPV then decreased rapidly in subsequent years until PPV was declared eradicated in Pennsylvania in 2009. In Pennsylvania, the incidence fell below 0.05% in 2001, and stayed below that level until no new PPV-positive trees were detected in 2007. In Ontario, the incidence of PPV was 1.86% at its peak in 2000. Incidence then decreased to less than 1% in 2002, and further decreased to less than 0.5% in 2007. In 2008 and 2009 incidence fell below 0.05%.

Plum pox virus incidence for Pennsylvania and Ontario was transformed using a negative exponential model to obtain a linear relationship between time (year) and

incidence in both Pennsylvania ($F = 12.93$, $P = 0.0156$) and Ontario ($F = 86.29$, $P < 0.0001$) (Fig. 3 and 4). The coefficient of determination (R^2) was 72.1% for Pennsylvania PPV incidence, and 91.5% for PPV incidence in Ontario. Based on the slopes of the regression lines, incidence decreased approximately 1.5 times faster in Pennsylvania than in Ontario.

Spatial distribution of PPV-positive *Prunus* blocks. *Plum pox virus*-positive *Prunus* blocks in Pennsylvania in 2000 were significantly more clustered than the underlying spatial pattern of all blocks for distances of 0.7 – 4.3 km, 4.5 – 4.7 km, and at 4.9 km (Fig. 5). At distances of 0.2 – 0.6 km, 4.4 km, and 4.8 km the pattern of PPV-positive blocks was not significantly different from the pattern of all *Prunus* blocks (i.e., PPV-positive blocks were randomly distributed among all *Prunus* blocks at these distances). Blocks were regularly distributed at distances of 0.1 km.

In Ontario, PPV-positive blocks were significantly more clustered than the underlying spatial patterns of all *Prunus* blocks for distances of 1.0 to 25.0 km in all four years (2006-2009) (Fig. 6). Using this measure of spatial dependence, the pattern of PPV-positive blocks in Ontario did not change over this time period.

Applying the bivariate form of Ripley's K function to consecutive years of survey data in Ontario, PPV-positive blocks in 2007 were found to be significantly clustered around PPV-positive blocks from 2006 at distances of 0.1 to 3.0 km (Fig. 7A). In 2008, PPV-positive blocks were clustered around PPV-positive blocks in 2007 between distances of 0.1 and 17.0 km (Fig. 7B). Finally, in 2009, PPV-positive blocks were clustered around blocks that were PPV-positive in 2008 for distances of 0.1 and 17.4 km (Fig. 7C).

The distances from PPV-positive blocks to 50% of new positive blocks the ensuing year (D_{50}) in Pennsylvania increased each year from 1.3 km in 2000 to a maximum of 17.1 km in 2005 (Fig. 8). In 2006, D_{50} decreased to 6.3 km. In 2001, D_{95} initially increased to a maximum of 34.6 km, followed by a decrease to 11.2 in 2004, and then increased in both 2005 and 2006 to 19.7 km. In Ontario, D_{50} values increased each year from 2007 to 2009 (Fig. 9), whereas D_{95} values decreased between 2007 and 2008, and then increased in 2009.

Spatial pattern of PPV-positive trees within *Prunus* blocks in Ontario. The spatial patterns of PPV-positive trees within rows were random for nine of the 12 *Prunus* blocks analyzed (Table 1). In the remaining three blocks, PPV-positive trees within rows were clustered. Across rows, the spatial patterns of PPV-positive trees in 10 of 12 *Prunus* blocks were random. In the remaining two blocks, PPV-positive trees were clustered.

Discussion

Spatial pattern of PPV-positive blocks. Marked point pattern spatial analyses of PPV-positive *Prunus* blocks revealed that the spatial dynamics of PPV epidemics in Pennsylvania and Ontario at the block scale are quite different. The L function calculated for individual years of data revealed that PPV-positive blocks were clustered over relatively short distances in Pennsylvania (0.7 – 4.3 km) in 2000, whereas clustering was observed over larger distances in Ontario (1 – 25 km) in 2006 – 2009. This discrepancy in spatial dependence suggests that the dissemination processes determining the distribution of PPV-positive blocks in Pennsylvania and Ontario were quite different. One of the factors that may have influenced block scale spatial dynamics is block

incidence (no. PPV-positive *Prunus* blocks/total no. *Prunus* blocks surveyed x 100). Although block incidence was comparable in 2000 in Pennsylvania (3.33%) and Ontario in 2006 (3.79%), the frequency of PPV-positive blocks in Ontario was approximately 6 times higher than in Pennsylvania. The large number of PPV-positive blocks in Ontario likely contributed to the large-scale clustering of PPV-positive blocks in Ontario, compared to the relatively few PPV-positive blocks in Pennsylvania, where smaller-scale clustering was detected.

The density of *Prunus* blocks may also have contributed to the clustering observed in Pennsylvania and Ontario. In Ontario, the density of *Prunus* blocks is much larger than the density of blocks in Pennsylvania. In Ontario, *Prunus* production is limited to the Western border of Lake Ontario, while in Pennsylvania *Prunus* is grown throughout the state. Moreover, the Ontario *Prunus* industry is much larger than the Pennsylvania industry. Consequently, the density of *Prunus* blocks is much greater in Ontario than in Pennsylvania, which may lead to greater alloinfection from block-to-block than in Pennsylvania (40). Thus, both the large number of PPV-positive blocks and the high density of *Prunus* blocks in Ontario are likely contributing to the large scale clustering observed Ontario.

The extent of spatial dependence found in our analyses is consistent with previous studies concerning the spatial dependence of blocks in Pennsylvania and Ontario. Gottwald (10) has also reported the presence of spatial dependence for PPV at the orchard scale up to a distance of 4.3 km in Ontario (after accounting for the underlying pattern of all orchards). This distance is very similar to what was found when the L function was applied to PPV-positive blocks in 2000 in Pennsylvania. These findings

may suggest that the distribution of PPV-positive blocks in Pennsylvania and Ontario was initially similar during the early stages of both epidemics. However, as the epidemics progressed in Ontario, the spatial distribution of PPV-positive blocks changed, and by 2006, PPV-positive blocks were clustered up to distances of 25.0 km, indicating that PPV-positive blocks are now having an impact on the PPV health status of other *Prunus* blocks up to a distance of 25.0 km.

Applying the L function to consecutive years of survey data, it was found that the location of PPV-positive blocks were spatially dependent upon the locations of PPV-positive blocks the previous year. This analysis provides a quantitative measure of PPV clustering that carries over from one year to subsequent years. Thus, even when PPV-positive trees were removed from a block, previous PPV-positive blocks were still having an impact on the health status of other *Prunus* blocks in subsequent years. Spatial dependence, with respect to the location of PPV-positive blocks the previous year differed between 2007 and 2009. In 2007, clustering was observed up to 3 km, while in 2008 and 2009, clustering was observed up to approximately 17 km. The small-scale clustering observed in 2007 was likely the result of the high PPV incidence in 2006, resulting in future PPV-positive blocks being closer to the previous years' PPV-positive blocks that were detected in 2007. Clustering in 2007 and 2008 were likely similar because the incidence and distribution of PPV-positive blocks in both of the previous years were very similar.

The $D_{50/95}$ values integrate distance and time as dimensions to quantifying the spatial dynamics of PPV-positive blocks from year-to-year. Because $D_{50/95}$ is based on the nearest neighbor, it provides a conservative measure of spread, as the nearest

neighbor (PPV-positive block) from the previous year is not necessarily the source of inoculum for new PPV-positive blocks. Despite this, D_{50} and D_{95} measures may be useful in determining what sampling and eradication distances are most appropriate to achieve success. For example, D_{95} values may serve as a guide to establish the distance from previous PPV-positive blocks that should be intensively surveyed for new positives. In Pennsylvania, following 2001, 95% of new positives were between 10 and 20 km of a PPV-positive block from the previous year's. This suggests that surveying efforts should be more intensive up to 20 km away from the previous year's positive blocks, when relatively few new positives are thought to exist. Furthermore, the finding that newly detected PPV-positive blocks in Ontario are spatially closer to previously detected PPV-positive blocks, may suggest that PPV-positive blocks should be removed, along with those found within a certain distance, such as the 500 m buffer removal policy used in Pennsylvania.

The concept of determining the distance from previous PPV-positive trees to newly detected positive trees has been used in other pathosystems to provide a science based protocol for tree and block removal. Gottwald et. al. (12) determined that a previous 125 ft. removal distance of citrus trees around citrus canker-infected trees was insufficient, based on findings that the distance to newly detected citrus canker trees was much greater than 125 ft. Similarly, our findings suggest that the current Canadian PPV eradication protocol that removes only PPV-positive blocks above a certain threshold (0.5% PPV incidence) may be insufficient to successfully eradicate PPV because it does not take into account the spatial dynamics of PPV.

The successful eradication of PPV in Pennsylvania was in part due to an aggressive eradication program. Removing all potential hosts within 500 m of an infected tree effectively limited the occurrence of new PPV-positive trees/blocks (lowered initial inoculum), and thus slowed the dispersal of PPV. While it has been suggested that similar removal buffers may also have eradicated PPV in Ontario (22), these more strict quarantine/eradication measures would have been highly detrimental to Canada's *Prunus* industry. In 2006, a year of relatively high PPV incidence, nearly 50% of *Prunus* blocks in Ontario would have been required to be removed, if a 500 m tree removal (eradication) protocol was utilized (data not shown). Still, block removal thresholds in Ontario that currently allow PPV-positive *Prunus* blocks to remain in production for up to three years, likely allow PPV-positive blocks to act as potential sources of PPV inoculum for both *Prunus* trees within and beyond the PPV-infected blocks. This is evidenced by the fact that approximately 34% of PPV-positive blocks in 2007 were also found to have PPV-positive trees within those blocks the previous year. Hence, a balance needs to be struck between removing potential hosts, while at the same time preserving as much of the existing *Prunus* industry as possible.

Spatial distribution of PPV-positive trees within blocks. The random spatial patterns of PPV-positive trees observed in nine of the 12 *Prunus* blocks in Ontario is consistent with other within-block analyses of the spatial pattern of PPV-positive trees in Pennsylvania that were conducted in 1999 and 2000. These findings have a potential impact on the sampling design that should be employed when scouting for new PPV-positives. Because the distribution of trees within blocks was sometimes clustered (25%), it would be best to utilize a systematic sampling design for all *Prunus* blocks as

this design can be used for both clustered and random spatial patterns of diseased plants (27).

The previous study of the within-block spatial pattern of PPV-positive in Pennsylvania, as well as our findings in Ontario are also consistent with a within block study of PPV-positive tress in Spain (9), which reported that PPV-positive trees were randomly distributed in most plots. However, these findings differ from a study conducted in France, which found that PPV-positive trees were aggregated in 15 of 18 blocks when analyzed within or across rows (6). In a larger context, our findings are supportive of the notion that PPV strain, aphid vector presence and abundance, and environmental factors are important in the epidemiology of PPV, and in particular the spatial and temporal dynamics of PPV epidemics.

Acknowledgements

Funding for this research was provided by the USDA-ERS. Thanks are given to Nancy Richwine and Ruth Welliver (Pennsylvania Department of Agriculture) and Violet Galvin, Eric Wierenga, Laura Maurice, and Charlene Green (Canadian Food Inspection Agency) for providing us with spatial data, as well as access to *Prunus* blocks for mapping.

