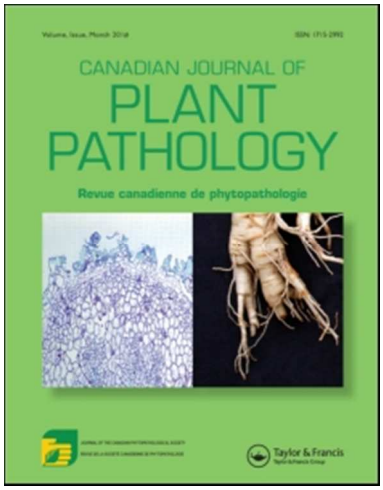

Studies on the impact of Plum pox virus (PPV-D) infection on peach tree growth, productivity and bud cold hardiness.

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Studies on the impact of *Plum pox virus* (PPV-D) infection on peach tree growth, productivity and bud cold hardiness.

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Abstract: In 2000, the Dideron (D) strain of *Plum pox virus* (PPV) was detected in commercial peach and nectarine orchards in the Niagara region of Ontario where most of Canada's stone fruit crops are produced. As part of a disease management research program, peach (*Prunus persica* L. Batsch) trees in a commercial orchard at Niagara-on-the-Lake were assayed for PPV annually for 3 years. The orchard consisted of two blocks of the cultivars 'Allstar' and 'Brighton', of which 4 of 288 and 5 of 252 trees, respectively, were infected with PPV. The growth and health of these PPV-infected and non-infected trees were evaluated based on the annual growth rates, vigour (chlorophyll content) and bud winter hardiness. Comparative fruit quantity and quality index values were based on total yield and marketable yield per tree, fruit size and weight, fruit pH, titratable acidity, total soluble solids, flesh firmness, and fruit skin colour. Results from these preliminary studies showed that trees infected with this mild Ontario isolate of PPV produced slightly more fruit of smaller size that ripened earlier than non-infected trees. However, yield efficiencies based on weight of fruit relative to the trunk cross sectional area did not differ statistically. Screenhouse studies on three graft-inoculated fresh market peach cultivars ('Babygold', 'Catherina', and 'Garnet Beauty') similarly did not demonstrate any differences in growth or fruit production in the 2nd and 3rd year post inoculation, but fruit on infected trees matured somewhat earlier.

Key Words: *Plum pox virus*, fruit quality, chlorophyll content, yield, bud hardiness.

Introduction

Approximately 25,500 tonnes of peaches (*Prunus persica* L. Batsch) with a farm gate value of C$ 32.6 million are produced in Canada annually (Statistics Canada, 2012). The Niagara region of southern Ontario is the primary production area with over 85% of the total peach acreage.

Plum pox virus (PPV) is one of the most damaging viral diseases of stone fruit (*Prunus* spp.) worldwide (Nemeth 1986; Cambra et al. 2006; Wijkamp et al. 2011). Different strains of PPV significantly limit production of peaches, plums (*P. domestica* L.), apricots (*P. armeniaca* L.), and other stone fruits in areas where they are established. Economic losses from these strains can be catastrophic to stone fruit production in large geographic areas (Agrios 1990), with estimated losses exceeding €10 billion worldwide (Cambra et al. 2006). Induced symptoms vary depending on the virus strain and host cultivar. Leaves may have pale green spots, lines and rings, vein clearing or yellowing bordering secondary or tertiary veins, leaf puckering, crinkling and curling. Colour breaking may occur in flowers on some cultivars. Infected fruit may have rings, chlorotic spots, blotches, and deformities. Trees may exhibit reduced growth and vigor and be less tolerant to drought and cold (Cambra et al. 2012). The virus is transmitted through infected propagation material and by numerous aphid species in a non-circulative, non-persistent manner (Labonne et al. 1994, 1995; Gildow et al. 2004; ; Lowery et al. 2009, 2015).

In 1999, the Dideron strain of PPV (PPV-D) was first detected in North America in several peach and plum orchards in Pennsylvania (Levy et al. 2000; Damsteegt et al. 2001). The following year, PPV was found in nectarine, peach and ornamental *Prunus* spp. in Ontario and in peach in Nova Scotia, Canada (James & Upton 2001; Thompson et al. 2001). An eradication

program was implemented between 2000 and 2011 (Gougherty & Nutter 2012; Gottwald et al. 2013; CFIA 2014; Treasury Board of Canada Secretariat 2015). Orchards identified as having infected trees were partially or fully removed and growers received compensation from a government program (CFIA 2014). Propagative spread is believed to account for the introduction and much of the initial dissemination of the virus in Ontario (Gottwald 2006). The D strain of PPV is generally thought to be not as effectively transmitted by aphids as other strains and tends to spread more slowly (Brunt et al. 1997; Wijkamp & van der Gaag 2011; Šubr & Glasa 2013). However, atypical isolates of PPV-D that spread more rapidly within and between peach orchards have been reported from France (Dallot et al. 1998) and Chile (Herrera 2013). In Canada, the eradication program was replaced in 2011 with a disease management and monitoring program that maintained a quarantine zone within a 1.5 km monitored buffer zone. By 2011 most of the processing peach cultivars (primarily clingstone cultivars) had been removed, in part because of higher disease incidence and symptomatic fruit, but mostly resulting from closure in 2008 of the last remaining fruit canning facility in southern Ontario. For this reason, our research was limited to fresh market freestone cultivars.

