Pyracantha ‘Mohave’ Fruit Infection by *Phytophthora ramorum* and Transmission of the Pathogen from Infected Fruit to Roots of *Viburnum tinus*

Paul W. Tooley, Marsha Browning, and Nina Shishkoff, United States Department of Agriculture–Agricultural Research Service Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD 21702

Abstract

Tooley, P. W., Browning, M., and Shishkoff, N. 2016. *Pyracantha* ‘Mohave’ fruit infection by *Phytophthora ramorum* and transmission of the pathogen from infected fruit to roots of *Viburnum tinus*. Plant Dis. 100:555–560.

Colonization of the fleshy fruit of *Cornus florida*, *C. kousa*, *Laurus nobilis*, *Malus hupehensis*, and *Pyracantha* ‘Mohave’ was observed following inoculation with sporangia of *Phytophthora ramorum*. However, abundant production of chlamydospores was only observed in the fruit of *Pyracantha* ‘Mohave’. *Pyracantha* ‘Mohave’ fruit that had been inoculated with a *P. ramorum* sporangia suspension were placed in pots containing rooted cuttings of *Viburnum tinus* in a misting tent or in water-filled trays in a climate-controlled greenhouse. Runoff was collected for 24 to 30 days, and roots were plated after the final collection. Mean percent recovery from infected roots was

not significantly different ($P = 0.05$, Tukey’s test) between bottom-watered treatments in trays and misted treatments, averaging 58% for bottom-watered and 54% for mist treatments. The number of CFU collected in runoff from bottom-watered plants was consistently lower than that obtained from plants held under mist, likely due to desiccation of the fruit. The results show that root infection of *V. tinus* can occur by *P. ramorum* via infected fruit of *Pyracantha* ‘Mohave’. This phenomenon represents a pathway of infection for *P. ramorum* not previously reported, which may play a role in disease epidemiology.

Phytophthora ramorum was first observed causing sudden oak death in California forests in the mid-1990s (Rizzo et al. 2002, 2005) and was described as a new species in 2001 (Werres et al. 2001). *P. ramorum* has now not only caused significant losses to forest resources in the Western United States (Frankel 2008; Grünwald et al. 2012; Hansen 2015) but also causes sudden larch death in the United Kingdom (Brasier and Webber 2010; Webber et al. 2010). Ramorum blight is a major problem affecting nursery stock as well, with quarantine restrictions currently in place to prevent interstate movement of the pathogen (APHIS 2015; California Oak Mortality Task Force 2015; Dart and Chastagner 2007; Goss et al. 2009). To date, there exist four evolutionary lineages of *P. ramorum* (Grünwald et al. 2009; Van Poucke et al. 2012).

A number of *Phytophthora* spp. are known to infect the fruit or seed of several host species (Erwin and Ribeiro 1996). Avocado fruit can be infected by *P. cinnamomi* and also by *P. citricola* and *P. hevea* (Koike et al. 1987; Perez-Jimenez 2008). Ando et al. (2009) described infection of various cucurbit fruit by *P. capsici*. Upstone (1978) showed that *P. syringae* can cause rotting of apple fruit. Graham et al. (1998) described citrus brown rot caused by *P. palmivora* and *P. nicotianae*. Several workers (Awuah and Frimpong 2007; Jackson and Newhook 1978; Kumi et al. 1996) have shown that infected cacao pods are an important source of inoculum and that *P. palmivora* can be systemically transmitted from infected pods to cacao seedlings. Kumi et al. (1996) also showed that *P. palmivora* could be isolated from symptomless cacao pods on infected trees. Fruit infection also provides inoculum for *P. botryosa* on *Hevea* spp. (Chee 1969). Wen et al. (2000) showed that soybean seed

resulting from inoculation of young seed pods can be infected by *P. sojae* and, when sown, can infect nearby plants.