Literature Cited

1. Atanassov, D. 1932. Plum Pox: A new virus disease. Annals of the University Sofia, Faculty of Agriculture and Silviculture 11:49-69.
2. Boulila, M. and Ravelonandro, M. 2006. *Plum pox virus* (PPV) in Tunisia. EPPO Bull. 36:216-216.
3. Caglayan, K. 2006. *Plum pox virus* (PPV) in Turkey. EPPO Bull. 36:216-217.

4. Cambra, M., Capote, N., Myrta, A., and Llacer, G. 2006. *Plum pox virus* and the estimated costs associated with sharka disease. EPPO Bull. 36:202-204.

5. Cambra, M. A., Serra, J., Cano, A., and Cambra, M. 2006. *Plum pox virus* (PPV) in Spain. EPPO Bull. 36:215-215.

6. Dallot, S., Gottwald, T., Labonne, G., and Quiot, J. B. 2003. Spatial pattern analysis of sharka disease (*Plum pox virus* strain M) in peach orchards of southern France. Phytopathology 93:1543-1552.

7. Di Terlizzi, B. and Boscia, D. 2006. *Plum pox virus* (PPV) in Italy. EPPO Bull. 36:210-210.

8. Dixon, P. M. 2002. Ripley's *K* function. Pages 1796 - 1803 in: Encyclopedia of Environmetrics. A. H. El-Shaarawi and W. W. Piegorsch, eds. Wiley & Sons, Chichester.

9. Gottwald, T., Avinent, L., Llácer, G., and Hermoso, D. E. M. 1995. Analysis of the spatial spread of sharka (*Plum pox virus*) in apricot and peach orchards in eastern Spain. Plant Dis. 79:266-278.

10. Gottwald, T. R. 2006. Epidemiology of sharka disease in North America. EPPO Bull. 36:279-286.

11. Gottwald, T. R., Dallot, S., and Favin, R. 2003. Regional Spatial Pattern Analyses of *Plum Pox Virus*. NE1006: Eradication, Containment and/or Management of Plum Pox Disease (Sharka). Shepardstown, WV.

12. Gottwald, T. R., Sun, X., Riley, T., Graham, J. H., Ferrandino, F., and Taylor, E. L. 2002. Geo-referenced spatiotemporal analysis of the urban citrus canker epidemic in Florida. Phytopathology 92:361-377.

13. Habili, N. and Nutter Jr, F. W. 1997. Temporal and spatial analysis of grapevine leafroll-associated virus 3 in Pinot Noir grapevines in Australia. Plant Dis. 81:625-628.

14. Ismaeil, F. 2006. *Plum pox virus* (PPV) in Syria. EPPO Bull. 36:216-216.

15. Jarausch, W. 2006. *Plum pox virus* (PPV) in Germany. EPPO Bull. 36:209-209.

16. Levy, L. 2006. *Plum pox virus* (PPV) in the United States of America. EPPO Bull. 36:217-218.

17. Levy, L., Damsteegt, V., and Welliver, R. 2000. First report of *Plum pox virus* (Sharka disease) in *Prunus persica* in the United States. Plant Dis. 84:202.

18. Levy, L., Damsteegt, V., Scorza, R., and Kolber, M. 2000. Plum plox potyvirus disease of stone fruits. http://www.apsnet.org/online/feature/plumpox/top.html.

19. Llacer, G. and Cambra, M. 2006. Hosts and symptoms of *Plum pox virus*: fruiting *Prunus* species. EPPO Bull. 36:219-221.

20. Madden, L., Louie, R., Abt, J., and Knoke, J. 1982. Evaluation of Tests for Randomness of Infected Plants. Phytopathology 72:195-198.

21. Matić, S., Durić, G., and Myrta, A. 2006. *Plum pox virus* (PPV) in Bosnia and Herzegovina. EPPO Bull. 36:205-206.

22. Mitham, P. 2010. Ontario faces challenges to *Plum pox virus* eradication. Good Fruit Grower. April 2010.

23. Mumford, R. A. 2006. *Plum pox virus* (PPV) in the United Kingdom. EPPO Bull. 36:217-217.

24. Muñoz, M. and Collao, M. 2006. *Plum pox virus* (PPV) in Chile. EPPO Bull. 36:207-207.

25. NAPPO 2004. Guidelines for phytosanitary action following detection of *Plum pox virus*. NAPPO Regional Standards for Phytosanitary Measures http://www.nappo.org/Standards/Standards%28all%29/RSPM18-18-10-04-e.pdf.

26. Navratil, M., Safarova, D., Karesova, R., and Petrzik, K. 2006. *Plum pox virus* (PPV) in China. EPPO Bull. 36:207-207.

27. Nutter, F. W. 1997. Quantifying the temporal dynamics of plant virus epidemics: a review. Crop Protection 16:603-618.

28. Ortego, J., Zotto, A. D., Caloggero, S., Raigón, J. M., Gasparini, M. L., Ojeda, M. E., and Ducasse, D. A. 2006. *Plum pox virus* (PPV) in Argentina. EPPO Bull. 36:205-205.

29. Ripley, B. D. 1981. *Spatial statistics*. Wiley, New York

30. Speich, P. 2006. *Plum pox virus* (PPV) in France. EPPO Bull. 36:208-209.

31. Stamo, B. and Myrta, A. 2006. *Plum pox virus* (PPV) in Albania. EPPO Bull. 36:205-205.

32. Steinlage, T. A., Hill, J., and Nutter Jr, F. 2002. Temporal and spatial spread of Soybean mosaic virus (SMV) in soybeans transformed with the coat protein gene of SMV. Phytopathology 92:478-486.

33. Thompson, D. 2006. Control and monitoring: control strategies for *Plum pox virus* in Canada. EPPO Bull. 36:302-304.

34. Thompson, D. 2006. *Plum pox virus* (PPV) in Canada. EPPO Bull. 36:206-206.

35. Thompson, D., McCann, M., MacLeod, M., Lye, D., Green, M., and James, D. 2001. First report of Plum pox potyvirus in Ontario, Canada. Plant Dis. 85:97.

36. USDA 2007. Recovery plan for *Plum pox virus* (Sharka) of stone fruit. National Plant Disease Recovery System.

http://www.ars.usda.gov/SP2UserFiles/Place/00000000/opmp/Plum%20Pox%20PPV%2070222.pdf.

37. Varveri, C. 2006. *Plum pox virus* (PPV) in Greece. EPPO Bull. 36:209-210.

38. Wiegand, T. and Moloney, K. A. 2004. Rings, circles, and null-models for point pattern analysis in ecology. Oikos 104:209-229.

39. Youssef, S. A. and Shalaby, A. 2006. *Plum pox virus* (PPV) in Egypt. EPPO Bull. 36:208-208.

40. Zadoks, J. C. and Schein, R. D. 1979. *Epidemiology and plant disease management*. Oxford University Press, New York

41. Zamharir, M. G., Bashir, N. S., and Khakvar, R. 2006. *Plum pox virus* (PPV) in Iran. EPPO Bull. 36:210-210.

Table 1. Spatial pattern analyses of PPV-positive *Prunus* trees within twelve PPV-positive *Prunus* blocks in Ontario, Canada.

		Within row		Across row	
Block	Incidence (%)	*Z*-score[A]	Spatial pattern	*Z*-score[A]	Spatial pattern
1	0.55	0.15	Random	0.15	Random
2	1.28	0.20	Random	0.20	Random
3	1.50	0.27	Random	0.27	Random
4	0.90	0.15	Random	0.15	Random
5	1.19	-6.72	Clustered	0.16	Random
6	0.66	-2.82	Clustered	-2.82	Clustered
7	0.41	0.13	Random	0.13	Random
8	0.61	0.26	Random	0.26	Random
9	0.24	0.13	Random	0.13	Random
10	1.80	0.26	Random	1.78	Random
11	1.00	-7.78	Clustered	-7.78	Clustered
12	1.45	0.07	Random	0.07	Random

[A]The spatial pattern of PPV-trees was considered clustered within a *Prunus* block if the *Z*-score was < -1.64.

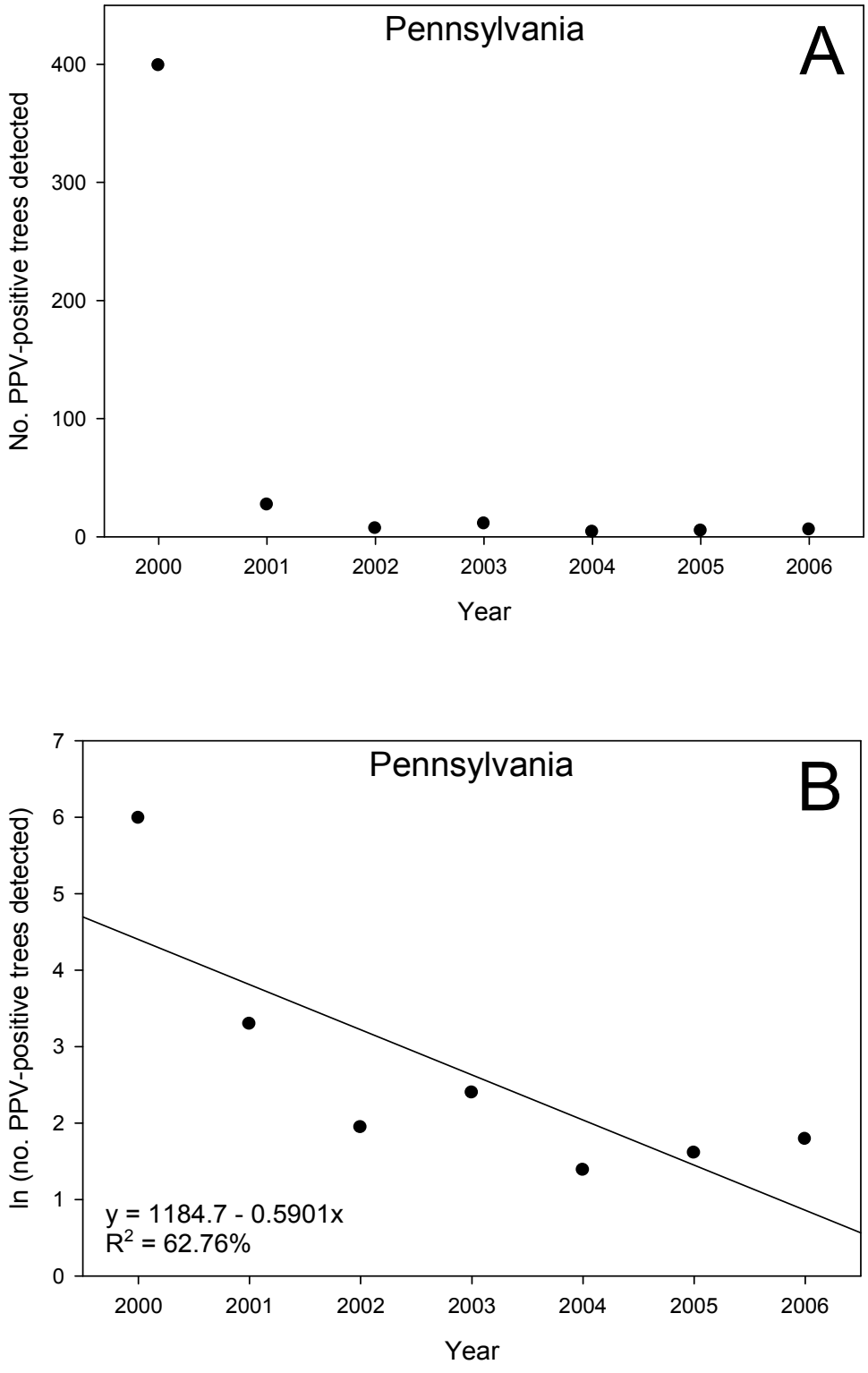

Figure 1. The number of PPV-positive *Prunus* trees detected each year in Pennsylvania (A) plotted over time (year) and (B) transformed using an exponential model.

