

# Assessments of *Rhagoletis pomonella* (Diptera: Tephritidae) infestation of temperate, tropical, and subtropical fruit in the field and laboratory in Washington State, U.S.

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## ABSTRACT

To understand the likelihood of any risk of apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), to domestic and foreign fruit export markets, knowledge of its host plant use is needed. Here, assessments of *R. pomonella* infestation of temperate, tropical, and subtropical fruit were made in the field and laboratory in Washington State, U.S. In field surveys in 2010–2017 in central Washington, 6.7% of *Crataegus douglasii* and 6.1% of feral *Malus domestica* trees (both temperate plants) in fly-managed (insecticide-treated) sites were infested by larvae. In unmanaged sites, 54.1% of *C. douglasii* and 16.3% of feral *M. domestica* tree samples were infested. In field surveys of 36 types of temperate fruit in 2015–2018 in southwestern Washington, new host records for *R. pomonella* were one species and three hybrids of *Crataegus*, as well as *Prunus domestica* subsp. *syriaca* – all of which produced adult flies. In addition, *Prunus avium* was a new host record for Washington State, producing one adult fly. *Prunus armeniaca* x *Prunus salicina* and *Vitis vinifera* exposed to flies in the laboratory produced adult flies. Of 37 types of tropical and subtropical fruit hung in fly-infested *M. domestica* trees in southwestern Washington, only *Mangifera indica* produced puparia. Out of nine tropical and subtropical fruit types in laboratory tests, *Musa acuminata* x *balbisiana* produced puparia but no adult flies. Results provide a basis for further research and hypotheses concerning host use by *R. pomonella* and its potential impact on protecting both U.S. and tropical and subtropical fruit markets.

## INTRODUCTION

The apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), is a quarantine pest of cultivated apple (*Malus domestica*) (Rosaceae) in western North America whose ancestral hosts are hawthorns, *Crataegus* spp. (Rosaceae) (Bush 1966). Native to eastern North America and Mexico, *R. pomonella* in western North America was first detected in *M. domestica* in 1979 in Portland, Oregon, in the Pacific Northwest (PNW) of the U.S. (AliNiazee and Penrose 1981). It is now found throughout the PNW west of the Cascade Mountain Range, a relatively humid region with little commercial tree fruit production. However, arid or semi-arid regions in the PNW east of the range in central Washington State (Washington), Oregon, and Idaho, as well as in British Columbia, Canada (Canadian Food Inspection Agency 2016), where commercial apples are grown are mostly free of the fly. Preventing fly movement from infested to pest-free areas across the PNW and preventing increases in fly numbers within quarantine areas are high priorities for U.S. state departments of agriculture. To date, there have been no reports of commercially grown apples from the PNW infested by *R. pomonella* larvae (Washington State Department of Agriculture 2018).

Washington is the PNW's biggest apple producer. It exports about 30% of its crop, which is valued at ≈US\$2.26 billion a year (NASS 2017), to overseas markets (Anonymous 2018). About 20 of 60 export markets have requirements or restrictions for apple import due to *R. pomonella*. These markets include China, Japan, South Korea, India, South Africa, Indonesia, Australia, Brazil, Chile, and Mexico. Some of these countries include regions with humid, subtropical climates. Although *R. pomonella* is a temperate species, ecological niche models indicate there are marginal to favorable habitats for the fly in such climates (between 15°N and 30°N latitude; e.g., southern China, northern Laos, Vietnam, and the Philippines) (Kumar *et al.* 2016). Furthermore, low temperatures are not a requirement for adult emergence (AliNiazee 1988). Thus, *R. pomonella* might become established if it were introduced into a subtropical country.

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To understand the likelihood of any risk of *R. pomonella* to domestic and foreign fruit export markets, knowledge of its host-plant use is needed. *Rhagoletis pomonella* is known to develop in at least 60 plant taxa (Yee and Norrbom 2017), therefore movement of these taxa needs to be restricted. However, more knowledge of its host-plant use could help to further reduce the perceived risk. Areas in which greater knowledge is needed include (1) frequencies of infestations of *Crataegus douglasii* (black hawthorn) and *M. domestica* trees, (2) additional host plants infested by the fly, and (3) fly infestation of tropical and subtropical fruit.

With respect to (1), *C. douglasii* and feral *M. domestica* occur spottily around commercial apple orchards in central Washington, where <10% and <1%, respectively, of trees were found to be infested by *R. pomonella* in 2004–2006 (Yee 2008). This suggests frequencies of infestations of both species are low, but that *C. douglasii* – which is native to the region – is more frequently infested and thus a greater source of flies. However, reassessments of infestation frequencies of the two plants over time may show that the frequencies change and thus can affect the trees' importance in fly control. In addition, Washington State Department of Agriculture (WSDA) and county pest boards detect *R. pomonella* in *C. douglasii* and feral *M. domestica* trees at sites near apple orchards using traps and then treat fly-positive trees with insecticides, but these entities do not control flies in *C. douglasii* and feral *M. domestica* trees at sites farther from commercial orchards. Whether differences in frequencies and patterns of larval infestations of *C. douglasii* versus *M. domestica* trees in fly-managed (insecticide-treated) and unmanaged sites occur has yet to be determined.

With respect to (2), there may be host plants of *R. pomonella* that are not yet identified that could increase the risk of the fly spreading should their fruit be moved from infested to uninfested areas within the PNW. *Crataegus* (hawthorn) species would be likely candidates, as 30 of the 60 plant taxa that are hosts for *R. pomonella* belong to this genus (Yee and Norrbom 2017).