The impact of PPV on fruit production depends on many factors, including the virus strain and isolate, tree cultivar and age, rootstock, cultural practices, time and mode of infection, presence of vector species and climatic conditions (Usenik et al. 2014). Little is known about the impact of the Ontario isolate of PPV-D on peach vigour, fruit production, fruit quality, and winter hardiness under cool temperate conditions. Further, the Ontario PPV-D strain is different from other reported PPV-D isolates based on molecular mapping studies (Theilmann et al. 2006; Maejima et al. 2011; Schneider et al. 2011; James et al. 2015) and transmission efficiency (Lowery & Vickers 2007; Lowery et al. 2009, 2015). Isolates from the US, Canada and Chile are

believed to be more closely related to each other than to European isolates. The two US isolates from Pennsylvania were transmitted more efficiently by *M. persicae* than a European D isolate (Schneider et al. 2011). The first isolates of PPV strain W (isolate W3174) and PPV-Rec described in *Prunus* in Niagara were discovered on residential properties; neither isolate has been detected in commercial stone fruit orchards in Canada. These two isolates appear to represent separate and distinct introductions by homeowners (James and Varga 2005; James et al. 2015). Increasing levels of PPV inoculum in the industry may be a potential reservoir for intra-strain variation. PPV strains are characterized by relatively low intra-strain diversity (reaching 1.1%–3.9% at the nucleotide level for full-length genomes, except for PPV-W, where the divergence reaches 7.9%) and by comparatively high between-strain diversity (4.4%–22.8%; Glasa et al. 2012). Although forming monophyletic groups, PPV-M, PPV-D, PPV-Rec, PPV-T and PPV-W are evolutionarily linked by recombination events, including an ancestral recombination affecting the 5' part of PPV-M, PPV-D and PPV-Rec strains (Garcia et al. 2014). Such mutations could alter pathogenicity and host range determinants (Glasa et al. 2012; Garcia et al. 2014).

The objective of this study was to monitor and assess the effects of the Ontario PPV-D isolate on two fresh market peach cultivars in an orchard over three consecutive years. We also examined early infection on newly planted trees and its impact on production and tree viability. The findings will address some of the controversy in the industry regarding the impact of PPV at the farm gate and on tree viability.

Materials and methods

A. Plant material

Field study

At the termination of the federal PPV eradication program in 2011, it was difficult to locate and gain access to sites with infected trees. An extensive survey (11,740 trees) was conducted in the 2011-2012 dormant season, with follow up sampling in the 2012 growing season throughout commercial peach orchards in Niagara, Ontario, with a previous history of PPV infection. Five trees of PPV-infected cv. 'Brighton' (ca. 4 yr old) and four trees of cv. 'Allstar' (ca. 7 yr old) were identified in an orchard in Niagara-on-the-Lake. All infected trees of both fresh market cultivars used in our field study between 2012 and 2015 were uniformly infected with virus throughout the tree scaffolds. 'Allstar' and 'Brighton' infected with the local isolate of PPV-D were mildly symptomatic, with faint line patterning. This is similar to group 2 (tolerant cultivars) defined by Polak et al (2003) for the classification of peach cultivar susceptibility to PPV. Basal leaves on shoots consistently tested highly positive for PPV by both ELISA and PCR. No symptoms were present on the fruit. For negative controls, 10 non-infected trees of each cultivar located randomly throughout the orchards were selected. For each cultivar, trees used in the study were planted at the same time (cvs 'Allstar' in 2006 and 'Brighton' in 2009) and were similar in growth and appearance, and free of any obvious diseases such as peach canker. Trees were spaced 3 m within rows and 4 m between rows and trained to an open centre with three to five main scaffolds. Cultural management practices and pesticide applications were made according to standard commercial practices for the area (OMAF 2006). Annually at leaf-out (April - May), 4 – 5 scaffolds of each study tree were mapped into at least 20 branches per scaffold, and 12 basal leaves were collected from each branch and stored for no more than 48 h at 4^{o}C until assayed. Leaves were macerated in ELISA extraction buffer (EEB) (1:6, tissue:buffer) (Clark & Adams 1977). The suspension was further diluted using Direct Plant Extraction Buffer (DiPEB) (1:9, macerate in EEB:DiPEB) and assayed by Direct Reverse

Transcriptase Real Time Polymerase Chain Reaction Assay (DRT-qPCR) (Kim et al. 2008). Overall, infection was uniformly distributed in the test trees, suggesting that the trees were not newly infected.

Screenhouse study

To study the impact of early PPV infection of peach nursery stock on production and viability of trees, a 465 m^2 screenhouse was modified to containment level 2 standards and certified by the Canadian Food Inspection Agency (CFIA) for PPV studies. Two screened overhead ventilation fans provided air circulation and annual whitewash applications kept temperatures close to ambient levels over most of the growing season. In June 2011, 15 two year old peach trees certified free of PPV of each of three peach cultivars ('Babygold', 'Catherina', 'Garnet Beauty') were planted in a randomized complete block design with 1.7 m x 1.5 m spacing between rows and trees respectively. The screenhouse was maintained free of aphids and monitored by regular inspections and yellow sticky cards. Trees were drip irrigated and maintained according to standard commercial practice. In September 2012, 10 trees of each cultivar were T-bud grafted with 5 PPV-infected buds from infected peach stock. Trees were tested for virus in the spring and summer from 2013-2015. By 2014, 8, 5, and 9 of the inoculated trees of 'Baby Gold', 'Catherina' and 'Garnett Beauty' respectively were PPV infected. Based on Polak's definitions (Polak 2003), 'Catherina' and 'Garnett Beauty' were considered to be in Group 2 (tolerant) and 'Babygold' in Group 3 (medium susceptible cultivar, Polak 2003). These trees and the five uninoculated trees per cultivar were subsequently monitored in 2014 and 2015 for growth, yield and fruit quality as previously described.