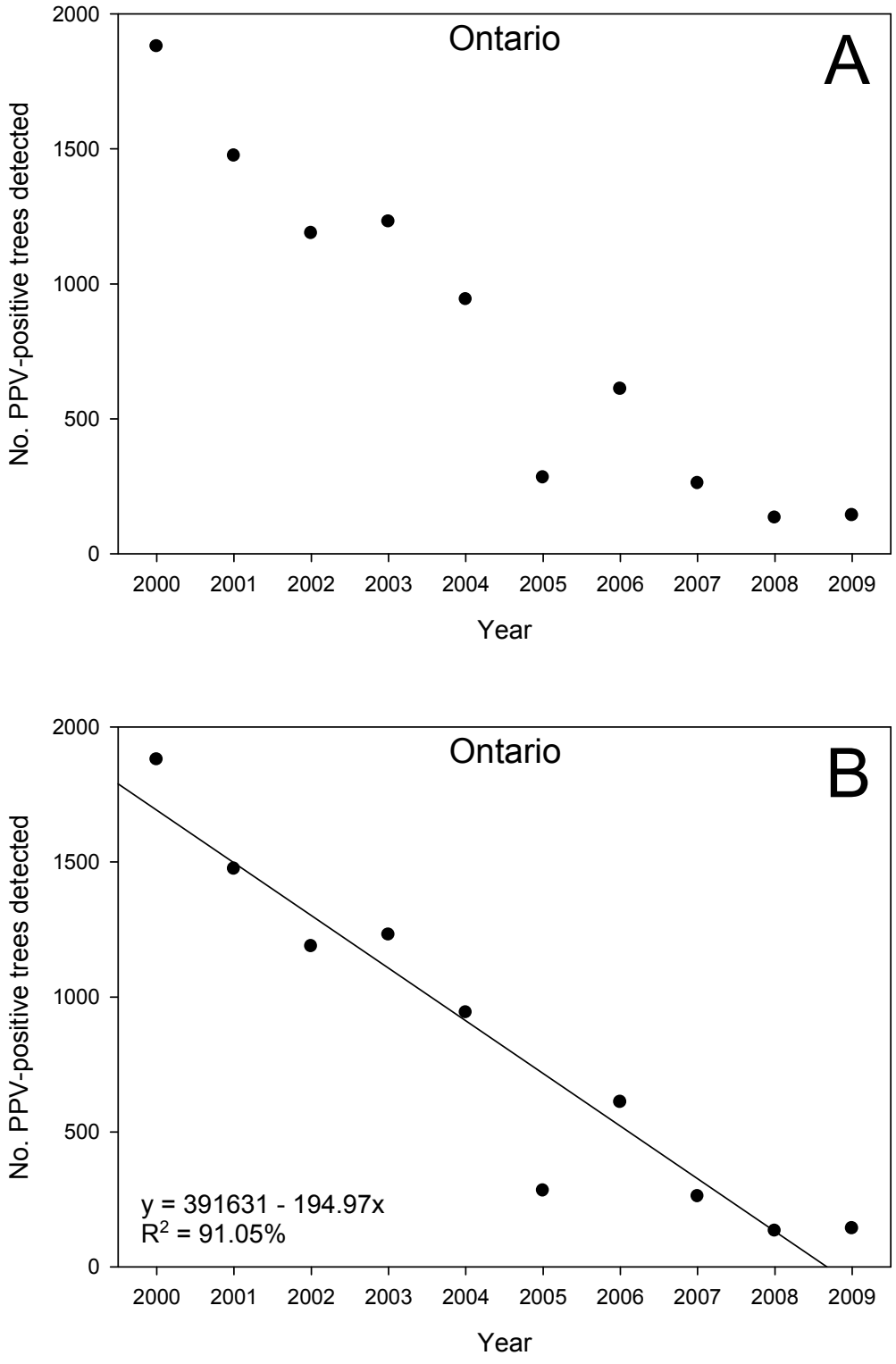

Figure 2. The number of PPV-positive *Prunus* trees detected each year in Ontario (A) plotted over time (year) and (B) transformed using an exponential model.

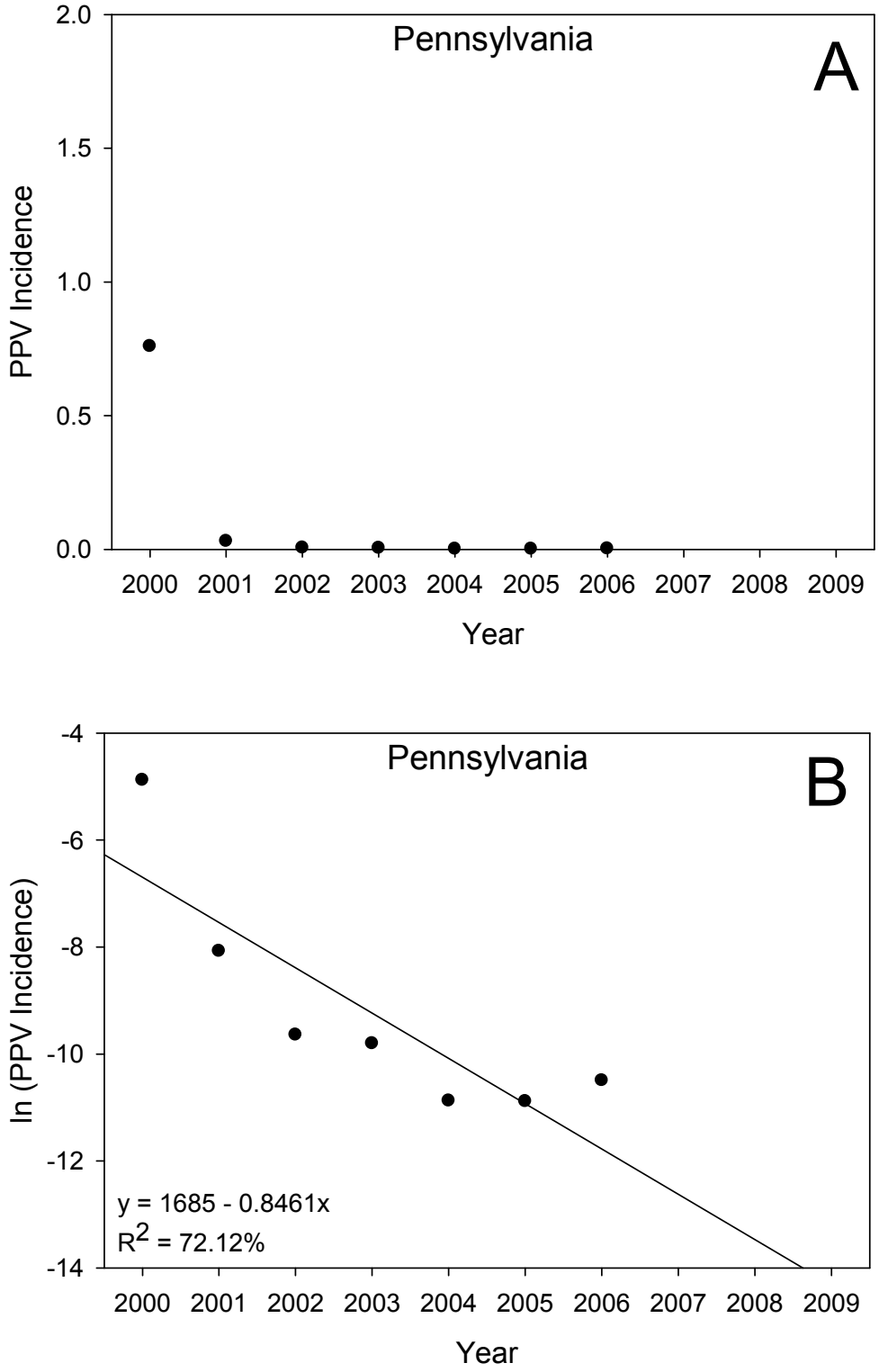

Figure 3. (A) Incidence of PPV-positive *Prunus* trees in Pennsylvania plotted over time (year) and (B) PPV incidence transformed using an exponential model plotted over time.

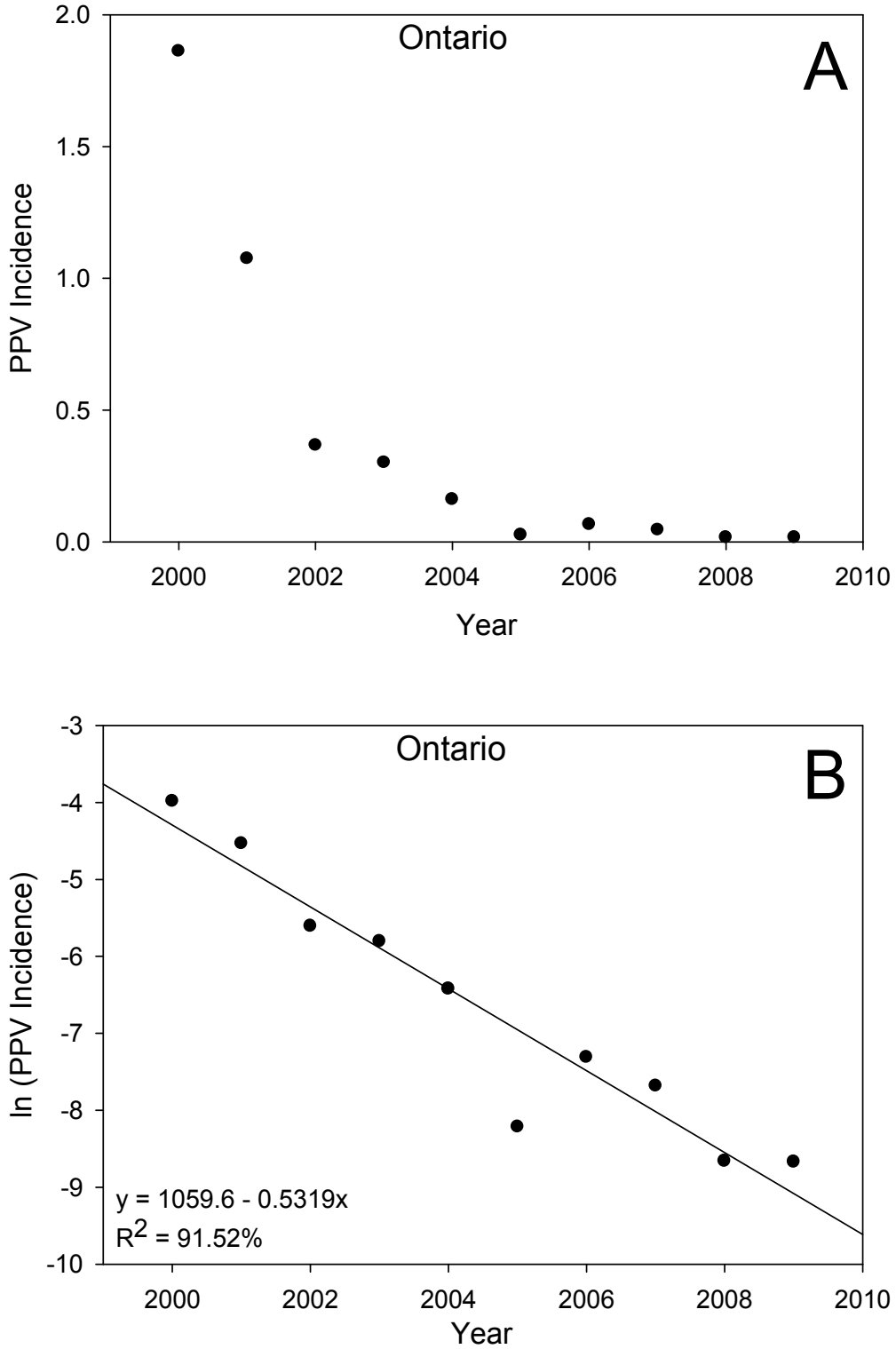

Figure 4. (A) Incidence of PPV-positive *Prunus* trees in Ontario plotted over time (year) and (B) PPV incidence transformed using an exponential model plotted over time.