With respect to (3), *R. pomonella* attacks *Mangifera indica* (mango) (Anacardiaceae) and *Carica papaya* (papaya) (Caricaceae) hung in *M. domestica* trees in the field and in the laboratory and are suitable hosts that produced adult *R. pomonella* (Yee and Goughnour 2017). However, suitability of other tropical and subtropical fruit has not yet been determined. No commercial tropical and subtropical fruit belong to the Rosaceae, and because they differ from *Crataegus* and *Malus* species in many respects, they may not be attractive to *R. pomonella*. If that is the case, there may be no (zero) or minimal threat of *R. pomonella* attacking most tropical or subtropical fruit in subtropical environments.

Here, our objective was to assess *R. pomonella* infestation of temperate, tropical, and subtropical fruit through surveys and tests in the field and laboratory in Washington. Specific goals were to determine (1) *R. pomonella* infestation frequencies of *C. douglasii* versus feral *M. domestica* in central Washington, both in fly-managed and unmanaged sites; (2) whether there are unrecorded host plants of the fly in southwestern Washington; and (3) whether various tropical and subtropical fruit are suitable as developmental hosts for *R. pomonella*.

## MATERIALS AND METHODS

**Field surveys.** In July to September 2010–2017 in central Washington, field surveys were conducted of infestation by *R. pomonella* of *C. douglasii* versus *M. domestica*. Fruit from *C. douglasii* and feral *M. domestica* were collected at 10 sympatric sites (Appendix 1). Within each site, trees of the two species were ~10–5,000 m apart, with numbers of each varying widely across sites. Sites were in sagebrush, bunchgrass, or ponderosa pine ecosystems (Lyons and Merilees 1995). The three fly-managed sites – in arid sagebrush and bunchgrass habitats – had an active fly detection and control program using insecticides run by WSDA and county pest boards. The seven unmanaged sites – mostly in less arid ponderosa pine habitat – had no history of fly control or had no control for up to 20 years before surveys. Each site was sampled for 1 to 3 years from 2010–2015. Exceptions were Klickitat, which was sampled only in 2010 and 2012, and Nile, where an additional *C. douglasii* sample took place in 2017. Both tree species occurred along creeks, along roadsides beside ditches, in meadows, along trails in wooded areas away from creeks, and in pastures. Fruit from both species were collected when ripe: *C. douglasii* from mid-July to late August, and *M. domestica* from mid-August to early October. About 800 *C. douglasii* fruit were picked per tree, depending on fruit load. About 50 *M. domestica* fruit were collected from beneath each tree about 1 week after they had dropped.

In July to November 2015–2018 in southwestern Washington, field surveys were conducted of 36 types of temperate fruit – mostly non-native species – including hybrids, subspecies, and varieties (see Table 1 for a list of temperate fruit surveyed or tested for infestation by *R. pomonella*). The focus was on fruit of unrecorded hosts, but fruit of known hosts were also collected for comparison. Fruit types collected included most of the accessible fruit present at the 10 sites in the coast forest ecosystem (Appendix 1). Collections were made in parks, along roadsides, in urban areas, and in demonstration tree plantings. All sites were unmanaged, except for the Vancouver Orchard. *Malus domestica* fruit were collected from the ground beneath trees, whereas other ripe fruit were collected off trees or bushes, except the fruit of *Prunus persica*, which were collected off the ground at the Vancouver Orchard. To gain additional information, the colour, diameters, and weights of at least 20 individual ripe fruit of newly identified *Crataegus* hosts were recorded. Fruit were collected for measurements in November 2018 from the same trees that had produced fruit positive for *R. pomonella* larvae in previous years.

**Table 1.** Temperate fruit sampled or tested for *Rhagoletis pomonella* infestation in field surveys or in the laboratory in southwestern Washington State, U.S.

Common name	Scientific name	Family
Apple <sup>a</sup>	<i>Malus domestica</i> Borkhausen	Rosaceae
Dolgo Crabapple	<i>Malus x 'Dolgo'</i>	Rosaceae
Fruiting Crabapple	<i>Malus</i> sp., unknown cultivar	Rosaceae
Harvest Gold Flowering Crabapple	<i>Malus x 'Harvest Gold'</i>	Rosaceae
Black Hawthorn	<i>Crataegus douglasii</i> Lindley	Rosaceae
Red Sun Chinese Hawthorn	<i>Crataegus pinnatifida</i> Bunge	Rosaceae
Autumn Glory Hawthorn	<i>Crataegus laevigata</i> (Poiret) de Candolle x <i>Crataegus mexicana</i> Mocino & Sessé ex de Candolle	Rosaceae
Lavalle Hawthorn	<i>Crataegus x lavallei</i> Hérincq ex Lavallée (hybrid of <i>C. mexicana</i> x probably <i>C. calpodendron</i> (Ehrhart) Medikus)	Rosaceae
Toba Hawthorn	<i>Crataegus x mordensis</i> Boom (hybrid of <i>Crataegus laevigata</i> (Poiret) de Candolle x <i>Crataegus succulenta</i> Schrader ex Link)	Rosaceae
Cockspur Hawthorn	<i>Crataegus crus-galli</i> Linnaeus	Rosaceae
Washington Hawthorn	<i>Crataegus phaenopyrum</i> Borkhausen	Rosaceae
Sweet Cherry	<i>Prunus avium</i> (Linnaeus) Linnaeus	Rosaceae
Tart Cherry	<i>Prunus cerasus</i> Linnaeus	Rosaceae
Mirabelle Plum	<i>Prunus domestica</i> Linnaeus subsp. <i>syriaca</i>	Rosaceae
Italian Plum	<i>Prunus domestica</i> Linnaeus	Rosaceae
French Petite Plum	<i>Prunus domestica</i> Linnaeus	Rosaceae
Friar Black Plum	<i>Prunus domestica</i> Linnaeus	Rosaceae
Cherry Plum	<i>Prunus cerasifera</i> Ehrhart	Rosaceae
Japanese Plum	<i>Prunus salicina</i> Lindley	Rosaceae
Choke Cherry	<i>Prunus virginiana</i> Linnaeus	Rosaceae
Peach (White, Mexican, California) <sup>a</sup>	<i>Prunus persica</i> (Linnaeus) Batsch	Rosaceae
Dapple Dandy Pluot <sup>a</sup>	~30% apricot ( <i>Prunus armeniaca</i> Linnaeus); ~70% plum ( <i>Prunus salicina</i> )	Rosaceae