P. ramorum has been reported to sporulate on asymptomatic fruit of several plant species, including *Crataegus monogyna*, *Laurus nobilis*, *Quercus ilex*, *Rosa sempervirens*, and *Smilax aspera* (Denman et al. 2008). Fruit inoculation assays were also conducted by Moralejo et al. (2006), who found that fruit of *Arbutus unedo*, *L. nobilis*, *Pistacia lentiscus*, *Rhamnus alaternus*, and *Viburnum tinus* were susceptible to *Phytophthora ramorum*. They also observed that *P. ramorum* was able to colonize the mesocarp and endocarp of berries of *Pistacia lentiscus* and *R. alaternus*, which are both dispersed by birds.

Each year, *Phytophthora ramorum* is detected in nurseries in the Eastern United States as the result of movement of plants in the nursery industry (Stokstad 2004). In 2014, *P. ramorum* was reported in 19 host nurseries located in three regulated states (California, Oregon, and Washington) and four nonregulated states (Maine, New York, Texas, and Virginia) (Hebbar 2014). In Spring 2010, *P. ramorum* was detected on seedlings of *L. nobilis* (Sweetbay or European bay laurel) in a nursery in Lancaster, PA, which had been grown from seed obtained from a *P. ramorum*-infested and Animal and Plant Health Inspection Service (APHIS)-regulated county in California (Hebbar 2010a,b). At that time, APHIS released a note clarifying that the infected plants were found in seedling flats in a commercial greenhouse and not in the forestry environment. It was also stated that the Confirmed Nursery Protocol was completed and that subsequent plant and soil samples were all negative for *P. ramorum* (Hebbar 2010b). In addition, trace-forward investigations of over 45 samples from both Eastern and Western states tested negative (Hebbar 2010a).

L. nobilis has a fleshy fruit and is currently on the APHIS “List of Regulated Hosts and Plants Proven or Associated with *Phytophthora ramorum*”, as is *Cornus kousa* (kousa dogwood), another species with a fleshy fruit. A wide variety of plant species, including some with fleshy fruit, are commonly shipped within the nursery industry, and it is desirable to know whether seed or fruit of such plants may be able to transmit *P. ramorum* and provide inoculum that can infect aboveground plant parts as well as roots of susceptible host species. Both seed and fruit shipped interstate as well as plants containing seed or fruit potentially infected with *P. ramorum* could be of concern. Because of the positive 2010 detection of *P. ramorum* in the

Corresponding author: P. W. Tooley; E-mail: paul.tooley@ars.usda.gov

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Accepted for publication 14 September 2015.

<http://dx.doi.org/10.1094/PDIS-03-15-0369-RE>

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Lancaster, PA nursery, APHIS was interested in determining whether a *P. ramorum* pathway could exist via fleshy-seeded plant species shipped from the Western to Eastern United States via the nursery trade. Thus, APHIS requested that we perform research to determine the propensity of such materials to become infected by *P. ramorum* and transmit the disease. In this study, we tested the potential for selected plant species with fleshy fruit to sustain fruit infection by *P. ramorum*. Because only *Pyracantha* 'Mohave' fruit supported an abundance of *P. ramorum* chlamydospores following inoculation, we further conducted a study to investigate the potential for *P. ramorum* to be transmitted to roots of a susceptible (*V. tinus*) host via infected *Pyracantha* 'Mohave' fruit.

Materials and Methods

Inoculum production and inoculation. Sporangia from six *P. ramorum* isolates (Table 1) were produced in 1% soil extract (Tooley et al. 2004). Spores were harvested and combined into a single spore suspension, with the concentration adjusted to 2,000 sporangia/ml. Fruit of selected plant species known to contain fleshy fruit were collected from local sources (*C. florida*, *C. kousa*, *Malus hupehensis*, and *Pyracantha* 'Mohave'), while *L. nobilis* fruit were obtained from colleagues in California. Additional *Pyracantha* 'Mohave' fruit was collected from plants obtained commercially and maintained in a greenhouse. Collected fruit were held in Ziploc bags at 4°C until use. Batches of approximately 100 fruit were surface disinfested in 0.525% sodium hypochlorite for 5 min, triple rinsed in sterile reverse osmotic (RO) water, then immersed in either 250 ml of a *P. ramorum* sporangia suspension or 1% soil extract control for 1 h. Fruit were then collected and transferred to Ziploc bags containing moistened vermiculite. The bags were held at 20°C for 2 days to allow for infection and then stratified in the dark at 4°C for 200 to 300 days. Fruit were rinsed clean of vermiculite prior to use.