B. Vegetative growth and fruit yield evaluations

Field Study

Mature orchard trees were monitored to determine the effects of PPV on tree vigour, growth, yield, fruit quality and winter bud hardiness. Examinations were made in the field and in the laboratory at AAFC in Vineland Station, ON. Tree circumferences (30 cm above ground) were measured early in the growing season each year, and increases in trunk girth calculated for each year of the three year study period. Trunk cross sectional area (TCSA) was calculated to compare the vegetative growth of infected and non-infected trees per cultivar (Lepsis & Blanke 2006). At marketable fruit maturity as determined by the grower and in accordance with provincially-regulated minimum fruit sizes for fresh market peaches (OTFPMB 2014), fruit was harvested into separately labeled baskets and weighed to obtain total fruit yield per tree. This required 2-3 picks per cultivar. Fruit was then stored at 4^{o}C and 50-60% RH in a cold storage chamber for 24 h before being assessed for quality.

Screenhouse study

The young trees were studied similarly to the field trees with the following exceptions. No winter bud hardiness data were collected, trunk circumferences were measured in mid-August annually and fruit was harvested and assessed the same day.

C. Fruit quality assessment

Field study

Fruit was first graded using a GREEFA grading machine (A3-UP Greefa®, the Netherlands) into seven size classes (<57, 57-64, 64-70, 70-76, 76-83, 83-89, >89 mm diameter for cv. Brighton; two lowest classes for Allstar were <54 and 54-64, the other categories were the same). Marketable fruit size was based on provincially-regulated minimum fruit sizes for fresh market peaches (OTFPMB 2014). The minimum fruit size was 57 mm for early-season peaches (e.g. 'Brighton') and ~60 mm for main season peaches (e.g. 'Allstar'). However, because of limited

sales opportunities for small size fruit the cooperating grower considered fruit less than 64 mm unmarketable. Total fruit numbers and total fruit weight for each class were recorded. For each tree, a sample of 10 market-size peaches per scaffold were chosen for fruit quality assessments, which included diameter, fruit skin colour, firmness, total soluble solids, juice pH and titratable acidity. Maximum fruit diameter was measured in two directions using an electronic caliper with LCD readout (Mastercraft®, 58-6800-4, Canadian Tire). Colorimetric measurements of fruit skin colour were determined from both sides of each fruit using a Chroma meter CR-400 (Konica Minolta Sensing Inc., Japan) that measured light reflectance ('L' value) and the intensity of the red or green ('a' value) and yellow or blue ('b') colours (Voss 1992). The 'C' values measured the Chroma and the 'h' values measures the hue angle. Fruit firmness was measured using a digital Fruit Texture Analyser (FTA; GUSS-GS-14®, South Africa) with a 12 mm diameter stainless steel cylinder probe that was applied to opposite sides of each fruit on an area with the skin removed to expose the fresh flesh. A fruit and vegetable extractor (Health Smart 67900, Hamilton Beach®, Picton, ON) was used to obtain juice from a composite of the 10-fruit sample per scaffold. The extracted juice was collected in 20 ml labelled plastic cups from which two or three drops were placed on the prism of a digital refractometer (PR-32α, Atago®, Japan; range 0-32^{o}Brix) for measurements of total soluble solids (TSS). Titratable acidity (TA) of the extracted juice was measured using a pH meter (Fisher Scientific®, Ottawa, ON). A 7.5 ml aliquot of juice was then diluted with 92.5 ml de-ionized water and titrated with 0.1 N sodium hydroxide to a pH 8.1 (± 0.2) end point, using the 860 compact Titrosampler, (Metrohm®, Switzerland), and the volume of NaOH used was recorded as the TA volume.

Screenhouse study

Fruit was manually graded with a minimum circumference of 15 cm accepted for the study. This translates into a minimum diameter of 4.8 cm (circumference x 10/π). Peaches were smaller than those collected from older orchard trees. A total of 20 fruit were taken from each tree and their quality assessed as described excluding titratable solids measurements.

D. Foliar chlorophyll content and cold hardiness assessments

Field study

Chlorophyll content of leaves of PPV-infected and non-infected trees, which is closely associated with plant nitrogen status, was measured using a SPAD (SPAD-502 plus, Konica Minolta Co., Ltd., Japan) chlorophyll meter that measures the transmission of radiation through the leaf at wavelengths near 650 and 940 nm. During the growing season, an average of three readings per leaf was recorded from six or seven leaves per tree selected as the third or fourth fully expanded leaves from the shoot apex. A total of four PPV-infected and four non-infected trees per cultivar were monitored over three years.

As described by Mills et al. (2006), differential thermal analysis (DTA) based on freezing point of water in plant tissues was used to detect the lethal low-temperature exotherm (LTE) for dormant peach flower buds. During the winter, 10-15 pencil-thick (ca. 1 cm diameter) shoots per tree scaffold from both PPV positive and negative trees were collected, labelled, and stored at 4^{o}C until processed within a week. The DTA consisted of the thermoelectric modules (TEMs) (model CP1.4-127-045L; Melcor Corporation, Trenton, NJ), the Keithley Multimeter Data Acquisition System (DAS) (model 2700-DAQ-40; Keithley Instruments, Cleveland, OH) and a programmable freezer (Tenney Environmental Test Chamber model T2C, Thermal Product Solutions, Williamsport, PA), and output computer software (Bud LTE 1.2.3; Brock University, St. Catharines, ON). The remaining dormant buds and cambium tissues were macerated and

diluted in EEB and DiPEB as previously described and the tissue suspensions assayed for the presence of PPV by DRT-qPCR.

Screenhouse study

No SPAD and DTA readings were conducted on the screenhouse trees.

E. Statistical analyses

Data from each experiment was checked for normality and homogeneity of variance. Where necessary, Box Cox Y transformations, or arcsine square root transformations in the case of proportional data, were applied to the data to satisfy the assumption of normality. All statistical tests were performed using JMP version 10 (SAS Institute Inc., Cary, North Carolina), with all statistical error rates pre-set with an $\alpha = 0.05$.

Statistical analysis of field data. Results were analyzed with a three-way mixed-model ANOVA with between-subjects factors (infection status and cultivar) and repeated measures on one factor (year), with interactions. Post hoc contrasts followed the analysis to compare data between 2012 and each of the other years (2013, 2014).