European Quince	<i>Cydonia oblonga</i> Miller	Rosaceae
Pineapple Quince	<i>Cydonia oblonga</i> Miller	Rosaceae
Kosui Asian Pear	<i>Pyrus pyrifolia</i> (Burman) Nakai	Rosaceae
20th Century Asian Pear	<i>Pyrus pyrifolia</i> (Burman) Nakai	Rosaceae
Bartlett Pear	<i>Pyrus communis</i> Linnaeus	Rosaceae
Winter Pear	<i>Pyrus communis</i> Linnaeus	Rosaceae
Bosc Pear	<i>Pyrus communis</i> Linnaeus	Rosaceae
Cotoneaster	<i>Cotoneaster</i> sp.	Rosaceae
Western Mountain Ash	<i>Sorbus scopulina</i> Greene	Rosaceae
Red Currant	<i>Ribes rubrum</i> Linnaeus	Grossulariaceae
White Currant	<i>Ribes rubrum</i> Linnaeus (albino of red currant)	Grossulariaceae
Goumi Berry	<i>Elaeagnus multiflora</i> Thunberg	Elaeagnaceae
Twinberry Honeysuckle	<i>Lonicera involucrata</i> (Richardson) Banks ex Sprengel	Caprifoliaceae
Highbush Blueberry	<i>Vaccinium corymbosum</i> Linnaeus	Ericaceae
Red Globe Grape <sup>a</sup>	<i>Vitis vinifera</i> Linnaeus	Vitaceae
Jiro Fuyu Persimmon	<i>Diospyros kaki</i> Linnaeus the Younger	Ebenaceae

<sup>a</sup> used in laboratory tests. The only plants native to Washington are *C. douglasii*, *P. virginiana*, *S. scopulina*, *R. rubrum*, and *L. involucrata*; the rest originated from eastern North America, Europe, or Asia.

For both central and southwestern Washington surveys, precise fruit counts were made after collections. Fruit from individual trees were held in separate tubs at ~15–27 °C outdoors for 2–3 months for larval emergence. Tubs were checked for puparia every 1–3 days. Puparia were counted and identified using puparial traits (Yee and Goughnour 2016). In addition, for the southwestern Washington surveys, puparia from any fruit not previously recorded as a host were placed in cups with moist soil, held at 3–4 °C for ~4 months, and then at 21–23 °C for adult fly emergence. Adult flies were identified as *R. pomonella* using morphological characters (Bush 1966). Voucher specimens of reared adult flies from select hosts are held at the USDA-ARS Temperate Tree Fruit & Vegetable Research Unit in Wapato, Washington.

**Infestation of tropical and subtropical fruit in the field, southwestern Washington.** Field tests of infestation by *R. pomonella* of tropical and subtropical fruit were conducted in 2015, 2016, 2017, and 2018, in southwestern Washington at T.G., Devine, and Woodland sites (three of the same sites that were included for temperate fruit surveys; Appendix 1). These sites had high *R. pomonella* populations; use of these sites was intended to increase the chances fruit would be attacked.

A total of 37 types of tropical and subtropical fruit were hung 3–4 m above ground, according to the methods described in Yee and Goughnour (2017), in *M. domestica* trees infested by *R. pomonella* (see Table 2 for a list of tested tropical or subtropical fruit types, including cultivars of the same species, if known; *Psidium guajava* [guava apple and giant guava] were tested in the laboratory but not the field). *Malus domestica* (Gala variety) fruit were hung in the same trees as tropical and subtropical fruit to serve as positive controls. At each site, there were 14–30 *M. domestica* trees (3–9 m tall and 3–8 m wide), each with 20–60 test fruit at any one time. Fruit were hung ≥1 m apart.

Fruit were tested in July and August, when adult flies were most abundant. Over the four years, 5–189 total fruit per type (1,708 total fruit) were exposed to flies. As *M. indica*, *C. papaya*, and *Citrus* spp. are particularly important commercial tropical or subtropical fruit, emphasis was placed on testing these species. After 2–3 week exposures, fruit were removed, placed in tubs, and monitored for larval emergence.

**Table 2.** Tropical and subtropical fruit tested for *Rhagoletis pomonella* infestation in the field or laboratory in southwestern Washington State, U.S.