Runoff assay. We used the system described by Shishkoff (2011), which employs host species *V. tinus* (Ginetti et al. 2014) to quantify the amount of *P. ramorum* inoculum present in runoff (Fig. 1). Stem cuttings of *V. tinus* were rooted in Turface MVP (calcined, montmorillonite clay; Profile Products LLC, Buffalo Grove, IL) under

mist. Containers were Anderson plant bands (square pots; 5.5 cm wide at the top, 5.0 cm wide at the bottom, and 7.5 cm high) fitted with a plastic mesh liner to hold in the Turface. Once the cuttings had rooted (2 to 3 months), 15 plants (1 per pot) were placed in a mist tent outfitted with fogging nozzles set to activate for 15 s every 12 min, and 15 plants were set up in trays in a climate-controlled greenhouse at 20°C. Water levels in trays were maintained at a depth of approximately 2 to 3 cm. Six plants at each location received 10 inoculated fruit or 10 noninoculated fruit. The remaining three plants served as negative controls. Runoff from roots in the stored fruit experiments was collected at 1, 3, 6, 8, 10, 13, 15, and 24 days. A 50-ml conical centrifuge tube was positioned at a corner of the pot and, with the pot tilted toward the tube, 15 ml of water was added to the medium surface. Runoff was collected, tilting the pot to collect from each corner, until a volume of 15 ml was reached. When necessary, the pot was agitated gently up and down to increase the volume of runoff. This process was repeated, collecting runoff from each pot. For the plants held in trays, excess water was allowed to drain from the pots prior to flushing the Turface medium with additional water and collecting the runoff. Tubes containing runoff were vortexed, and 1 ml of the suspension was added to each of three 100-by-15-mm petri dishes containing PARPH (pimaricin-ampicillin-rifampicin-pentachloronitrobenzene-hymexazol) selective medium (Jeffers and Martin 1986). The dishes were tilted to distribute the suspension over the medium surface. Plates were incubated unwrapped in a 20°C incubator in the dark. Plates were examined and colonies marked on the underside of the dish over a 7-day period. The number of colonies of *P. ramorum* observed on each plate was recorded, combined, and

Table 1. Isolates of *Phytophthora ramorum* used in these studies

Isolate	Host genus	Source	Lineage ^z
NS-5C	<i>Camellia</i>	California	NA1
WSDA 1772	<i>Viburnum</i>	Washington State	NA1
09-053	<i>Rhododendron</i>	California	NA2
05-166	<i>Rhododendron</i>	Washington State	NA2
BBA 9/95	<i>Rhododendron</i>	Germany	EU1
06-021	<i>Pieris</i>	Oregon	EU1

^z Clonal lineage as described by Grünwald et al. (2009)

Fig. 1. Healthy (left) and diseased (right) fruit of *Pyracantha* 'Mohave' on surface of Turface containing plants of *Viburnum tinus* for assay to determine amount of *Phytophthora ramorum* inoculum present in runoff. Bar represents 1 cm.

Fig. 2. Number of CFU per plant contained in runoff from *Viburnum tinus* plants inoculated with *Phytophthora ramorum*-infected fruit of *Pyracantha* 'Mohave' for stored (top) and fresh fruit (bottom). Data are means of three experiments, with six plants assayed in each experiment. Bars represent standard errors.

multiplied by 5 to give the number of CFU in the total 15 ml of runoff collected from each plant. Three experiments were performed, and runoff data are presented as the total number of CFU observed per plant for each date. Three experiments were also performed using freshly harvested fruit rather than stored fruit. Fruit was immersed in the *P. ramorum* sporangia suspension (2,000 sporangia/ml) for 24 h, then transferred to the Turface surface. Runoff was collected as described above on days 3, 6, 8, 10, 13, 15, 24, and 30, and roots were plated after collecting and plating runoff on day 30.