Statistical analysis of screenhouse data. Change in trunk circumference for each year combination was compared with a two-factor (infection status, cultivar) analysis of variance (ANOVA) with an interaction. All other screenhouse data were compared using a three-factor ANOVA (infection status, cultivar, time) with two-way interactions between all factors. ANOVA tests were followed by a Student's t separation of the means if warranted.

Results

The mean trunk cross sectional area (TCSA) of trees naturally infected with PPV in the field did not differ significantly ($P>0.05$) from that of non-infected trees at the beginning of the study (data not shown). Considered for both cultivars over all three years, growth of PPV-infected

versus non-infected trees, as measured by annual increases in TCSA, did not differ statistically (P= 0.423) (Table 1). There was no consistent difference in growth rates for the two classes over the three years. Analysis of TCSA values by cultivar showed no statistically significant differences between PPV-infected and non-infected trees for any of the three years (P>0.05) (Figure 1). Likely reflecting their younger age, the growth of PPV-infected and non-infected 'Brighton' trees was significantly higher over each subsequent year of study. Growth of infected 'Allstar' trees did not differ between years, but the yearly increase in TCSA of non-infected trees was significantly higher in 2014 compared with the previous two years. Similar to the field results, growth of peach trees in the screenhouse that had been inoculated with PPV did not differ from that of healthy trees during the first year (P=0.977), second year (P=0.183), or over all three years (P=0.353) of the study (Table 2).

Measurements of leaf chlorophyll that are associated with nitrogen levels provide another indicator of tree vigour. PPV-infected and non-infected peaches in the commercial orchard did not differ (P=0.866) with respect to leaf chlorophyll (SPAD meter readings) (Table 1). Infection status also did not affect cold hardiness of fruit buds assessed in mid-winter in 2013 or 2014 (P>0.05); there was also no difference (P=0.330) between the two cultivars over both years (Table 1). Regardless of virus infection, 100 % of the fruit buds of both cultivars were killed at a temperature of about -19 ^{o}C.

For both cultivars, yield efficiencies over the three years based on fruit production (kg) relative to the TCSA were not significantly different (P=0.952) for trees infected with PPV in the field compared with non-infected trees (Table 1). Mean values were similar for infected and non-infected trees during the first two years. PPV-infected trees produced approximately 10% more fruit relative to their trunk cross sectional areas in 2015, but the difference was not significant

(P>0.05). Analysis of yield efficiencies separately for each cultivar showed no significant differences (P>0.05) between PPV-infected and non-infected trees for either cultivar in any year (Figure 2). The yield efficiencies of 'Brighton' and 'Allstar' trees were significantly lower during 2013 compared with 2012 regardless of infection status, and both cultivars produced significantly more fruit per TCSA during 2014 compared with the previous two years. In the screenhouse, healthy trees produced more fruit on average (8.03 kg/tree/yr) than infected trees (6.02 kg/tree/yr), but the difference was not statistically significant (P=0.89) (Table 2).

For both cultivars combined, trees infected with PPV in the field produced significantly larger numbers of fruit (P=0.008) during the two years of assessment than the non-infected trees (Table 1). However, the average fruit size and average fruit mass were significantly lower (P=0.019 and P=0.035, respectively) than fruit from healthy trees (Table 1). The production of more fruit of smaller size for the infected trees compared with the healthy trees accounts for a lack of difference in yield efficiency between the two infection categories. Average yield varied between years for both cultivars, perhaps due to environmental conditions and variation in agricultural practices such as pruning and thinning. Grading of fruit into size categories suggested that more fruit from PPV-infected trees sorted into the smaller grade 2 category (57-64 mm dia.) relative to non-infected trees, but there were no significant differences (P>0.05) between infected and non-infected trees in the proportions of total fruit assorted to the seven size categories (Figure 3). In the screenhouse, trees not infected with PPV produced individual fruit that weighed slightly more (157.7 vs. 141.7 g; P= 0.121) on average and were larger in size (avg. circumference = 34.9 vs. 32.5 cm; P=0.154) compared with fruit from trees that had been bud-inoculated with PPV, but the differences were not statistically significant (Table 2).

At the commercially mature stage as determined by the producer, there were no significant differences ($P > 0.05$) between infected and non-infected trees in the recorded fruit ripeness indices (Table 1). Over each of the three years, yearly average titratable acidity (TA) values for the two cultivars ranged from 6.85-8.95 g/L for PPV-infected trees and 7.07-8.88 g/L for non-infected trees (P=0.871). Yearly average juice pH values over the course of the study ranged from 3.56-3.72 for infected and from 3.51-3.67 for non-infected trees (P=0.371). Soluble solids content (SSC) measured as ^{o}Brix ranged from 10.8-12.9% and 10.6-12.8% for fruit from infected and non-infected trees, respectively (P=0.616). There were no consistent differences in any of the measured fruit ripeness values between fruit from infected and non-infected trees over the three years of study (Table 1). Measurements of fruit firmness were higher in every year for fruit from non-infected trees, with values from 9.0-21.5% higher than for infected trees (Table 1). These yearly average values were not statistically different (P>0.05), however, and there was also no difference in fruit firmness when all years and both cultivars were considered together (P=0.36). Chroma and 'L' fruit colour assessments which relate to advancing fruit maturity were slightly higher in every year for fruit from PPV-infected trees than for non-infected trees (Table 1), but the differences were not statistically significant (P>0.05). Analysis of the main effect of infection status for cultivars and years combined showed no overall differences for Chroma (P=0.771) or 'L' values (P=0.076). Differences in fruit Hue angle were not consistent across years and there was no overall difference (P=0.650) between fruit from infected and from healthy trees (Table 1).