Common name	Scientific name	Family
Yellow Mango (Ataulfo)	<i>Mangifera indica</i> Linnaeus	Anacardiaceae
Red Mango <sup>a</sup>	<i>Mangifera indica</i> Linnaeus	Anacardiaceae
Carambola <sup>b</sup>	<i>Averrhoa carambola</i> Linnaeus	Oxalidaceae
Blue Java Banana <sup>b</sup>	<i>Musa acuminata</i> Colla × <i>balbisiana</i> (ABB Group) 'Blue Java'	Musaceae
Red Banana	<i>Musa acuminata</i> Colla (AAA Group)	Musaceae
Cavendish Banana	<i>Musa acuminata</i> Colla Cavendish subgroup of the AAA Group	Musaceae
Pineapple	<i>Ananas comosus</i> (Linnaeus) Merrill	Bromeliaceae
Pink Pineapple	<i>Ananas comosus</i> (Linnaeus) Merrill	Bromeliaceae
Passion Fruit	<i>Passiflora edulis</i> Sims	Passifloraceae
Cherimoya <sup>b</sup>	<i>Annona cherimola</i> Miller	Annonaceae
Hawaiian Papaya <sup>b</sup>	<i>Carica papaya</i> Linnaeus	Caricaceae
Mamey Sapote	<i>Pouteria sapota</i> (Jacquin) Harold Emery Moore & Stearn	Sapotaceae
Pineapple Guava	<i>Acca sellowiana</i> (Otto Berg) Burret	Myrtaceae
Mexican Guava	<i>Psidium guajava</i> Linnaeus	Myrtaceae
Pink Guava	<i>Psidium guajava</i> Linnaeus	Myrtaceae
Guava Apple <sup>b</sup>	<i>Psidium guajava</i> Linnaeus	Myrtaceae
Thai Guava	<i>Psidium guajava</i> Linnaeus	Myrtaceae
Giant Guava <sup>b</sup>	<i>Psidium guajava</i> Linnaeus	Myrtaceae
Horned Melon	<i>Cucumis metuliferus</i> Ernst Meyer	Cucurbitaceae
Mangosteen <sup>b</sup>	<i>Garcinia mangostana</i> Linnaeus	Clusiaceae
Hass Avocado	<i>Persea americana</i> Miller	Lauraceae
Red Dragon Fruit	<i>Hylocereus costaricensis</i> (Frederic Albert Constantin Weber) Britton & Rose	Cactaceae
Yellow Dragon Fruit	<i>Hylocereus megalanthus</i> (Karl Schumann ex Vaupel) Ralf Bauer	Cactaceae
Orange	<i>Citrus</i> × <i>sinensis</i>	Rutaceae
Blood Orange <sup>b</sup>	<i>Citrus</i> × <i>sinensis</i>	Rutaceae
Navel Orange	<i>Citrus</i> × <i>sinensis</i>	Rutaceae
Valencia Orange	<i>Citrus</i> × <i>sinensis</i> pummelo x mandarin orange	Rutaceae
Daisy Mandarin	<i>Citrus reticulata</i> Blanco	Rutaceae
Mandarin Orange <sup>b</sup>	<i>Citrus reticulata</i> Blanco	Rutaceae
Tangerine	<i>Citrus tangerina</i> Tanaka (hybrids)	Rutaceae
Murcott Orange	Mandarin x sweet orange hybrid	Rutaceae

Ortanique Tangerine	<i>Citrus reticulata</i> x <i>C. sinensis</i> hybrid	Rutaceae
Clementine Orange	<i>Citrus</i> × <i>clementina</i> Mandarin orange x sweet orange	Rutaceae
Grapefruit	<i>Citrus</i> × <i>paradisi</i> Macfadyen	Rutaceae
Lemon	<i>Citrus limon</i> (Linnaeus) Osbeck	Rutaceae
Lime	Hybrid of <i>Citrus</i> spp.	Rutaceae
Key Lime	<i>Citrus aurantifolia</i> Swingle	Rutaceae
Fuzzy Kiwifruit	<i>Actinidia deliciosa</i> (Auguste Chevalier) Chou-Fen Liang & Allan Ross Ferguson	Actinidiaceae
Smooth Skin Kiwifruit	<i>Actinidia chinensis</i> (Golden)	Actinidiaceae

<sup>a</sup>Includes Tommy Atkins, Kent, and Palmer varieties.

<sup>b</sup>Used in laboratory tests.

A caveat to results from tropical and subtropical fruit tests in the field and in the laboratory tests of temperate, tropical, and subtropical fruit described in the next section of this paper is that the fruit used were obtained from markets rather than from the field. Thus, there is a possibility insecticides in fruit killed the larvae and, therefore, no infestation was detected. However, in laboratory studies, non-organic *M. indica* and *M. domestica* exposed to *R. pomonella* adults consistently produce larvae (Yee and Goughnour 2017; W.L.Y., unpublished). Thus, levels of any insecticides in market fruit must have been at sufficiently low levels as to be harmless to at least some larvae. Also, no insecticide-based method is accepted for disinfecting *M. domestica* of *R. pomonella* larvae for export; only cold treatment is accepted as a method for killing larvae (Canadian Food Inspection Agency 2017). Thus, it is unlikely any residues would have killed larvae. Finally, there is a possibility that adult flies were prevented from ovipositing into market fruit due to insecticide residues on the fruit surfaces. However, the fact that larvae were produced from market fruit (see results) and that no research has shown insecticide residues on market fruit are effective oviposition deterrents reduce this possibility. Nevertheless, the use of market fruit needs to be kept in mind when interpreting results.