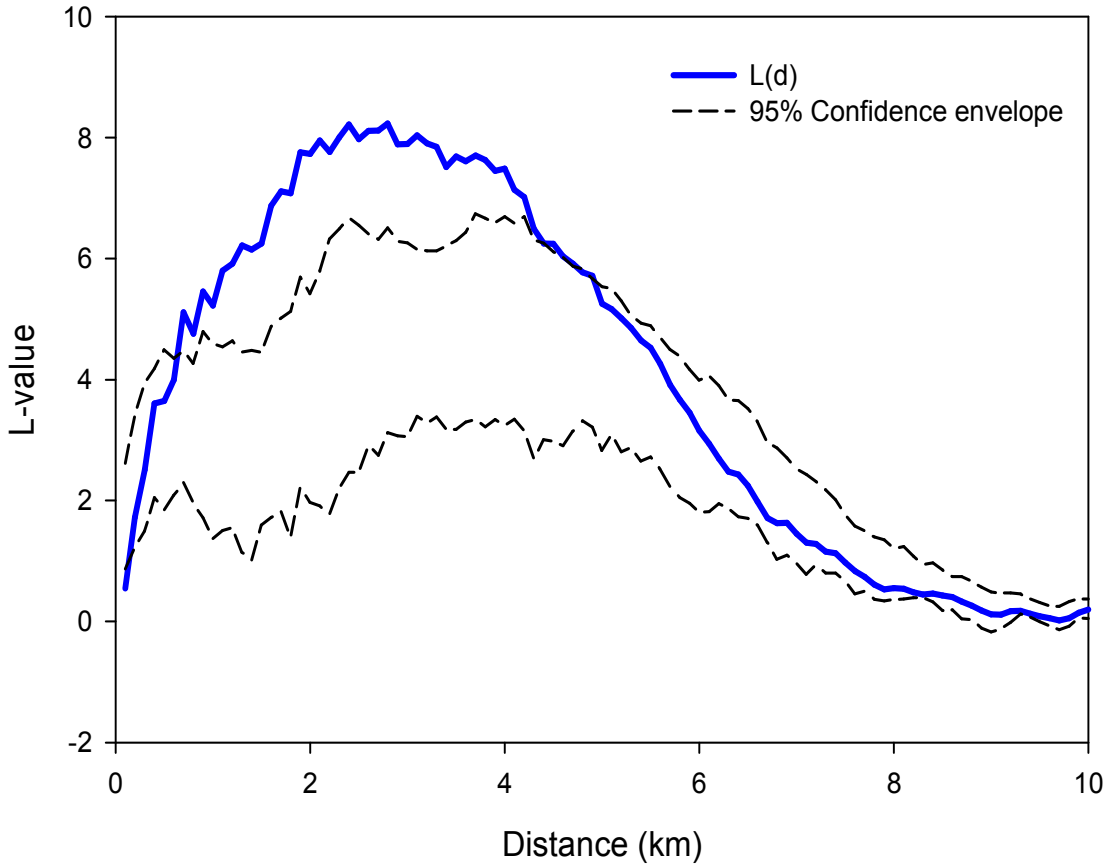

Figure 5. Ripley's L function, with a random labeling null hypothesis, performed on the PPV-positive *Prunus* blocks detected in Pennsylvania in 2000.

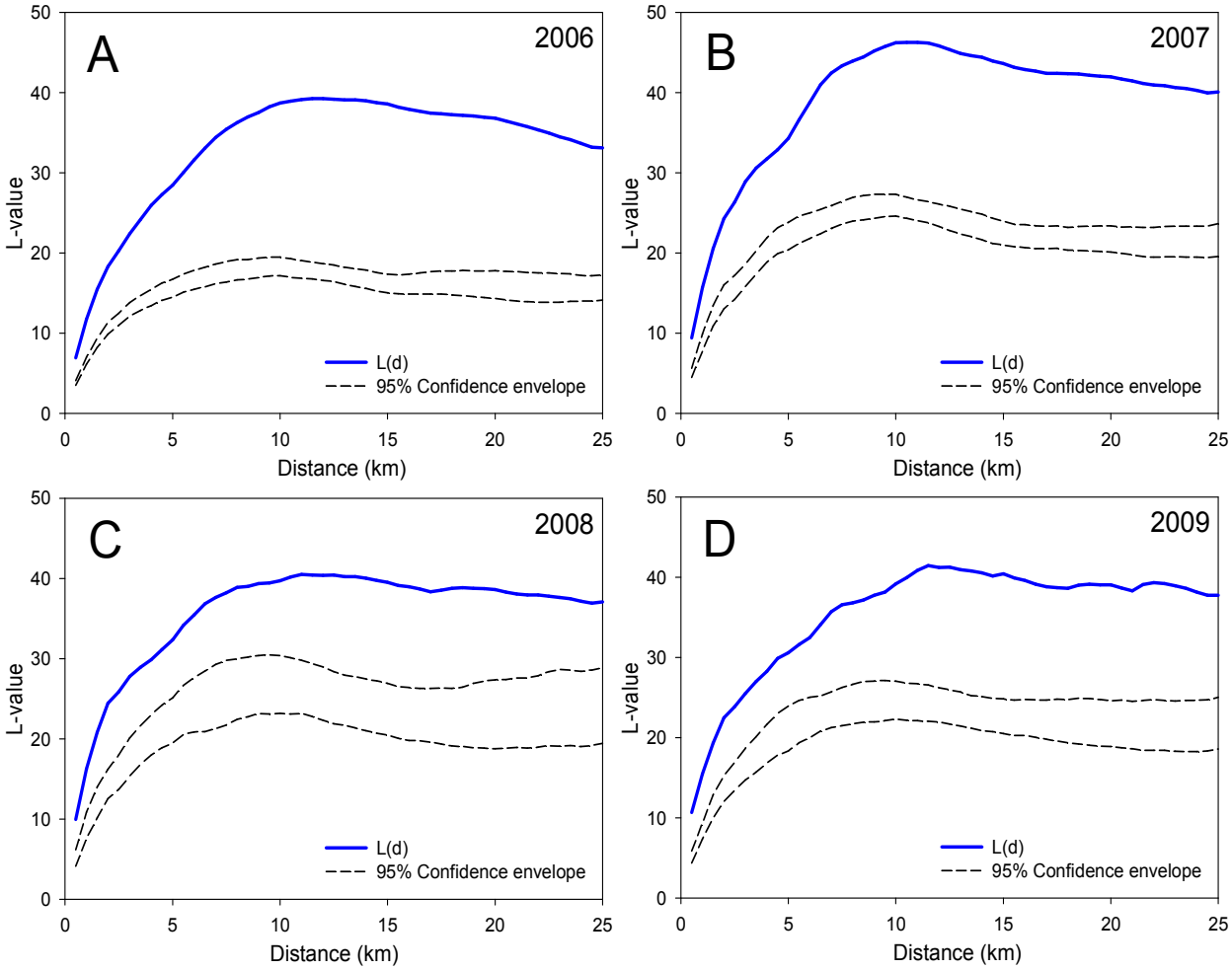

Figure 6. Ripley's L function, with a random labeling null hypothesis, performed on the PPV-positive *Prunus* blocks detected in Ontario in (A) 2006, (B) 2007, (C) 2008, (D) 2009.

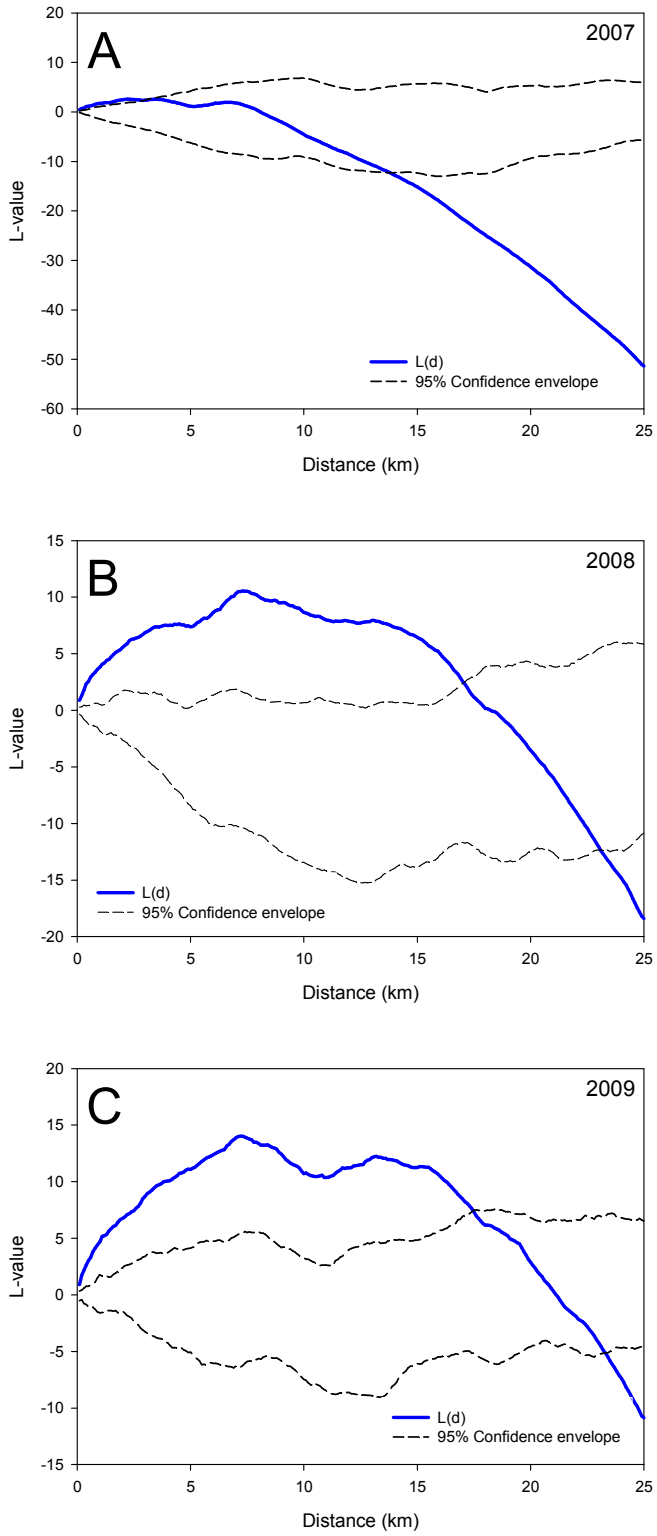

Figure 7. Bivariate Ripley's L function, with a random labeling null hypothesis, performed on consecutive years of PPV-positive *Prunus* block in Ontario in (A) 2007, (B) 2008, and (C) 2009.