Root assay. Following the final collection and plating of runoff, roots were rinsed clean of Turface, surface sterilized in 0.26% sodium hypochlorite for 5 min, and triple-rinsed in RO water. Seven 1-cm root-tip segments were collected at random from each root system and plated on each of three 9-cm-diameter petri dishes filled with PARPH selective medium, with 2% clarified, buffered V8 juice added, for a total of 21 root segments per plant. Plates were incubated at 20°C in darkness and examined for the presence of *P. ramorum* over a 21-day period. Recovery data are presented as the mean percentage of root segments plated per plant from which *P. ramorum* was recovered.

Chlamyospore counts. Direct counts of chlamyospores in 10 stored fruit were made by removing skin and seed and squashing the pulp between a glass slide and coverslip, two squash mounts per fruit. Three batches of 10 diseased fruit (stored, inoculated fruit only) were also homogenized, diluted, and plated on PARPH selective medium. The 10 fruit in each batch were homogenized in a blender in 40 ml of sterile RO water (eight quick bursts) and the volume was then brought up to 80 ml using additional sterile RO water. Then, 1 ml was plated on 9-cm-diameter petri dishes of PARPH, and five counts were performed per batch of fruit. To confirm the ability of *Pyraacantha* 'Mohave' fruit and its contents to support chlamyospore production and germination by *P. ramorum*, an extract was prepared from fruit and inoculated with *P. ramorum*-infested plugs of 10% V8 juice agar. *Pyraacantha* fruit was removed from storage at 4°C in Ziploc bags, surface disinfested with a 0.5% sodium hypochlorite solution for 10 min, then triple rinsed with sterile RO water. Approximately 150 cm³ of fruit was placed in a 1-liter beaker and the volume was increased to 400 ml with RO water. The suspension was heated in a microwave for 2 min, then covered with foil and left on a benchtop for a week. The softened fruit was crushed with the back of a spoon and left on a benchtop for one more day. The suspension was then filtered through four layers of Kimwipes and autoclaved. Petri dishes (60 by 15 mm) were filled with 10 ml of *Pyraacantha* 'Mohave' fruit extract (0, 2, 5, 10, 20, and 50% [vol/vol]), four dishes per concentration. Dishes were inoculated with single agar fragments (approximately 2 mm in diameter) from a 10% V8 juice agar culture (approximately 14 days old) of *P. ramorum* isolate NS-5C (Table 1). Plates were wrapped and placed in the dark at 20°C for a week, then examined. After 2 weeks of incubation, chlamyospores were mechanically harvested from colonies growing in 2% fruit extract. Approximately 100 chlamyospores were pipetted in a 1-ml suspension onto five plates of PARPH selective agar medium. Plates were incubated unwrapped in a 20°C incubator for 3 days. Counts of germinated chlamyospores were performed, with only those colonies that could be traced back to a germinated chlamyospore being counted.

Results

P. ramorum was recovered from inoculated fleshy fruit of *C. florida*, *C. kousa*, *L. nobilis*, *M. hupehensis*, and *Pyraacantha* 'Mohave' but no chlamyospores were observed in the fruit pulp. Also, no chlamyospores were observed in noninoculated fruit of any plant species. Examination of the fleshy pulp revealed an abundance of chlamyospores only in the fruit of *Pyraacantha* 'Mohave'. Dilution plating results revealed an average of 1,099 CFU/fruit (standard error 26 CFU), while direct counts revealed an average of 1,707 chlamyospores/fruit (standard error of 150 chlamyospores). *Pyraacantha* fruit exposed to *P. ramorum* sporangia appeared russet brown in color and the pulp was quite soft, whereas fruit which had been exposed to soil extract only and then transferred to vermiculite for cold storage was still red and mostly firm.