**Laboratory tests.** Laboratory tests of infestation of temperate fruit by *R. pomonella* were conducted in March to May 2017. Fruit were obtained from markets in Vancouver, Washington. Test flies were 14 d post-emergence in age, and were reared from larvae collected from naturally infested *M. domestica* in 2016 in southwestern Washington. Six types of temperate fruit, including *M. domestica* (Gala variety) as a positive control and three types of peaches (Table 1; a superscripts), were exposed to two to five females and three to five males per 1.9-litre (16.2 cm inner diameter x 10.5 cm inner height) paper container. One individual fruit or two fruits (*Vitis vinifera*) were exposed to flies at any one time inside a container with dry food (80% sucrose:20% yeast extract, wt:wt) on a paper strip and water at 22–25 °C, 40–50% relative humidity, and 16:8 L:D. Two or three successive one- or two-fruit exposures took place, each for 2 weeks. Fruit were removed after a 2-week period and held for larval emergence. Tests were replicated 10 or 15 times for each fruit type.

Laboratory tests of infestation of tropical and subtropical fruit were similarly conducted from December 2016 to June 2017. All fruit were obtained from markets in Vancouver or Yakima, Washington. Nine types of fruit including apple (Table 2; b superscripts) were exposed to flies following methods described for temperate fruit in tests above. There were three to 17 replicates per fruit type.

**Statistics.** Frequencies of *C. douglasii* and feral *M. domestica* trees infested with *R. pomonella* in central Washington surveys within fly-managed and unmanaged sites were compared using a test of two proportions (Zar 1999). Within fly-managed and unmanaged site categories, the total number of infested *C. douglasii* or *M. domestica* trees were divided by the total number of *C. douglasii* or *M. domestica* trees sampled. The frequencies for the two tree species within fly-managed and unmanaged tree categories were compared. Within tree species, the frequencies of trees infested in fly-managed versus unmanaged sites were similarly calculated. In addition, to summarize data and provide a descriptive measure of

variability (rather than raw data from each site per year), mean frequencies of infestation  $\pm$  SE were calculated. Frequencies of infested trees across years within a site were averaged, and means of the frequencies across sites – with each site serving as an observation – were generated. For laboratory tests, tests of two or more proportions or Fisher's exact test were conducted to compare percentages of larvae-positive fruit types.

## RESULTS

**Infestation of *C. douglasii* and *M. domestica*, central Washington field surveys.** In central Washington, both *C. douglasii* and *M. domestica* were infested in some sites in all survey years. In the three fly-managed (insecticide-treated) sites, 6.7% of *C. douglasii* and 6.1% of *M. domestica* were infested ( $\chi^2 = 0.01$ ;  $P = 0.90$ ). In fly-managed sites, the mean frequency of *C. douglasii* trees that were infested was numerically greater than the mean frequency of *M. domestica* (Table 3) trees infested, due to one site (Ellensburg) where 40.0% of trees were infested. In the seven unmanaged sites, 54.1% of *C. douglasii* versus 16.3% of *M. domestica* samples were infested ( $\chi^2 = 63.77$ ;  $P < 0.0001$ ) (because fruit from some of the same survey trees were collected in more than one year, numbers of fruit samples were slightly greater than those of trees). In unmanaged sites, the mean frequency of infested *C. douglasii* trees was numerically greater than the mean frequency of infested *M. domestica* trees (Table 3). The frequency of *C. douglasii* samples infested in fly-managed sites was lower than in unmanaged sites ( $\chi^2 = 37.71$ ;  $P < 0.0001$ ), but for *M. domestica*, the difference was not significant ( $P = 0.07$ ).

**Table 3.** Surveys of infestation by *Rhagoletis pomonella* in *Crataegus douglasii* (black hawthorn) and feral *Malus domestica* (apple) trees at three fly-managed (insecticide-treated) and seven fly-unmanaged sites in central Washington State, U.S., 2013–2017.

Host plant	Years sampled	Nos. trees sampled	Nos. fruit	Nos. puparia	Nos. puparia/ fruit	Mean frequencies of trees infested $\pm$ SE
<u>Fly-managed sites (n = 3)</u>						
<i>Crataegus douglasii</i>	2013, 2014	60	51,815	5	0.000096	13.3 $\pm$ 13.3
<i>Malus domestica</i>	2013, 2014	33	3,405	6	0.001762	5.0 $\pm$ 2.9
<u>Fly-unmanaged sites (n = 7)</u>						
<i>Crataegus douglasii</i>	2010, 2012, 2013, 2015, 2017	139 <sup>a</sup>	224,249	5,515	0.024593	36.7 $\pm$ 13.6
<i>Malus domestica</i>	2010, 2012, 2013, 2015	123 <sup>a</sup>	13,668	366	0.026778	10.5 $\pm$ 5.9

<sup>a</sup>Some trees were sampled multiple times across years. Frequencies of infested trees across years within a site were averaged, and means of these frequencies across sites (each site as an observation) were generated.

**Infestation of temperate fruit, southwestern Washington field surveys.** In southwestern Washington, *Malus* x 'Dolgo' (Dolgo crabapple), *Malus* sp. (unknown cultivar; fruiting crabapple), and *C. douglasii* – all known hosts – produced *R. pomonella* puparia (Table 4).