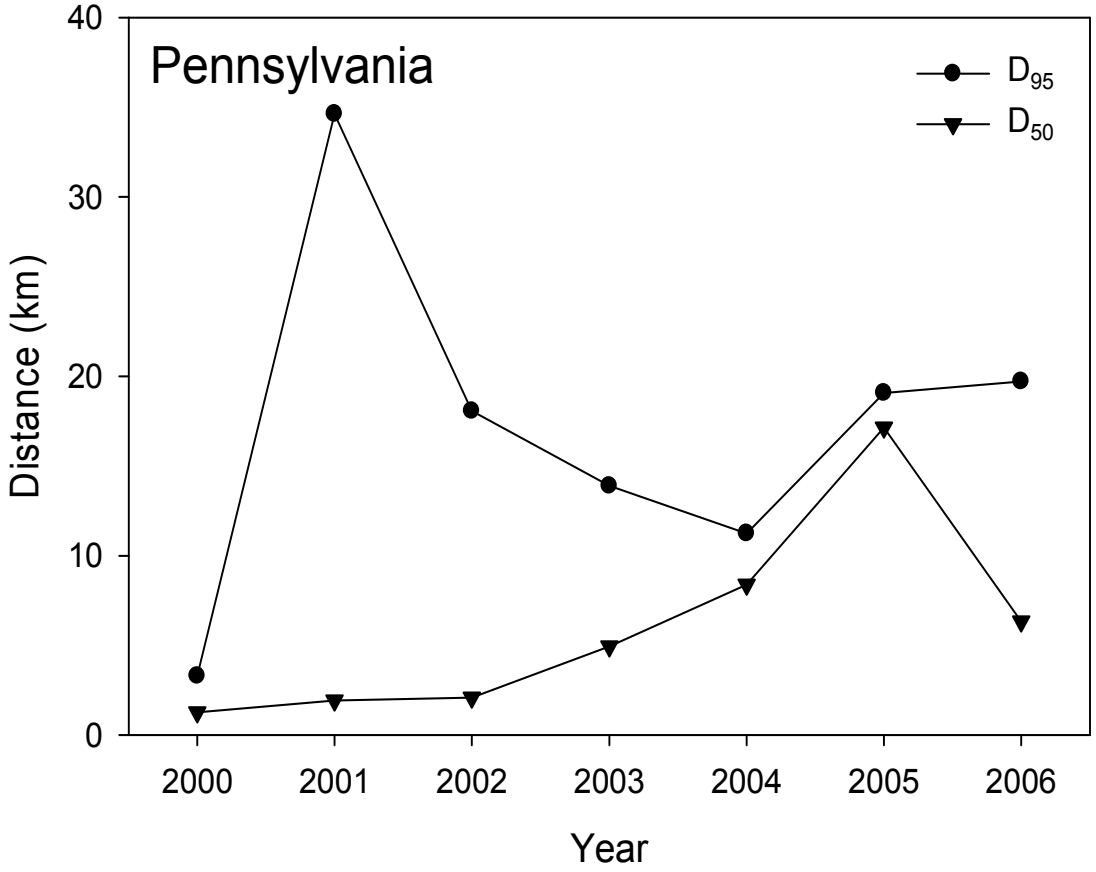

Figure 8. The distance to 50% (D_{50}) and 95% (D_{95}) of new PPV-positive *Prunus* blocks in Pennsylvania plotted over time. Values represent the 50% and 95% quantiles for the minimum distances between PPV-positive *Prunus* blocks and the nearest PPV-positive *Prunus* block from the previous year.

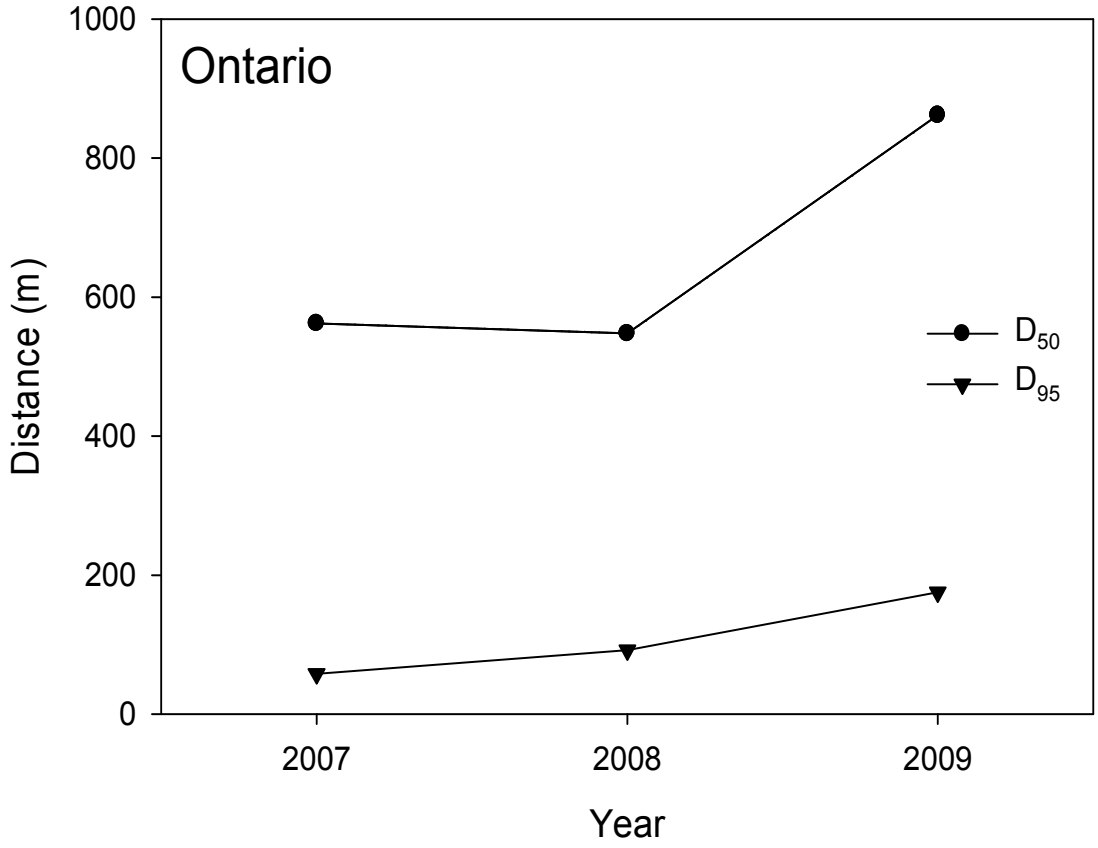

Figure 9. The distance to 50% (D_{50}) and 95% (D_{95}) of new PPV-positive *Prunus* blocks in Ontario plotted over time. Values represent the 50% and 95% quantiles for the minimum distances between PPV-positive *Prunus* blocks and the nearest PPV-positive *Prunus* block from the previous year.

CHAPTER 3.

COMPARING THE SAMPLING AND TESTING SYSTEMS OF THE US AND CANADIAN *PLUM POX VIRUS* ERADICATION PROGRAMS

Manuscript prepared for submission to *Phytopathology*

Abstract

Plum pox virus (PPV) was first detected in the US in Pennsylvania in 1999 and in Ontario, Canada in 2000. In 2009, PPV was officially declared eradicated in Pennsylvania, following the implementation of a ten-year PPV survey and eradication program. Despite implementing a similar eradication program in Canada, PPV has yet to be eradicated in Ontario. The US and Canadian Eradication Programs, though similar, differ in a number of ways. These include: (i) the number of leaves sampled and tested per tree, (ii) the ELISA test kit used to test *Prunus* leaves, and (iii) the removal (eradication) protocols that are enforced when a PPV-positive tree is detected. The goal of this research, therefore, was to compare how the US and Canadian PPV Eradication Programs affect the probability of detecting PPV-positive leaves, scaffolds, tree, and blocks. A simulation model was developed to quantify and compare the components of the US versus Canadian PPV detection efficiency. The sampling and detection protocols used in the US (tree scale) had a detection efficiency of approximately 72%, compared to 41% for Canada's. Employing a stratified (by scaffold) random sampling design did not improve PPV detection efficiency compared to using a simple random sampling design to sample leaves from *Prunus* trees. Detection efficiency increased with increasing sample

size (leaves/tree); however, the gain in detection efficiency diminished as sample size increased. There was good agreement between the ELISA kits used in the US and Canadian at the leaf and scaffold scales (based upon Chi-square analysis), but not at the tree scale. This indicates that the results of the two ELISA test kits at the tree scale were independent of one another. The results from this study have important implications with regard to the two Eradication Programs, and their impacts on successfully detecting and eradicating PPV infected *Prunus* trees and blocks.

Introduction

Plum pox virus (PPV) is one of the most damaging plant viruses affecting *Prunus* worldwide, causing severe reductions in both yield and fruit quality (2, 6). *Plum pox virus* was first detected in North America in 1999 in a peach orchard (cv. Encore) located in Adams County, Pennsylvania (5). Following the initial detection of PPV in Pennsylvania, PPV was detected in the Niagara peninsula of Ontario, Canada, as well as in Nova Scotia, Canada in 2000 (16). In response to these findings, both the US and Canadian governments developed and implemented National *Plum pox virus* Eradication Programs in an effort to quarantine and eradicate this virus before it could causes significant losses to the *Prunus* industry (14, 17). Ten years after the establishment of the US Plum Pox Eradication Program, PPV was officially declared eradicated in Pennsylvania in 2009, per North American Plant Protection Organization (NAPPO) guidelines (9, 10). However, PPV is still present in Ontario, and as of 2006, PPV is also present in New York State (within eight km of the Ontario PPV epidemic) (11).

The eradication programs implemented in Pennsylvania and Ontario differ in a number of important regulatory features, including: (i) sample size (number of leaves

sampled per tree), (ii) the commercial ELISA test kit used to test leaf samples, and (iii) the detection thresholds that determine where, when, and how *Prunus* trees/blocks will be eradicated following the detection of a PPV-positive *Prunus* tree. At present, it is not known how these program differences have affected the epidemiology and success in eradicating PPV in the two countries.

The US PPV Survey and Eradication Program. In Pennsylvania, survey crews visit all *Prunus* blocks within the state each growing season. In areas where the status of PPV is unknown or the virus has yet to be detected (non-quarantine areas), a systematic sampling design is used (whereby approximately 25% of the *Prunus* trees within each block are sampled) (4). In areas where PPV has been previously detected (quarantined areas), all *Prunus* trees within all *Prunus* blocks are sampled (17). Eight leaves per tree are sampled, with survey crews being instructed to sample leaves from each scaffold. Each 8-leaf sample is bulk-tested for PPV using a commercially available double antibody sandwich (DAS) enzyme linked immunosorbent assay (ELISA) test kit (Agdia, Elkhart, IN). It is assumed that only one of the eight leaves in a bulk sample needs to be positive for PPV for the bulk sample to be detected as positive. When a PPV-positive tree is detected in the US, this tree and all other *Prunus* trees within 500 m of the PPV-positive tree (diseased or not) are removed (eradicated) (3, 17).