Similar to the field results, fruit produced by PPV-infected trees grown in the screenhouse did not differ from fruit of non-infected trees with respect to pH (P=0.835) or ^{o}Brix values (P=0.485) (Table 1). For all three cultivars over both years, average pH values were

nearly identical and average °Brix readings only 1.5% higher for fruit from healthy trees compared with fruit from trees infected with PPV. Pressure readings for infected fruit were significantly lower (P=0.023) than for healthy fruit. Fruit Chroma values for 'Garnet Beauty' differed from the other two cultivars with respect to infection status (infection x cultivar interaction) and had to be analyzed separately. While there was no significant difference (P=0.385) in Chroma values for infected versus healthy 'Garnet Beauty' fruit (Table 2), infection of 'Catherina' and 'Baby Gold' trees in the screenhouse resulted in significantly higher (P=0.003) Chroma values compared to healthy trees. For cultivars and years combined, there were no significant differences in 'L' (P=0.116) or Hue (P=0.177) readings between fruit from PPV-infected versus healthy trees (Table 2).

Discussion

Most plant diseases have adverse effects on plant growth and productivity, which can range from relatively minor reductions to total crop loss and death of the host plant. When PPV was first discovered in North America little was known about the impact of PPV-D on crop production, fruit quality, and tree vigor and hardiness. While PPV-D infection has been linked with unmarketable fruit, tree mortality, and up to 100% crop loss for certain *Prunus* species, details about the impact on peach production are limited (Anonymous 2013). Following the termination of the Canadian eradication program in 2011, an opportunity was presented to compare the impact of the Ontario isolate of PPV-D on infected peach trees with healthy trees in a commercial orchard. A survey of over 11,000 peach trees during 2011-2012 in orchards in close proximity to formerly heavily infected orchard blocks only identified nine PPV-positive trees for use in our study. This was due in large part to the near success of the eradication program and a

compensation program that encouraged voluntary block removals in orchards adjacent to PPV-infected blocks.

Viral diseases of fruit trees can often take many years before symptoms are expressed throughout the canopy. Irregular movement and distribution of virus in PPV in newly infected *Prunus* spp. is well documented (Dosba et al. 1986; Ferri et al. 2002; Dicenta et al. 2003). Sampling and mapping of individual scaffolds and branches of the infected field study trees in this study demonstrated that the canopies were uniformly infected, suggesting that the trees had become infected with PPV more than one or two years previously (Sutic 1971; Wijkamp & Van der Gaag 2011, Rimbald et al. 2015). Young trees usually have higher virus concentrations one year post-inoculation than older trees (infected >40 yrs) (Polak 1998).

Results obtained over the three years of our field study showed few significant differences between PPV-positive and negative control trees for all of the measured attributes. There was no difference in vigour as expressed by comparisons of TCSA, yield efficiency, chlorophyll measures, or cold hardiness of fruit buds (Table 1). Fruit at marketable maturity did not differ with respect to pH, soluble solids, firmness, titratable acidity or measured colour parameters. Infected trees did, however, produce larger numbers of fruit of significantly smaller size (Table 1).

Results from our screenhouse trial support those from the field. PPV-infected and healthy trees did not differ with regard to growth rate, fruit production, or in most measures of fruit quality or maturity (Table 2). Fruit from infected trees had lower pressure readings. Infected 'Catherina' and 'Baby Gold' trees produced fruit with higher colourimeter measurements (Chroma) on the fruit skin. These measures can reflect a stress response and advanced ripening. This is in agreement with results from a preliminary study in 2003 demonstrating a slightly

earlier maturation date of infected fruit of two fresh market peach cultivars, 'Redhaven' and 'Early Redhaven' (Errampalli et al. 2003). Additionally, taste preference tests of healthy and infected fruit indicated an overall 74% preference for the infected fruit, likely influenced by slightly higher sugar levels.

PPV was not detected in the screenhouse nursery trees until the year following infection, with over one half of the trees testing strongly positive by the second year. Three years post-inoculation, no significant differences were seen in any of the measured parameters between healthy and infected trees other than some degree of advanced ripening. Visually, trees infected with PPV in the field or the screenhouse were indistinguishable from healthy trees in overall appearance. None of the cultivars showed flower breaking symptoms or any distinct leaf spots, streaks, or deformities. Anecdotal reports from some growers implied that the infected trees often appeared more vigorous and healthier.

More severe strains (PPV-M and Rec) reported infecting peach in other countries have a more devastating impact on production and fruit quality (Nemeth 1994; Glasa et al. 2012). To our knowledge, no other comparable impact studies were found in Europe or Chile that exclusively addressed PPV-D infection in peach (Herrera, 2013). While it has been estimated that yield reductions of up to 15% could occur based on European experience, supporting evidence is not available (CFIA 2004). The lack of significant major differences between PPV-positive and PPV-negative trees is an important finding from both field and screenhouse studies. Our findings, while admittedly based on a small number of trees naturally infected in the field, suggest that this mild strain of PPV-D has little measureable impact on several commercial peach cultivars growing in a cool temperate climate in Ontario. Infected trees continued to produce good yields of high quality fruit with no impact on tree vigor and bud hardiness in both the field

and screenhouse studies. Based on anecdotal reports from growers over the 11 years that the eradication program was in effect, despite varying degrees of symptom expression on different fresh market peach cultivars and different growing conditions, they did not experience losses in crop yield or increased mortality from winter kill. Long term infection by a Spanish isolate of PPV-D has been shown to produce oxidative stress, and the authors suggest that an antioxidative metabolism imbalance may be related to the progress of PPV infection and symptoms in peach trees (Hernandez et al. 2004). It would be of interest to compare such changes in the Canadian PPV-D isolate which is less aggressive, with mild to absence of symptoms on many peach cultivars.