In addition, four *Crataegus* that had never been reported previously as hosts were identified. These were *C. pinnatifida* (Red Sun Chinese hawthorn), *C. laevigata* x *C. mexicana* (Autumn Glory hawthorn), *C. lavalleyi* (Lavalle hawthorn), and *C. x mordensis* (Toba hawthorn), the latter three being hybrids. Adult *R. pomonella* were reared from all four respective hosts: two males and one female; six females; one male and three females; and one female. Larvae-infested fruit were collected on the following dates: *C. pinnatifida* – 9 and 28 September and 2 November 2015; *C. laevigata* x *C. mexicana* – 2 and 28 September 2015; *C. lavalleyi* – 28 September and 2 November 2015; *C. x mordensis* – 2 and 28 September 2015. Mean diameter (mm) and weight (g)  $\pm$  se, respectively, of fresh ripe fruit (n = 20) were: *C. pinnatifida* – 32.3  $\pm$  0.5 and 12.06  $\pm$  0.43; *C. laevigata* x *C. mexicana* – 19.7  $\pm$  0.5 and 3.92  $\pm$  0.28; and *C. lavalleyi* – 16.4  $\pm$  0.4 and 2.81  $\pm$  0.16. Measurements of *C. x mordensis* fruit were not made, but they were ~10 mm in diameter. *Crataegus crus-galli* (cockspur hawthorn) – a known host – was also infested, but *C. phaenopyrum* (Washington hawthorn) – not a known host – was not.

**Table 4.** Infestation of temperate fruit in field surveys by *Rhagoletis pomonella* in southwestern Washington State, U.S., 2015–2018.

Fruit	Site <sup>a</sup>	Fruit collection date	Nos. plants	Nos. fruit	Nos. puparia	Puparia/fruit
<i>Malus domestica</i> (mixed varieties)	H. Farm	July-Sep 2015;	1-12	505	123	0.2436
	Devine	July 2017	4	392	228	0.5816
<i>Malus</i> sp. (Dolgo, others)	Woodland	Aug 2017; Aug 2018	1, 10	5,043	17,352	3.4408
	H.O.	18 Aug, Nov 2015	4	640	104	0.1625
<i>Crataegus douglasii</i>	T.G.	Jul 2015; Aug 2017	1	853	71	0.0832
	Devine		13	4,173	1,716	0.4112
<i>Crataegus pinnatifida</i>	H.O.	Sep, Nov 2015	1	644	7	0.0109
<i>Crataegus laevigata</i> x <i>Crataegus mexicana</i>	H.O.	Sep 2015	1	1,155	32	0.0277
<i>C. mexicana</i> x <i>C. calpodendron</i>	H.O.	Sep, Nov 2015	1	645	69	0.1070
<i>Crataegus x mordensis</i>	H. O.	Sep 2015	1	1,031	8	0.0078
<i>Crataegus crus-galli</i>	H.O., T.G.	Sep, Oct 2015	3	1,711	5	0.0029
<i>Crataegus phaenopyrum</i>	Leverage Park	Aug 2015; Sep 2015	3	1,005	0	0
	Salmon Creek				0	0
<i>Prunus domestica</i> <sup>b</sup>	T.G., H.O., Devine	July, Aug 2015; Aug, Sep 2017	5	1,063	153	0.1439
<i>Prunus cerasifera</i>	T.G.	July 2015	1	356	0	0
<i>Prunus salicina</i>	Cherry Grove	Aug, Sep 2015; Sep 2017	3	285	5	0.0175
<i>Prunus avium</i>	Cherry Grove	July 2017; June 2018	12, 9	6,388	1	0.0002
	Woodland (town)	June 2018	1	150	0	0
	Devine	June 2018	2	498	0	0
<i>Prunus cerasus</i>	Cherry Grove	July 2017	2-9	4,180	0	0
	Devine	June 2018	1	209	0	0
<i>Prunus virginiana</i>	T.G.	Sep 2017	3	743	0	0
<i>Prunus persica</i>	Vancouver Orchard	July, Aug 2017	20	672	0	0
<i>Cydonia oblonga</i> <sup>c</sup>	T.G.	Sep 2015	5	84	0	0
<i>Pyrus pyrifolia</i> <sup>d</sup>	T.G.	Sep, Aug 2015	4	107	0	0
<i>Pyrus communis</i> <sup>e</sup>	T.G.	Sep 2015	5	103	0	0
<i>Cotoneaster</i> sp.	H.O.	Sep 2015	10	2,567	0	0
<i>Sorbus scopulina</i>	Devine	Aug 2017	1	785	7	0.0089
<i>Ribes rubrum</i> <sup>f</sup>	H.O.	July 2017	2	1,841	0	0
<i>Elaeagnus multiflora</i>	T.G.	Sep 2017	5	660	0	0
<i>Lonicera involucrata</i>	Devine	July 2017	8	463	0	0
<i>Vaccinium corymbosum</i>	H. Farm, Upper Field	July 2017	12	10,992	0	0
<i>Diospyros kaki</i>	H.O.	Nov 2015; Sep 2016; Nov 2018	1	113	0	0

<sup>a</sup>Site names, coordinates, and elevations are in the appendix 1.<sup>b</sup>Comprising subsp. *syriaca*, and French Petite and Friar Black varieties of *P. domestica*.<sup>c</sup>European and pineapple quince.<sup>d</sup>Kosui and 20<sup>th</sup> Century.<sup>e</sup>Bartlett, Winter, and Bosc.<sup>f</sup>Red and albino.