For stored fruit, the number of CFU obtained from pots containing *V. tinus* plants and infected *Pyraacantha* 'Mohave' fruit watered using mist showed increases over time, except for decreases between 3 and 6 days and between 15 and 24 days (Fig. 2, top). CFU from pots watered from the bottom did not show a drop between 15 and 24 days but did show a drop between 6 and 8 days (Fig. 2, top). For fresh fruit (Fig. 2, bottom), the number of CFU per plant for the mist-watering treatment increased up to 24 days, then showed a decrease between 24 and 30 days. The number of CFU for the bottom-watered treatments decreased from day 3 through day 8, then increased from day 8 until day 24, then again showed a decrease to day 30 (Fig. 2, bottom).

Regression lines were fit and slopes for mist and bottom watering compared within the fresh and stored fruit datasets using analysis of covariance. For both stored and fresh fruit, regression slopes differed significantly ($P = 0.05$) between mist and bottom-watered treatments. Thus, the rate of increase in CFU obtained over days of collection was significantly higher for mist compared with bottom-watered treatments for both stored and fresh fruit. Intercepts did not differ significantly for either group.

For both stored and fresh fruit, results of analysis of variance for CFU showed that significant differences existed for irrigation (mist versus bottom watered) and days of collection but not for fruit (stored versus fresh), nor was there a significant fruit-irrigation interaction (Table 2). Misting treatment resulted in collection of significantly more *P. ramorum* CFU in runoff compared with bottom watering. Mean values shown for CFU showed no significant differences

Table 3. Mean values for CFU obtained in runoff from *Viburnum tinus* infected by *Phytophthora ramorum*-infected *Pyraacantha* 'Mohave' fruit

Fruit ^x	Irrigation method ^y	Mean CFU ^z
Stored	Mist	116.98 a
Fresh	Mist	110.94 a
Stored	Bottom	58.30 b
Fresh	Bottom	40.97 b

^x Freshly collected fruit versus fruit inoculated and stored at 4°C until use.

^y *V. tinus* plants placed under mist in a controlled-environment greenhouse cubicle or bottom watered in a containment greenhouse.

^z Means within a column followed by the same letter do not differ significantly via Tukey's test, $P = 0.05$ ($n = 144$).

Table 2. Analysis of variance for CFU counted based on independent variables fruit, irrigation, and days of runoff collection

Source	Degrees of freedom	Sequential sum of squares	Adjusted sum of squares	Adjusted mean square	F statistic	P value
Fruit ^x	1	257	17,208	17,208	1.23	0.267
Irrigation ^y	1	595,791	595,791	595,791	42.73	0.000
Days ^z	8	1,194,922	1,194,922	149,365	10.71	0.000
Fruit × irrigation	1	4,584	4,584	4,584	0.33	0.567
Error	564	7,864,274	7,864,274	13,944	...	...
Total	575	9,659,829	...	...	...	...

^x Freshly collected fruit versus fruit inoculated and stored at 4°C until use.

^y *Viburnum tinus* plants placed under mist in a controlled-environment greenhouse cubicle or bottom watered in a containment greenhouse.

^z Days over which runoff was collected from pots of *V. tinus* containing *Pyraacantha* 'Mohave' fruit infected with *Phytophthora ramorum*.

between stored and fresh fruit with the mist-watering treatment and no differences for the bottom-watering treatment but differences between mist and bottom watering (Table 3). No chlamydospores were observed in plated runoff samples.

In terms of percent recovery from infected *V. tinus* roots, an overall analysis of variance showed highly significant differences between stored and fresh fruit but no significant differences for irrigation (mist versus bottom watered), and no significant fruit–irrigation interaction (Table 4). The mean percent infected roots for fresh fruit was not significantly different by Tukey's test ($P = 0.05$) between mist- and bottom-watered treatments, averaging 58% for bottom-watered and 54% for mist-watered treatments (Table 5). Nor was the mean percent infected roots for stored fruit significantly different between mist- and bottom-watered treatments, averaging approximately 24% for both mist- and bottom-watered treatments (Table 5).