Our findings that the Ontario isolate of PPV-D causes minimal effects on fresh market peaches does not eliminate all concern. Increasing levels of PPV-D in the industry will adversely affect other susceptible and symptomatic crops such as plum and nectarine. As has happened in other countries, persistence of the virus in Ontario stone fruits over time is likely to result in mutations with greater pathogenicity. More severe isolates of the D strain have been described (Dallot et al. 1998) often with demonstrated differences in host range and aphid vectors. This is not surprising since small differences in the genetic sequence as a result of mutation can have drastic effects on Potyvirus biology (Schneider et al. 2011). Further studies should also be made to evaluate other peach cultivars, as well as other susceptible *Prunus* species, and include investigations on the phytochemical composition of the fruit. This is important since it influences their taste and quality. Co-infections of PPV with other viruses occurring in fruit trees may enhance the pathogenicity. This has been shown in mixed infections of PPV and *Prune dwarf Ilarvirus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) on some plum cultivars exhibiting growth reduction, bark canker and trunk malformation and tree mortality (Nemeth 1992). The

synergistic effect of PPV with other viruses has also been shown to reduce growth of seedlings by 2.9 – 69.1% (Nemeth 1992).

The continued spread of a single PPV-D isolate in Niagara provides a unique opportunity to examine both the epidemiology and impact at farm gate of this disease. More extensive cultivar studies over a longer period under varying climatic conditions and management regimes would benefit our understanding of this disease and potentially provide means to mitigate its impact to the industry. The latent nature of PPV-D infections, lower virus titre and reduced transient symptoms, as we have shown in this study, could explain why PPV-D strains have become much more widespread globally (Schneider et al. 2011; Wang et al. 2006).

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Table 1. Evaluation of tree vigour, fruit production and fruit ripeness of peach trees naturally infected (POS) with *Plum pox virus* (PPV) in the field as compared to non-infected (NEG) trees.

	Parameter measured	Infection status	N (trees)	Total no. of fruit sampled[a]	2012 Mean	2012 SE	2013 Mean	2013 SE	2014 Mean	2014 SE	*p*-value[b]
tree vigour	Increase in trunk cross-sectional area (cm^2) 2012 - 2014	POS	9	-	6.40	1.03	9.22	1.20	12.56	1.35	0.423
		NEG	20	-	5.25	0.65	7.54	1.04	14.02	0.89	
	Chlorophyll content (SPAD)	POS	32	384[e]	-	-	43.62	0.78	42.83	0.61	0.866
		NEG	28	336[e]	-	-	43.31	0.90	43.36	0.62	
	Bud hardiness (^oC)	POS	8	869[d]	-	-	-18.95	0.10	-18.78	0.23	0.330
		NEG	20	2115[d]	-	-	-18.58	0.10	-18.76	0.13	
fruit production	Yield efficiency (kg cm^{-2})	POS	9	-	0.45	0.02	0.34	0.02	1.72	0.32	0.952
		NEG	20	-	0.49	0.02	0.35	0.01	1.56	0.21	
	Fruit circumference (cm)	POS	9	1406	29.00	0.98	-	-	31.27	0.57	0.019 *
		NEG	20	2561	30.64	0.89	-	-	32.80	0.47	
	Fruit mass (g)	POS	9	1405	120.40	5.50	-	-	145.19	4.46	0.035 *
		NEG	20	2566	130.48	5.09	-	-	151.70	2.81	
	Total number of fruit per tree	POS	9	6929	280.67	16.12	212.89	19.00	254.11	18.67	0.008 **
		NEG	20	12617	228.45	18.88	178.45	9.88	223.95	12.98	
fruit ripeness	Fruit Chroma	POS	9	2043	20.81	1.11	21.42	0.79	24.29	0.75	0.771
		NEG	20	3793	21.94	0.76	22.79	0.47	25.67	0.73	
	Fruit "L"	POS	9	2043	23.92	0.84	24.30	0.77	26.38	0.76	0.076
		NEG	20	3793	24.80	0.56	25.39	0.45	27.30	0.66	
	Fruit Hue angle	POS	9	2043	22.78	0.16	22.24	0.55	24.11	0.62	0.650
		NEG	20	3793	22.84	0.23	21.93	0.32	24.50	0.54	
	Fruit firmness (kgf)	POS	9	2064	2.79	0.59	2.94	0.58	2.58	0.23	0.364
		NEG	20	3816	3.39	0.41	3.39	0.30	2.67	0.18	
	Fruit pH	POS	9	228[c]	3.72	0.03	3.56	0.02	3.56	0.02	0.371
		NEG	20	475[c]	3.67	0.02	3.51	0.01	3.61	0.03	
	Fruit Brix (^oBx)	POS	9	231[c]	12.89	0.42	11.76	0.32	10.78	0.10	0.616
		NEG	20	473[c]	12.75	0.37	11.90	0.14	10.56	0.10	
	Fruit Titratable acidity (g/L)	POS	9	232[c]	6.85	0.35	8.57	0.15	8.95	0.13	0.871
		NEG	20	474[c]	7.07	0.19	8.65	0.12	8.88	0.23	

[a] combined over all years of the experiment

[b] asterisk indicates level of significance: *p< 0.05, **p < 0.01; 3-way ANOVA with repeated measures on 'year' factor; post-hoc contrasts for mean

[c] number of samples tested, one per scaffold per harvest

[d] number of buds sampled

[e] number of leaves sampled

Table 2. Comparison of the growth, fruitfulness and fruit ripeness of peach trees graft-inoculated with *Plum pox virus* in the screenhouse compared with that of non-inoculated trees.