*Prunus domestica* was positive for *R. pomonella* puparia (Table 4). Of particular note, *P. domestica* subsp. *syriaca* was found to be infested for the first time on record, and adult flies were reared from it, although fly numbers were not recorded; infested fruit of this subspecies were collected 15 July 2015 and 19 and 30 July 2017. Infestations by *R. pomonella* were also detected in Italian, French Petite, and Friar Black varieties of *P. domestica*, as well as in *P. salicina* (Japanese plum) and *S. scopulina* (western mountain ash) (Table 4). In addition, a sample of Royal Anne *P. avium* (sweet cherry) collected 2 July 2017 was infested, with one adult female *R. pomonella* reared from it – the first record of *P. avium* being a host of the fly in the field in Washington. Black Republican and Bing varieties of *P. avium* in the same grove as Royal Anne *P. avium* did not produce puparia, nor did *P. cerasus* (tart cherry) (Table 4).

**Infestation of tropical and subtropical fruit, southwestern Washington.** Results from tropical and subtropical fruit tests at the three sites were combined, and some fruit varieties are pooled for presentation (Table 5). *Malus domestica* fruit that were hung in trees produced *R. pomonella* puparia, but of the tropical and subtropical fruit, only *M. indica* (red and yellow mangoes) produced puparia. In 2018, all tropical and subtropical fruit from the three sites tested negative – even at Woodland, where 17,287 *R. pomonella* puparia collected from 5,008 *M. domestica* fruit on the ground indicated high fly pressure at this site.

**Table 5.** Infestation of *Malus domestica* and tropical and subtropical fruit by *Rhagoletis pomonella* when hung in *M. domestica* trees at three sites with known populations of the insect in southwestern Washington State, U.S., 2015<sup>a</sup>, 2016, 2017, and 2018.

Fruit	Nos. tested	Nos. puparia	Nos. fruit positive (%)
<i>Malus domestica</i> (as control)	80	74	29 (36.2a)
<i>Mangifera indica</i> (Yellow, ataulfo)	157	9	7 (4.5b)
<i>Mangifera indica</i> (Red) <sup>a</sup>	116	5	2 (1.7b)
<i>Averrhoa carambola</i>	41	0	0
<i>Musa acuminata</i> × <i>balbisiana</i>	55	0	0
<i>Musa acuminata</i> (Red)	23	0	0
<i>Musa acuminata</i> (Cavendish)	189	0	0
<i>Ananas comosus</i>	61	0	0
<i>Ananas comosus</i> (Pink Pineapple)	4	0	0
<i>Passiflora edulis</i>	19	0	0
<i>Annona cherimola</i>	5	0	0
<i>Carica papaya</i>	120	0	0
<i>Pouteria sapota</i>	5	0	0
<i>Acca sellowiana</i>	25	0	0
<i>Psidium guajava</i> (Mexican, Pink, and Thai Guavas)	29	0	0
<i>Cucumis metuliferus</i>	5	0	0
<i>Garcinia mangostana</i>	5	0	0
<i>Persea americana</i>	131	0	0
<i>Hylocereus costaricensis</i>	10	0	0
<i>Hylocereus megalanthus</i>	10	0	0

<i>Citrus × sinensis</i> (Orange)	30	0	0
<i>Citrus × sinensis</i> (Blood Orange)	64	0	0
<i>Citrus × sinensis</i> (Navel Orange)	41	0	0
<i>Citrus × sinensis</i> (Valencia Orange)	52	0	0
<i>Citrus reticulata</i> (Daisy Mandarin)	5	0	0
<i>Citrus reticulata</i> (Mandarin Orange)	66	0	0
<i>Citrus tangerina</i>	5	0	0
Mandarin x sweet orange hybrid (Murcott Orange)	9	0	0
<i>Citrus reticulata</i> x <i>C. sinensis</i>	28	0	0
<i>Citrus × clementina</i>	10	0	0
<i>Citrus × paradisi</i>	28	0	0
<i>Citrus limon</i>	103	0	0
Hybrid of <i>Citrus</i> spp. (Lime)	114	0	0
<i>Citrus aurantifolia</i>	23	0	0
<i>Actinidia deliciosa</i>	30	0	0
<i>Actinidia chinensis</i>	10	0	0

<sup>a</sup>In 2015, 16 *M. indica* were hung. For positive fruit only:  $\chi^2 = 70.46$ ;  $df = 2$ ;  $P < 0.0001$ ; percentages with same letters are not significantly different ( $P > 0.05$ ).

**Laboratory tests.** In the laboratory tests using temperate fruit (Table 6), *M. domestica*, *P. persica* (white peach) and *P. armeniaca* x *P. salicina* (Dapple Dandy pluot) and *V. vinifera* (red globe grape) produced *R. pomonella* puparia. Adult flies were reared from *P. armeniaca* x *P. salicina*, but their precise numbers were not recorded. The percentages of *M. domestica* and *P. armeniaca* x *P. salicina* that produced puparia did not differ, but both were significantly greater than the percentage of *P. persica* that produced puparia (Table 6). One adult female *R. pomonella* was reared from the five puparia from *V. vinifera*.