In *Pyracantha* 'Mohave' fruit extract, colonies were of varying size, with the more concentrated fruit extracts producing the largest colonies. Colonies produced in 50, 20, and 10% extract were dense with hyphae and pigmented chlamydospores. Less concentrated fruit extracts (5 and 2%) produced smaller colonies with fewer mycelia and fewer spores. For the chlamydospores extracted from these colonies and plated on PARPH, nearly 100% germination was observed. For all experiments, data were analyzed using the general linear models and regression procedures within Minitab 17 (Minitab 2010).

Discussion

Colonization of the fleshy fruit of *C. florida*, *C. kousa*, *L. nobilis*, *M. hupehensis*, and *Pyracantha* 'Mohave' collected from local sources was observed following inoculation with sporangia of *P. ramorum*. However, an abundance of chlamydospores was observed only in the fruit of *Pyracantha* 'Mohave'. *Pyracantha* is a common evergreen shrub native to Europe and Southeast Asia which is sold commercially throughout the United States, and 'Mohave' has become a widespread *Pyracantha* cultivar within the nursery industry (U.S. National Arboretum 1999) and is propagated by some nurseries on the West Coast. Although 'Mohave' has never been reported as a host of *P. ramorum*, *Pyracantha koidzumii* was reported as a host (Briere et al. 2006) and is one of the parents in the cross that produced 'Mohave' (Egolf 1970). Because 'Mohave' is such a common cultivar and propagated on the West Coast of the United States where *Phytophthora ramorum* is established, it is possible that it could be exposed to *P. ramorum* and serve as an additional means of inoculum dispersal.

P. ramorum disease development is very dependent on environmental conditions (Tooley et al. 2009), and our results were obtained under artificial conditions that provided favorable conditions for the pathogen. In a natural setting, however, it is unclear what role *Pyracantha* 'Mohave' may play in the epidemiology of *P. ramorum*. Although the plant is common in many suburban environments, its propensity to come into contact with spores of *P. ramorum* may be limited, unless it is grown in commercial greenhouses or nurseries that also harbor infected *P. ramorum* host species. In the natural environment, it could become infected when growing in the vicinity of *P. ramorum* hosts because sporangia have been found to be generated in large numbers by a variety of host species (Tooley and Browning 2009), most notably California bay laurel (Davidson et al. 2008). It should also be noted that fruit of *Pyracantha* spp. tend

to remain on the plant throughout the winter months and not fall easily to the ground where infected fruit might provide chlamydospore or sporangial inoculum. Birds, however, are known to use *Pyracantha* berries as a food source, and could spread the pathogen, because it has been found that spores of other fungal species (Warner and French 1970) as well as chlamydospores of *P. cinnamomi* (Keast and Walsh 1979) can be disseminated by birds.

Although many *Phytophthora* spp. are known to cause fruit rots on different hosts (Erwin and Ribeiro 1996), the transmission of disease from infected fruit on the soil surface to host roots has not, to our knowledge, been shown. Our results show that root infection of a susceptible host can occur by *P. ramorum* via infected fruit of *Pyracantha* 'Mohave'. This phenomenon represents a pathway of infection for *P. ramorum* not previously reported, which may play a role in disease epidemiology. An average of 1,099 CFU/fruit were obtained when homogenized infected fruit was dilution plated, while 1,707 chlamydospores per infected fruit were counted by direct counting under the microscope. The number obtained by dilution plating may be lower than that obtained by direct counting due to the fact that chlamydospores would have had to germinate on the PARPH selective medium to be counted, and germination rates of *P. ramorum* chlamydospores are known to vary widely (Tooley et al. 2014). The direct count number may also underestimate the total number of chlamydospores present in infected fruit, because some chlamydospores adhered to or were obstructed by the skin of the fruit, rendering them uncountable. Also, the number of CFU collected in runoff from plants held in trays of water was consistently lower than from plants held under mist, likely due to desiccation of the fruit. Considerable air movement in the climate-controlled greenhouse where these experiments were performed resulted in some drying of the Surface medium surface.