	Parameter measured	N	Total no. of fruit sampled	PPV Positive		PPV Negative		p-value[a]	
				Mean	SE	Mean	SE		
growth rate	Increase in trunk circumference (cm) 2013-15	37	-	11.63	0.20	11.69	0.26	0.353	
	2013-14	37	-	3.34	0.14	3.12	0.18	0.977	
	2014-15	37	-	8.30	0.14	8.57	0.18	0.183	
fruit production	Yield per tree (kg)	76	-	6.02	0.88	8.03	1.18	0.089	
	Fruit mass (g)	73	1225	141.72	8.09	157.74	8.68	0.121	
	Fruit circumference (cm)	72	1211	32.51	1.25	34.94	1.38	0.154	
fruit ripeness	Fruit Chroma Garnet Beauty only	27	393	30.61	0.88	31.57	1.11	0.385	
	Fruit Chroma Catherina & Babygold	45	1085	46.45	0.80	45.74	0.82	0.003	**
	Fruit "L"	72	1478	31.26	0.66	32.98	0.72	0.116	
	Fruit Hue angle	72	1478	25.72	0.32	24.78	0.35	0.177	
	Fruit firmness (kgf)	75	1440	0.88	0.10	1.10	0.13	0.023	*
	Fruit pH	76	-	23.90	0.02	23.88	0.02	0.835	
	Fruit Brix (^{o}Bx)	77	-	10.64	0.22	10.80	0.28	0.485	

[a] asterisk indicates level of significance: *p< 0.05, **p < 0.01

Figure captions:

Figure 1. Annual increase in trunk cross sectional area (cm^2) for peach trees naturally infected (POS) with *Plum pox virus* in the field as compared to non-infected (NEG) trees. Mean values of bars marked with the same case letter are not significantly different (P>0.05)

Figure 2. Annual yield efficiency expressed as mass of fruit (Kg) per tree relative to the trunk cross sectional area (TCSA) (cm^2) for peach trees naturally infected (POS) with *Plum pox virus* in the field as compared to non-infected (NEG) trees. Mean values of bars marked with the same case letter are not significantly different (P>0.05)

Figure 3. Proportions of total fruit per gradeout size category for peach trees naturally infected (POS) with *Plum pox virus* in the field as compared to non-infected (NEG) trees. Mean values of bars marked with the same letter are not statistically different (P>0.05)

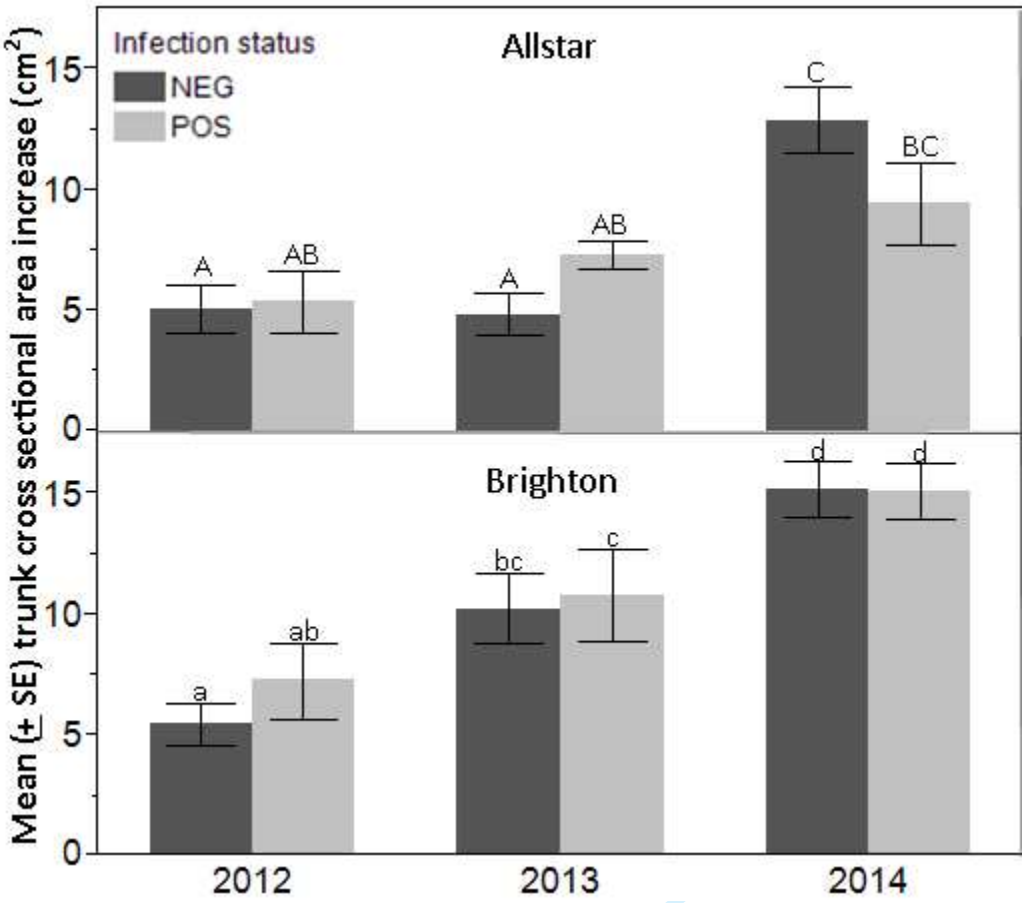

FIGURE 1.