The Canadian Survey and Eradication Program. As in Pennsylvania, *Prunus* blocks outside established quarantine areas are initially sampled less intensively than *Prunus* blocks that occur within established PPV quarantine zones, (i.e., where PPV-positive trees/blocks have been detected previously). In non-quarantine zones, leaf samples are collected from approximately 25% of *Prunus* trees within a *Prunus* block

(14). *Prunus* blocks that have previously had PPV-positive trees are censused (i.e., all trees within previously affected blocks are sampled and tested for PPV) (14). In Ontario, 20 leaves are sampled from each tree and these leaves are bulk-tested, with the assumption that at least two of the 20 leaves must have the virus present in order for the bulk sample to test positive for PPV. In previous years, twelve leaves were collected per sample (14). The Canadian PPV survey program uses a commercially available double antibody sandwich indirect (DASI) ELISA kit to test *Prunus* leaf samples for the presence of PPV (Durviz, Valencia, Spain). When a PPV-positive tree is detected in Ontario, only the PPV-positive trees are removed, unless the incidence of PPV within the block is >0.5%, in which case the entire block is removed. The threshold for block removal has deceased since the initial implementation of the eradication program. In 2000, the PPV incidence threshold for removal was set at 10%, which was similar to PPV removal thresholds utilized in some European countries (7, 8, 12). By 2006, the PPV removal threshold decreased to 1.5%, and is now set at 0.5%. It is believed that this threshold will eventually be decreased to 0%, whereby a single PPV-positive tree detected within a block will result in the removal of that entire block (14, 18). Another aspect that differs from the US program, is that an entire *Prunus* block is also removed if the block was positive for PPV for three consecutive years.

The successful eradication of PPV in Pennsylvania is thought to be due to the impact of implementing a large, intensive survey program that was coupled with an aggressive removal (eradication) policy. Although the eradication program in Ontario has been met with some success in terms of reducing the frequency of new PPV-positive trees over time, PPV has not yet been eradicated in Ontario. By evaluating and

comparing the sampling and testing efficiencies of the two National Eradication Programs, important information may be obtained to optimize present and future PPV eradication programs. Therefore, the objectives of this study were to: (i) compare the US and Canadian sampling and detection protocols (ii) quantify the level of agreement between the ELISA kits used in the US and Canada at the leaf, scaffold, and tree scales, and (iii) determine the most appropriate sample size and sampling design to detect PPV.

Material and Methods

Data collection. To determine the PPV-detection efficiency of the U.S. vs. Canadian sampling and testing protocols, 100-leaf samples were collected from 19 known PPV-positive peach (*Prunus persica*) trees in Ontario. The 19 trees initially tested positive as part of Canada's Plum Pox Survey and Eradication Program that is conducted under the auspices of the Canadian Food Inspection Agency (CFIA). The 19 trees were arbitrarily selected from the population of PPV-positive peach trees that were detected in Ontario over the course of the 2009 and 2010 growing seasons (two of the tree samples were collected in 2009, and the remaining 17 tree samples were collected in 2010). In collaboration with CFIA *Plum pox virus* survey teams, each of the 19 PPV-positive trees were re-sampled, collecting 100-leaf samples from each tree. These leaves were collected equally from each of the four scaffolds on each tree. Leaves were collected from the second year growth, as instructed by both the US and Canadian PPV sampling protocols, based upon reports that leaves from second year growth have the highest virus titer (13, 17). Of the 19 peach trees from which leaf samples were collected, 18 trees had 4 scaffolds, with 25 leaves being collected per scaffold. Leaf samples from the 19th tree,

however, were not stratified by scaffold when this tree was re-sampled, hence this tree was not included in statistical analyses performed at the scaffold scale.

ELISA testing. To determine the presence of PPV within individual leaves, each leaf was tested using enzyme linked immunosorbent assay (ELISA). Each leaf was tested using the ELISA test kits used in the US and Canada. This was facilitated by cutting each leaf down the midrib into two halves, one half of which was tested using the US PPV ELISA test kit protocol (Agdia, Elkhart, IN) and the other half-leaf was tested by PPV ELISA kit used in Canada (Durviz, Valencia, Spain). Both assays utilize the 5b universal monoclonal antibody, which can detect all known PPV strains (1). The two ELISA protocols, however, differ in a number of ways, including: (i) extraction buffer, (ii) an additional 2-hour blocking step with the Canadian protocol, and (iii) the US kit consisting of a double antibody sandwich (DAS) ELISA procedure, whereas the kit used in Canada consists of a double antibody sandwich indirect (DASI) ELISA.

Sap extraction. After leaves were cut into two halves (down the midrib) using a razor blade, each leaf-half was placed in a prelabled extraction bag.

U.S. test protocol. Leaf sap was extracted by adding approximately 10x vol./wt. general extraction buffer 4 (GEB4) to each extraction bag. Leaves were ground in extraction buffer using a pestle, sap samples were then transferred to 1.5 ml Eppendorf tubes and stored at -20^{o}C until testing. Ninety-six-well microtiter plates were coated with a 1:200 concentration of capture antibody diluted in carbonate buffer, and allowed to incubate overnight at 4^{o}C. Plates were then washed with phosphate buffer saline-Tween 20 (PBST), and wells were loaded with a 100 ul aliquot of leaf sample. Each plate was loaded with two positive control test wells, two negative control test wells, and two

buffer control wells. Plates were allowed to incubate overnight at 4°C. Following incubation, plates were again washed with PBST, and wells were loaded with 100 ul of 1:200 concentration enzyme conjugate diluted in RUB3, and allowed to incubate for 2 hours at room temperature. Plates were then washed a final time and loaded with 100 ul of 1 mg/ml p-Nitrophenol (PNP) solution and allowed to incubate in the dark for an hour before absorbance was read at 405 nm with a plate reader (Elx800, Bioteck Inc,Winooski, VT). Samples that were twice the mean of the absorbance of the negative control wells were deemed positive for PPV. Plates that did not have both positive control wells test positive for PPV were redone.

Canadian test protocol. In the Canadian PPV test protocol, approximately 10x vol/wt. of extraction buffer (PBS + 2% PVP + 0.2% skim milk powder) was added to each extraction bag. Leaves were ground in extraction buffer using a pestle, and sap samples were transferred to 1.5 ml Eppendorf tubes. Samples were stored at -20°C until tested. Plates were coated with 1:100 concentration capture antibody diluted in carbonate buffer and allowed to incubate overnight. Plates were then washed with PBST and loaded with 100 ul of leaf extract. A 100 ul aliquot of 1:1000 dilution PPV specific monoclonal antibody diluted in PBS, plus 0.5% bovine serum albumin was added to each well, and plates were then allowed to incubate for 2 hours. Plates were again washed with PBST and wells were loaded with a 1:1000 dilution of anti-mouse immunoglobulins conjugated with alkaline phosphatase diluted PBS, and allowed to incubate for 2 hours at room temperature. Plates were washed a final time with PBST and wells were loaded with 1 mg/ml PNP solution. After incubating an hour in the dark, absorbance was read at 405 nm, using a plate reader (Elx800, Bioteck Inc,Winooski, VT). Samples that were

twice the mean absorbance of negative control wells were deemed to be PPV positive, and plates that did not have both positive controls test positive for PPV were rerun.

Comparing test kits used in the US and Canada. The degree of agreement between the ELISA test kits used in the US and Canada at the leaf, scaffold, and tree scales were analyzed assessed using the chi-square option in PROC FREQ in SAS (Statistical Analysis System, SAS Institute, Cary, NC). Scaffolds and trees were considered PPV-positive if at least one leaf on a scaffold or one scaffold per tree (respectively) tested positive for PPV. Cohen's Kappa was used to determine how well the two ELISA tests agreed for the same sampling units. Kappa is a measure of concordance (3). Kappa is calculated by comparing the observed and expected frequencies of agreement of a contingency table, as:

$$\kappa = \frac{p_o - p_e}{1 - p_e}$$

,where p_o is the observed proportion of agreement and p_e is the proportion of agreement expected by chance. Kappa ranges from 0 to 1, though in some instances it can also be negative, where 0 indicates no correlation and 1 indicates perfect correlation (3). Kappa was calculated using the kappa option in PROC FREQ in SAS (Statistical Analysis System, SAS Institute, Cary, NC).

Comparison of sampling and detection efficiencies. A simulation model was developed to assess the detection efficiencies of: (i) the currently used US sampling and testing protocols versus the Canadian sampling and testing protocols, (ii) ELISA test kit protocol (US vs. Canada), (iii) sample size (leaves/tree), (iv) sampling design (simple random sample vs. stratified (by scaffold) random design), and (v) number of PPV-positive leaves assumed to be required for a bulk sample to test positive for PPV. The

simulation was run on the ELISA results from both the US and Canadian test kits protocols for the same leaves, scaffolds, and trees. Of the 19 trees re-sampled (100 leaves each), no PPV-positive leaves were detected by either the test kit used in the US or the kit used in Canada for two of the trees. Two additional trees were detected positive by the US test kit but not the Canadian, and 2 more were detected positive by the Canadian test kit but not the US test kit. Hence, only 13 of 19 trees had leaves detected positive by both the US and Canadian test kit protocols. The simulation, therefore, was run on the ELISA results from these 13 trees. The simulation model simulates the repeated sampling from the 13 known PPV-positive trees, using each of the different combinations of the sampling/testing criteria listed above. Ten leaf sample sizes per tree were utilized in the simulation, 4 to 40 by fours (i.e., 4, 8, 12, 16, etc. leaves per tree). Sample size increased in increments of four to allow for an equal number of leaves to be sampled from each scaffold when a stratified random sampling design was utilized. Two sampling designs were evaluated in the simulation: (i) a simple random sampling design and (ii) a random sampling design in which the leaves were stratified by scaffold. Utilizing the simple random sample, leaves were selected at random among all the leaves from all scaffolds of a tree, whereas the stratified random sampling design randomly selected an equal number of leaves from each of the four scaffolds. The number of PPV-positive leaves required for a bulk sample to test positive for PPV was varied from 1 to 5. For each of the 200 possible combinations of test kit, sample size, sampling design, and the number of PPV-positive leaves required for a bulk sample to test positive for PPV, was run for 500,000 iterations. Detection efficiency was calculated for each possible combination as:

$$\text{Detection efficiency} = \frac{\text{number of iterations resutling in PPV - positive tree}}{\text{total number of iterations}}$$

Results

Comparing detection efficiency of US and Canadian sampling systems.

US vs. Canadian sampling and testing systems. The US sampling/testing system (i.e., 8 leaf sample, 1 PPV-positive leaf required, stratified design, and Agdia test kit) had a higher detection efficiency than the Canadian sampling/testing system (i.e., 20 leaf sample, 2 PPV-positive leaves required, stratified random sample design, and Durviz test kit). The detection efficiency for the US system was approximately 71.7% (Fig. 1B), while the detection efficiency for the Canadian system was approximately 40.5% (Fig. 2B). This indicates that the US system detected 71.7% of PPV-positive trees, whereas the Canadian system detected approximately 40.5% of PPV-positive trees.

ELISA test kit results. The US test kit detected over twice as many PPV-positive leaves among the 19 trees sampled than the test kit used in Canada. In total, the US test kit detected 244 PPV-positive leaves (i.e., 13.2% of leaves tested), whereas the test kit used in Canada detected only 115 (6.2% of leaves tested). The incidence of PPV at the leaf scale among the 19 trees ranged from 0 to 44%, with an average PPV incidence of 13.3% using the US test kit, and from 0 to 35% for the test kit used in Canada with an average of 6.9%. In addition to a wide range in PPV leaf incidence among the 19 trees sampled (using either test kit), PPV-positive leaves were also found to be unequally distributed within each tree as well as unequally distributed among the four

scaffolds. Both the test kit used in the US and the kit used in Canada detected only four trees in which there was at least one positive leaf on each of the four scaffolds.