It is unclear how much of the inoculum gathered in runoff was due to production of propagules by *P. ramorum* in diseased fruit placed on the medium surface as opposed to the production of propagules by *P. ramorum* in diseased roots. Shishkoff (2009) reported that the production of *P. ramorum* inoculum from a diseased lilac (*Syringa vulgaris*) leaf maintained on the potting mix surface continued throughout the 22-day duration of the experiment when overhead irrigation was used. Recovery of *P. ramorum* from roots of *V. tinus* plants receiving diseased *Pyracantha* fruit confirms that the roots were colonized and, thus, it is likely that they also played a role in inoculum production. It is known, for example, that *P. ramorum*

Table 5. Mean values for percent recovery for roots of *Viburnum tinus* infected by *Phytophthora ramorum*-infected *Pyracantha* 'Mohave' fruit

Fruit ^x	Irrigation method ^y	Mean ^z
Fresh	Bottom	58.25 a
Fresh	Mist	54.13 a
Stored	Bottom	24.87 b
Stored	Mist	24.60 b

^x Freshly collected fruit versus fruit inoculated and stored at 4°C until use.

^y *Viburnum tinus* plants placed under mist in a controlled-environment greenhouse cubicle or bottom watered in a containment greenhouse.

^z Means within a column followed by the same letter do not differ significantly via Tukey's test, $P = 0.05$ ($n = 18$).

Table 4. Analysis of variance for percent recovery from infected roots, using adjusted sums of squares for tests

Source	DF	Sequential sum of squares	Adjusted sum of squares	Adjusted mean square	F value	P value
Fruit ^x	1	17,627.1	17,554.0	17,554.0	28.91	0.000
Irrigation ^y	1	83.2	85.3	85.3	0.14	0.709
Fruit × irrigation ^z	1	66.0	66.0	66.0	0.11	0.743
Error	67	40,685.6	40,685.6	607.2	...	...
Total	70	58,461.9	...	...	...	...

^x Freshly collected fruit versus fruit inoculated and stored at 4°C until use.

^y *Viburnum tinus* plants placed under mist in a controlled-environment greenhouse cubicle or bottom watered in a containment greenhouse.

^z Days over which runoff was collected from pots of *V. tinus* containing *Pyracantha* 'Mohave' fruit infected with *Phytophthora ramorum*.

sporangia form in abundance on infected roots (Shishkoff 2007). We observed the root systems of some of the infected plants to be so deteriorated that few roots were left to sample and plate. Although we do not have direct evidence, we presume that chlamydospores in fruit placed on the soil surface gave rise to sporangia that washed down and infected the roots, and that CFU obtained in runoff represented largely sporangia.

The finding that *P. ramorum* can infect fruit of and be transmitted to the root systems of susceptible host plants is significant, in that it represents a previously undescribed pathway of spread for this damaging pathogen. Regulators will have to consider fruit transmission of *P. ramorum* as a possibility allowing for spread of the disease, as we have shown to occur with *Pyracantha* 'Mohave', an ornamental commonly grown and transported within the nursery industry. Infected fruit containing numerous chlamydospores could serve as a mechanism for spreading the disease to new areas. Examination of the potential for colonization of the fruit of additional *P. ramorum* hosts (APHIS 2013) is desirable to determine whether they can serve as reservoirs of inoculum and contribute to disease epidemics in nurseries or natural ecosystems. Thus, the role of fruit infection in the epidemiology of diseases caused by other *Phytophthora* spp. also needs to be further explored.

Acknowledgments

We thank N. Grünwald, S. Werres, and G. Chastagner for providing some of the isolates of *P. ramorum* used in these studies; and L. Englander and K. Kosta for the collection of fruit.

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