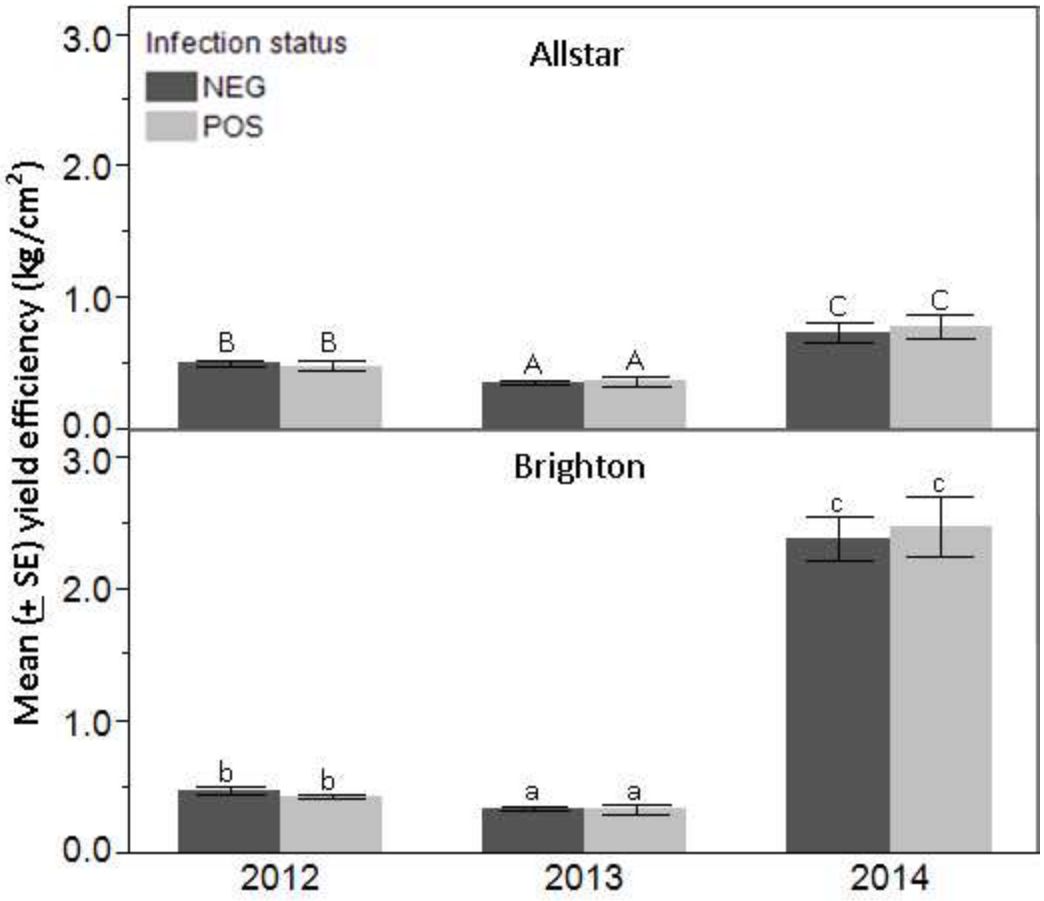

FIGURE 2.

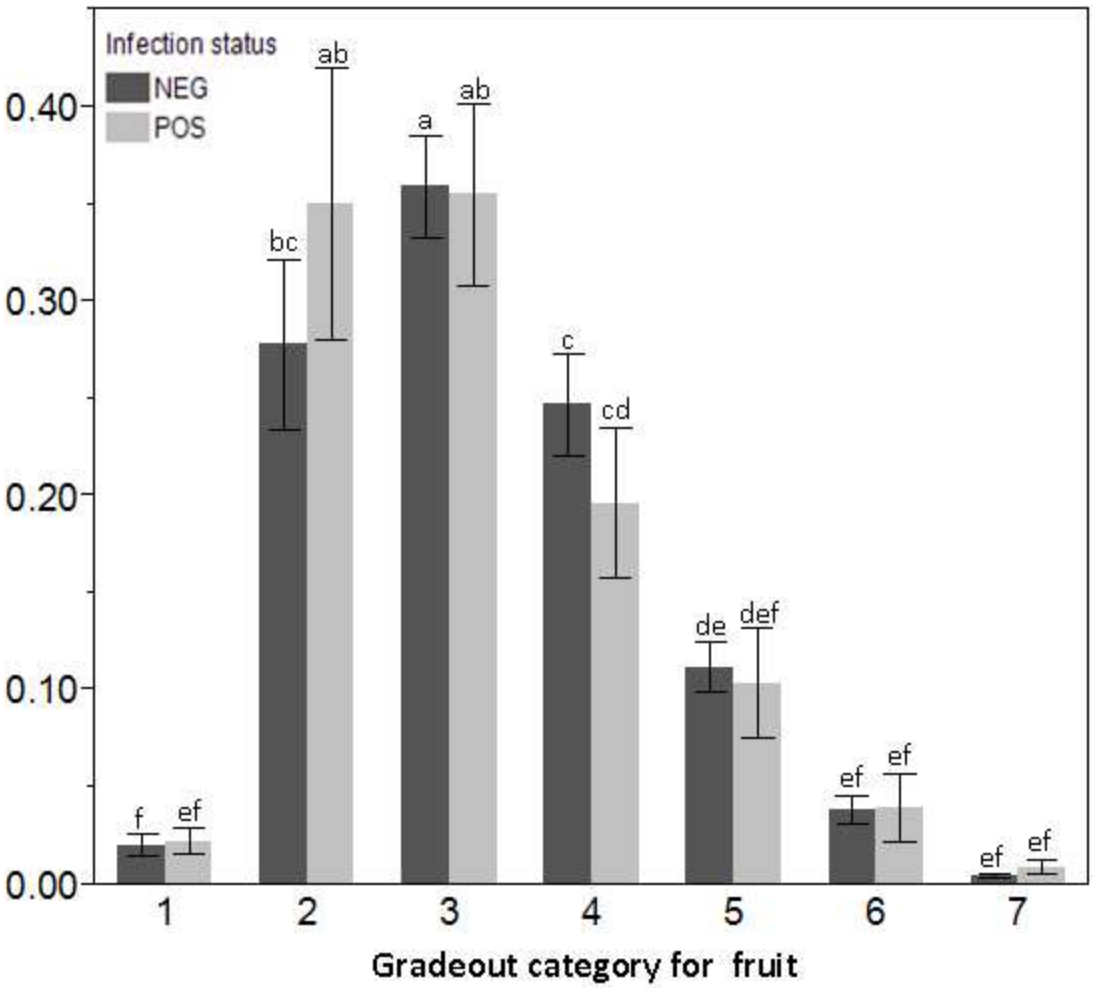

FIGURE 3.