Comparison of ELISA test kits used in the US and Canada. Chi-square tests for independence revealed that there was significant agreement between the US and Canadian ELISA kits at the leaf and scaffold scales ($P < 0.0001$ and $P = 0.0003$, respectively) (Table 1). The test for independence at the tree scale, however, was not significant ($P = 0.11$), indicating that the results of the two tests kits were independent of one another. Cohen's Kappa, a measure of concordance between the two test kits, was relatively low at all spatial scales. Kappa was highest at the scaffold scale, where K = 0.4036, followed by the leaf scale (K = 0.3822), and tree scale (K = 0.3667) (Table 1). This indicates that the correlation (agreement) between results of the test kit used in the US and kit used in Canada were highest at the scaffold scale, followed by the leaf and tree scales.

Sample size. Detection efficiency increased with increased sampling size for both the US and Canadian sampling and testing system. Although detection efficiencies increased with increased sample sizes, the benefit of increasing sample size diminished as sample size increased. This was true for both test kits and both sampling designs (Fig. 1 and 2).

Sample design. A simple random sampling design vs. a stratified (by scaffold) random sampling design did not appreciably differ in detection efficiency among ELISA test kits, sample sizes, or number of PPV-positive leaves required per bulk sample. For instance, utilizing the Canadian testing system with a bulk sample of 20 leaves, that requires two PPV-positive leaves in the bulk sample, the detection efficiency using a

random sampling design was 40.56%, whereas the stratified random sampling design had a detection efficiency of 40.47%. This was true for both test kits.

Number of PPV-positive leaves required for bulk leaf samples to test positive for PPV. Detection efficiency was highest for bulk samples that required only one positive leaf. Detection efficiency decreased with each additional PPV-positive leaf that was required for the bulk sample to test positive. As the number of PPV-positive leaves required increased, discrepancies in detection efficiency also decreased. That is, the discrepancy in detection efficiency between requiring one and two PPV-positive leaves is greater than the discrepancy between requiring four and five PPV-positive leaves. This was found to be true for each test kit, sample design, and sample size.

Test kit used in the US vs. test kit used in Canada. Detection efficiency was highest using the US ELISA test kit protocol than the Canadian test kit protocol for each combination of sample size, sample design, and number of PPV-positive leaves required in a bulk sample.

Discussion

Agreement between the test kit used in the US and test kit used in Canada. Though there was agreement between the two ELISA test kits protocols at the leaf and scaffold scales, the two tests protocols did not agree at the tree scale. The reason for this was likely due to the fact that for four of the trees, relatively few leaves (no more than five) tested positive for PPV using either the test kit used in the US or the test kit used in Canada test kit, while the other test kit did not detect any PPV-positive leaves. The lack of agreement between the two test kits is somewhat disconcerting, as PPV survey and tree removal protocols are dependent on the ability of a PPV sampling and testing

protocol to correctly detect PPV-positive trees. This further suggests that one potentially large reason for the disparity between the relative success of the two eradication programs is due, in part, to the fact that the US system had a higher diction efficiency at the leaf scale that would result in more PPV-positive trees being detected and removed.

US vs. Canadian sampling systems. The US sampling and testing system consistently had a higher detection efficiency than the Canadian sampling and testing system. One reason for this was that the US test kit detected many more positive leaves among the 19 trees sampled, than did the Canadian test. Consequently, because there was a higher probability of the simulation selecting one or more PPV-positive leaves using the US test kit (simply because these were more PPV-positive leaves detected by the US kit), the US test kit had a higher detection efficiency.

These findings are important with regard to eradication efforts. Ideally, detection efficiency of the US and Canadian systems would be close to 100%, as this would allow for the removal of nearly all positive trees. Though the US system detects less than 100% of all PPV-positive trees that were sampled and tested, the aggressive removal protocol (removing all *Prunus* trees within 500 m of PPV-positive trees) may have compensated for this by removing undetected local sources of PPV. Thus, by removing all *Prunus* trees within 500 m of a PPV-positive tree, it is likely that some PPV-positive trees that were not detected positive were removed simply because of their proximity to *Prunus* trees that were detected positive.

In Ontario, the detection efficiency of the Canadian sampling and testing system was low compared to the detection efficiency of the US system. This coupled with a less aggressive removal protocol, where trees are removed based on the basis of PPV

incidence at the tree scale within blocks and/or the number of consecutive years that a *Prunus* block has tested positive for the presence of PPV-positive trees, may be part of the reason PPV persists in Ontario. Our results show that a large number of PPV-positive trees are not being detected by the Canadian sampling/testing system. A study on the within-block pattern of PPV-positive trees in Ontario *Prunus* blocks found that many blocks had multiple years of PPV-positive trees. This indicating that either some PPV-positive are not being detected in Ontario *Prunus* blocks or that PPV-positive trees are having an effect on the health status of trees within the same block even after they are removed. This is troubling for an eradication program that is based on a single year PPV threshold being reached before eradication is implemented, as some of the PPV-positive trees that are sampled are not testing positive for PPV.

Sample size. Detection efficiency increased with increasing sample size for both the test kits used in the US and the test kit used in Canada, both sampling designs, and all number of PPV-positive leaves required for bulk samples to be detected positive. However, it is important to consider that as sample size increases, the number of PPV-positive leaves required for the bulk sample to be detected positive also increases. Based on the US assumption of requiring one PPV-positive leaf in an eight-leaf sample, and the Canadian assumption of requiring two PPV-positive leaves in a 20-leaf sample, as well as a published report indicating two PPV-positive leaves are required in a 16-leaf sample (4), it seems that approximately one additional PPV-positive leaf is required for an increase of 10 leaves/sample. So when considering sample size, with regard to the number of PPV-positive leaves required for the bulk test to be detected positive, there

may in fact be little benefit to having large sample sizes, as detection efficiency may not actually increase.

Sample design. Sampling design (random vs. stratified random (by scaffold)) was found not to affect detection efficiency. This result was consistent with our finding that both the US and Canadian testing systems detected only four of 19 trees in which each of the four scaffolds had at least one PPV-positive leaf.

Number of PPV-positive leaves required. The decrease in detection efficiency was observed to be greatest when requiring two PPV-positive leaves as opposed to one for a given sample size to be detected positive. Because of this, we feel that when considering different sample sizes, it may be best to require only one PPV-positive leaf for the sample to be detected positive. There are at least two ways to ensure only one PPV-positive leaf is required for the bulk sample to test positive, which are: (i) keeping sample sizes small, and (ii) collecting leaves when virus titer is the highest.

Plum pox virus titer in *Prunus* trees is known to vary throughout the growing season, often with an inverse relationship to temperature (13, 15, 17). That is, titer is lowest when temperature is highest. In a preliminary study, Thompson (15) found that PPV titer in Ontario was lowest between late July and late September. Despite this, both survey programs continue through the length of the summer months, though PPV titer is known to be at their lowest concentrations during the mid-to-late summer (14). This raises questions concerning the use of limited resources during the summer months to collect and test *Prunus* trees for PPV when the likelihood of detecting PPV-positive trees is reduced. The seasonality of PPV titer, combined with our finding that there is a significant drop in detection efficiency when more than one PPV-positive leaf is required

for a bulk sample to test PPV-positive suggests that *Prunus* leaf samples should be collected when PPV titer is at its highest concentration (in late spring/early summer). This is further supported by the temporal pattern of PPV-positive trees that were detection in both Pennsylvania and Ontario. The majority of PPV-positive trees in Pennsylvania from 1999 to 2006 and Ontario from 2006 to 2009 were detected in May and June (95.1% and 47.1%, respectively), when temperatures are relatively cooler, as opposed to July and August (0.7% and 52.8%, respectively), when seasonal temperatures were higher. These findings suggest that, because PPV detection efficiency may decrease during the mid-to-late summer month, sampling should be concentrated during the early summer months when virus titer is likely to be highest, and the fewest numbers of PPV-positive leaves would be required for a leaf bulk sample to test positive for PPV.

Acknowledgements

Funding for this project was provided by the USDA-ERS. We appreciate help from Karl Pazdernik and Dr. Mark Kaiser for helping to develop the simulation model, and interpreting the results. Many thanks are also given to CFIA personnel who collected and shipped leaf samples to us for testing. The help of lab members in processing leaf samples is also much appreciated.

Literature Cited

1. Cambra, M., Asensio, M., Gorris, M. T., Perez, E., Camarasa, E., Garcia, J. A., Moya, J. J., Lopez-Abella, D., Vela, C., and Sanz, A. 1994. Detection of plum pox potyvirus using monoclonal antibodies to structural and non-structural proteins. EPPO Bull. 24:569-577.

2. Cambra, M., Capote, N., Myrta, A., and Llacer, G. 2006. *Plum pox virus* and the estimated costs associated with sharka disease. EPPO Bull. 36:202-204.

3. Cohen, J. 1968. Weighted kappa: Nominal scale agreement provision for scaled disagreement or partial credit. Psychological bulletin 70:213.

4. Hughes, G., Gottwald, T. 2002. The use of hierarchical sampling in the surveillance program for Plum pox virus incidence in the United States. Plant Dis. 86:259.

5. Levy, L., Damsteegt, V., and Welliver, R. 2000. First report of *Plum pox virus* (Sharka disease) in *Prunus persica* in the United States. Plant Dis. 84:202.

6. Llacer, G. and Cambra, M. 2006. Hosts and symptoms of *Plum pox virus*: fruiting *Prunus* species. EPPO Bull. 36:219-221.

7. Mumford, R. A. 2006. Control and monitoring: control of *Plum pox virus* in the United Kingdom. EPPO Bull. 36:315-318.

8. Myrta, A., Di Terlizzi, B., Savino, V., and Martelli, G. P. 2006. Control and monitoring: monitoring and eradication of sharka in south-east Italy over 15 years. EPPO Bull. 36:309-311.

9. NAPPO 2004. Guidelines for phytosanitary action following detection of *Plum pox virus*. NAPPO Regional Standards for Phytosanitary Measures http://www.nappo.org/Standards/Standards%28all%29/RSPM18-18-10-04-e.pdf.

10. Redding, R. C. 2009. Revision of Quarantine and Program Orders; Relating to Plum Pox Virus. P. D. o. Agriculture Vol. 39, Number 46, 40 Pa.B. 7242

11. Snover-Clift, K. L., Clement, P. A., Jablonski, R., Mungari, R. J., Mavrodieva, V. A., Negi, S., and Levy, L. 2007. First report of *Plum pox virus* on plum in New York State. Plant Dis. 91:1512.

12. Speich, P. 2006. Control and monitoring: *Plum pox virus* quarantine situation in France. EPPO Bull. 36:307-308.

13. Stobbs, L. 2009. *Plum pox virus*: Studies on virus distribution and sampling methods. United States – Canada Plum Pox Virus Update Conference Niagara Falls, Ontario, Canada.

14. Thompson, D. 2006. Control and monitoring: control strategies for *Plum pox virus* in Canada. EPPO Bull. 36:302-304.

15. Thompson, D. 2003. Woody host range of Canadian PPV-D. NE1006: Eradication, Containment and/or Management of Plum Pox Disease (Sharka). Shepardstown, WV.

16. Thompson, D., McCann, M., MacLeod, M., Lye, D., Green, M., and James, D. 2001. First report of Plum pox potyvirus in Ontario, Canada. Plant Dis. 85:97.

17. USDA 2007. Recovery plan for *Plum pox virus* (Sharka) of stone fruit. National Plant Disease Recovery System. http://www.ars.usda.gov/SP2UserFiles/Place/00000000/opmp/Plum%20Pox%20PPV%2070222.pdf.

18. Wang, A., Sanfacon, H., Stobbs, L., James, D., Thompson, D., Svircev, A., and Brown, D. 2006. *Plum pox virus* in Canada: progress in research and future prospects for disease control. Can. J. Plant Pathology 28:182-196.

Table 1. Comparison of the US and Canadian ELISA test kits at the leaf, scaffold, and tree scales for *Plum pox virus* of *Prunus* using Chi-square analysis and Cohen's Kappa. Independence between the two test kits was rejected when $p < 0.05$. Kappa, a measure of correlation, can range from 0 to 1, where 1 is perfect correlation.

Scale	$\chi 2$	*P* value	Kappa
Leaf	318.8	<0.0001	0.3822
Scaffold	13.1	0.0003	0.4036
Tree	2.6	0.1100	0.3667

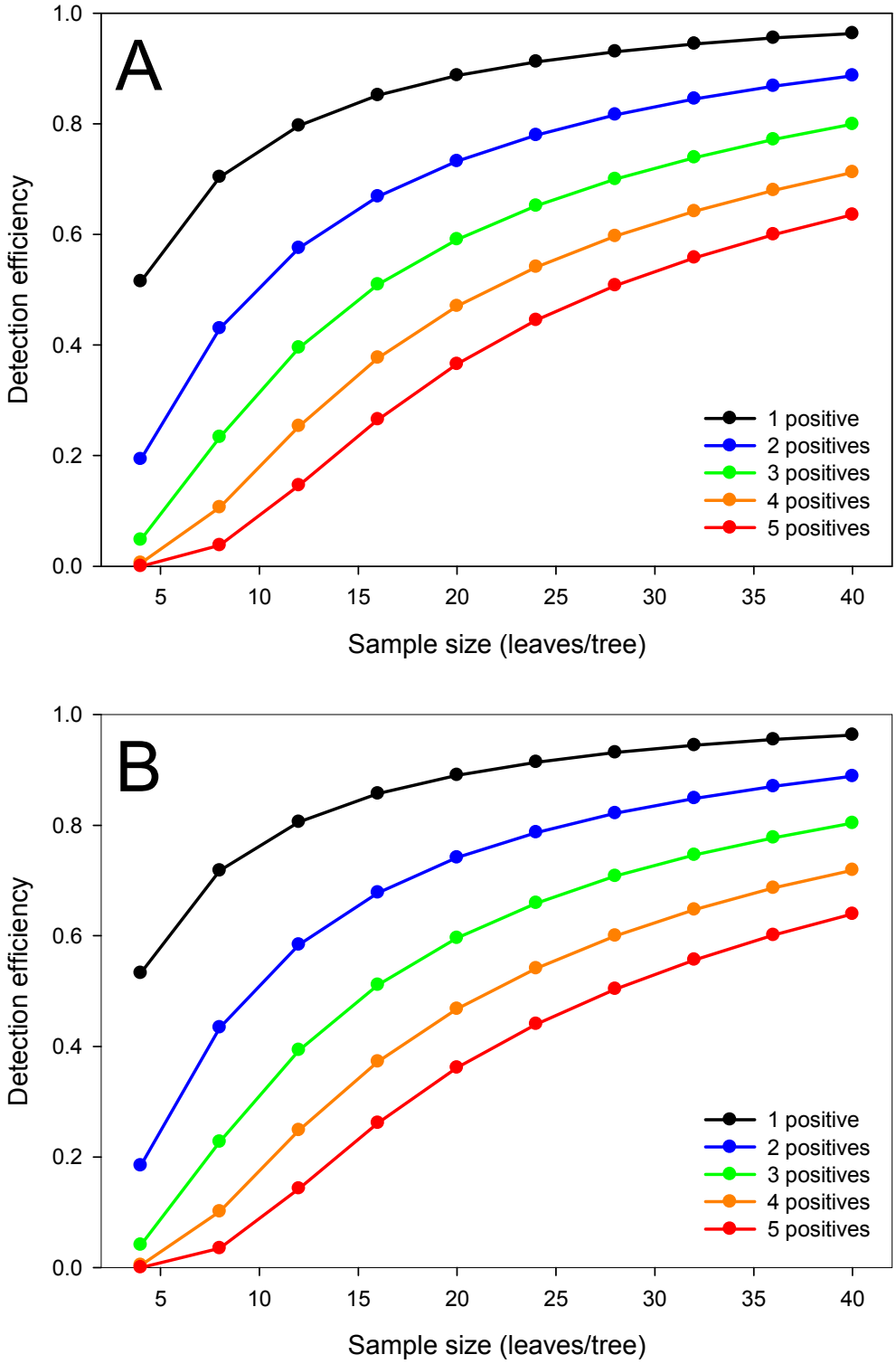

Figure 1. Simulation results using the US *Plum pox virus* ELISA kit using (A) a simple random sampling design, and (B) a stratified random (by scaffold) sampling design for repeated sampling (500,000 iterations) of 13 known PPV-positive *Prunus* trees in Ontario, Canada.

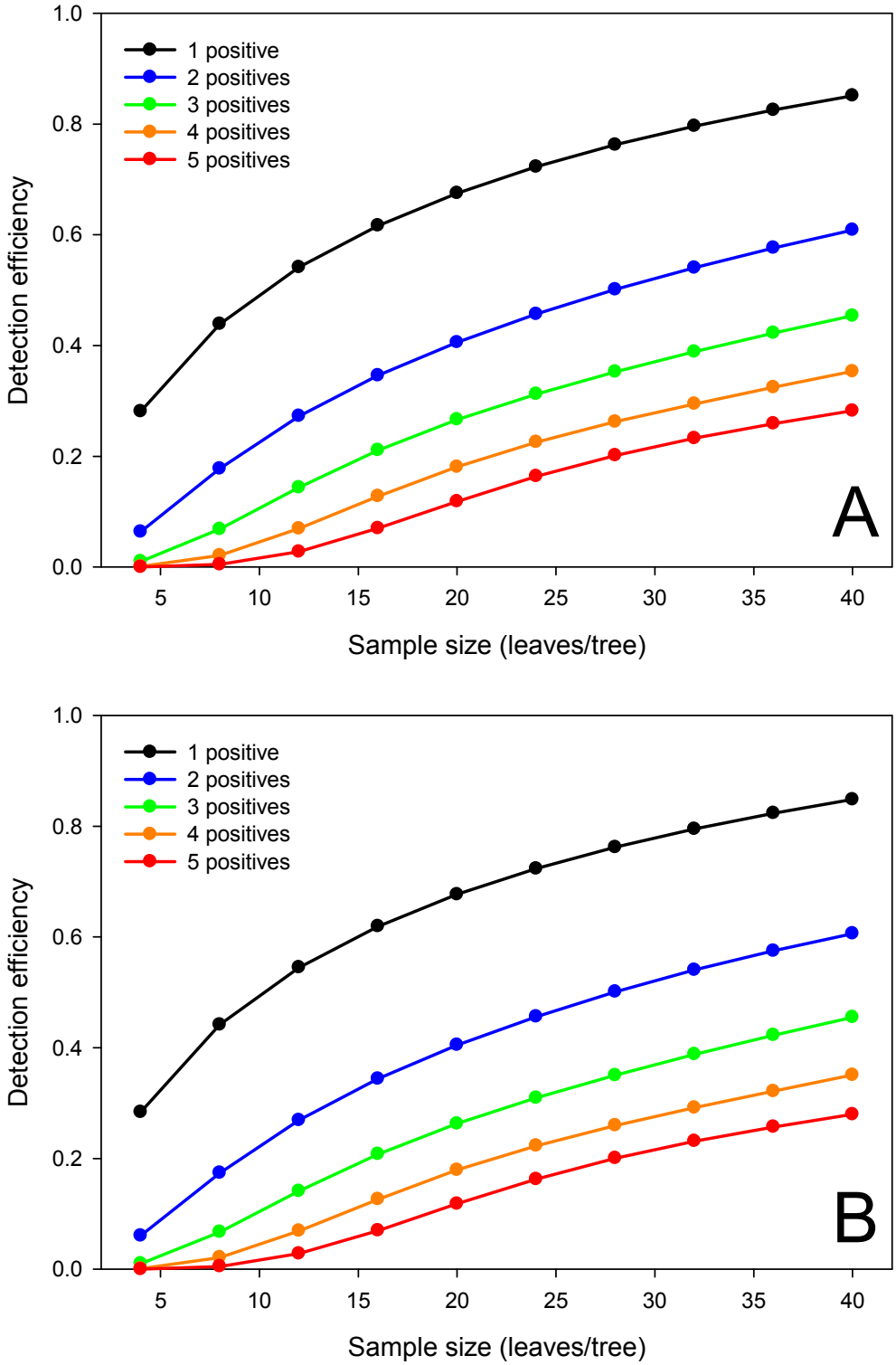

Figure 2. Simulation results using the *Plum pox virus* ELISA kit used in Canada using (A) a simple random sampling design, and (B) a stratified random (by scaffold) sampling design for repeated sampling (500,000 iterations) of 13 known PPV-positive *Prunus* trees in Ontario, Canada.

CHAPTER 4.

GENERAL CONCLUSIONS

The research conducted for this thesis showed that the *Plum pox virus* epidemics in Pennsylvania and Ontario were distinctly different from one another, and that the two respective eradication programs have had differing impacts on the epidemiology of the virus. Spatial analyses indicated that PPV-positive *Prunus* blocks were spatially clustered in both Pennsylvania and Ontario. This indicates that PPV-positive blocks are likely to be near other PPV-positive blocks, reinforcing the idea that surveying should be most intensive around previously detected PPV-positive *Prunus* blocks. In Ontario, where the density of *Prunus* blocks was very high, PPV-positive *Prunus* blocks were found to be very near PPV-positive *Prunus* blocks detected the year previous. This finding may suggest that a removal protocol based on the distance from PPV-positive *Prunus* blocks, such as that used in Pennsylvania, may better facilitate the eradication of PPV than the current policy of tree removal based on the incidence of PPV-positive trees within *Prunus* blocks or the number of consecutive years that PPV is present within a *Prunus* block. Based upon ordinary runs analyses, PPV-positive trees within blocks were found to be randomly distributed in nine of 12 *Prunus* blocks. This finding suggests that a systematic sampling design may be the most appropriate when nothing is known about the presence or absence of PPV within a block. A systematic sampling design will accommodate both random and clustered distributions of PPV-positive trees, but will also account for the occasional block in which PPV-positive trees are clustered.

Simulation model results indicate that the US *Plum pox virus* sampling and testing system had a higher detection efficiency than the Canadian sampling and testing system.

The large discrepancy in PPV detection efficiencies (approximately 30%) is troubling because this seems to indicate that the Canadian PPV detection program is misidentifying over 50% of the PPV-positive trees sampled and tested in Canada. Furthermore, because the identification of PPV-positive trees is required before eradication is imposed, there is a strong likelihood that these PPV-positive trees misidentified as negative are acting as a source of inoculum for new infections in subsequent years. This may have been part of the reason the US Eradication Program was successful. That is, the 500 m removal distance policy used in Pennsylvania likely removed PPV-positive trees that had not yet tested positive for PPV, simply because they were more likely to be in close proximity to a PPV-positive tree that did test positive. Results from simulation modeling also indicated that sample sizes of 8 to 12 leaves/tree that require just one PPV-positive leaf in a bulk sample would have the highest detection efficiency for PPV.

The findings from this research study have important implications for current (as well as future) survey and eradication programs, and provides science based information to revise and implement new PPV survey and eradication policies that will help to improve the probability of successfully eradication of PPV.