**Table 6.** Infestation of temperate fruit by *Rhagoletis pomonella* inside 1.9-litre containers in laboratory exposures in Vancouver, Washington State, U.S., 2017.

Fruit	Nos. replicates <sup>a</sup>	Nos. puparia	Nos. fruit tested	Nos. fruit positive (%) <sup>c</sup>
<i>Malus domestica</i>	10	48	30	11 (36.7a)
<i>Prunus persica</i> (White)	10	7	30	2 (6.7b)
<i>Prunus persica</i> (Mexican)	10	0	20	0
<i>Prunus persica</i> (California)	10	0	10	0
<i>Prunus armeniaca</i> x <i>P. salicina</i>	10	42	15	13 (86.7a)
<i>Vitis vinifera</i>	15 <sup>b</sup>	5	85	—

<sup>a</sup>Two to five females and three to five males per replicate container.

<sup>b</sup>Each with two fruit; number of fruit positive not recorded.

<sup>c</sup>For positive fruit only:  $\chi^2 = 12.11$ ;  $df = 2$ ;  $P = 0.002$ ; percentages with same letters are not significantly different ( $P > 0.05$ ).

In laboratory tests using tropical and subtropical fruit (Table 7), *M. domestica* produced *R. pomonella* puparia. Of nine tropical and subtropical fruit, only *M. acuminata* x *balbisiana* (Blue Java banana) produced puparia. However, none of the 11 puparia from *M. acuminata* x *balbisiana* produced adult flies.

**Table 7.** Infestation of tropical and subtropical fruit by *Rhagoletis pomonella* inside 1.9-litre containers in laboratory exposures in Vancouver, Washington State, U.S., 2016–2017.

Fruit	Nos. replicates <sup>a</sup>	Nos. puparia	Nos. fruit tested	Nos. fruit positive (%) <sup>b</sup>
<i>Malus domestica</i>	10	48	30	11 (36.7a)
<i>Averrhoa carambola</i>	17	0	53	0
<i>Musa acuminata</i> x <i>balbisiana</i>	9	11	17	3 (17.6a)
<i>Psidium guajava</i>	15	0	40	0
<i>Carica papaya</i>	13	0	21	0
<i>Annona cherimola</i>	3	0	6	0
<i>Citrus reticulata</i>	10	0	10	0
<i>Citrus</i> x <i>sinensis</i>	5	0	15	0
<i>Garcinia mangostana</i>	5	0	5	0

<sup>a</sup>Two to five females and three to five males per replicate container.

<sup>b</sup>For positive fruit only:  $\chi^2 = 1.88$ ;  $df = 1$ ;  $P = 0.17$ ; percentages with same letters are not significantly different ( $P > 0.05$ ).

## DISCUSSION

Results show that infestation of *C. douglasii* and feral *M. domestica* trees by *R. pomonella* in central Washington is not a rare occurrence, despite the region's dry habitat. In this study, 6.7% of *C. douglasii* and 6.1% of *M. domestica* trees were infested even in fly-managed (insecticide-treated) sites. Results suggest that, while frequencies of *C. douglasii* and *M. domestica* that are infested do not differ in fly-managed sites, *C. douglasii* is infested at a higher frequency in unmanaged sites, possibly due to several reasons. In fly-managed sites, fly populations were low due to control efforts. Also, the dry sagebrush ecosystem and climate is suboptimal for fly survival (Wakie *et al.* 2019), and *C. douglasii* and feral *M. domestica* trees are relatively rare and are often spaced far apart. The combination of disruption of fly populations caused by insecticide sprays, low fly numbers, and widely spaced trees may result in random infestations. In unmanaged sites, flies were not controlled; the ponderosa pine ecosystem with its milder climate is more suitable for fly survival (Wakie *et al.* 2019), resulting in higher fly populations, and more *C. douglasii* and feral *M. domestica* trees were spaced more closely together than in the sagebrush ecosystem. Any inherent preference by *R. pomonella* for *C. douglasii*, as suggested by frequencies of infested trees, would be more detectable under these conditions.

If the frequency of infested *C. douglasii* and feral *M. domestica* is a function of how many *R. pomonella* can survive in a region, then population increases in central Washington could result in more infested trees. *Rhagoletis pomonella* captures on WSDA survey traps from 2006 to 2017 increased 3–4 times. In 2006 and 2007, 0.0042 and 0.0029 flies/trap (4,260 and 4,482 traps), respectively, were caught. In 2014, 0.0122 flies/trap (4,673 traps) were caught (Yee *et al.* 2012; Klaus 2014). In 2017, 0.0384 flies/trap (5,420 traps) were caught (Klaus 2017). In previous *C. douglasii* and feral *M. domestica* surveys conducted in July, August, and September 2004–2006 at four or five sites (fly-managed and unmanaged, combined) in central Washington, means of 7.7% of *C. douglasii* trees and 0.2% of *M. domestica* trees were infested (based on mean frequency per site) (Yee 2008). The higher frequencies of infestation in the current study may be due in part to increased *R. pomonella* populations in central Washington in the years since the 2004–2006 surveys.

In southwestern Washington, most *Crataegus* and *Prunus* species appear to be suitable 'natural' hosts for *R. pomonella*. Specifically, *C. pinnatifida*, *C. laevigata* x *C. mexicana*, *C. lavalleyi*, *C. x mordensis*,

and *P. domestica* subsp. *syriaca* are newly recorded ‘natural’ hosts of *R. pomonella*. In addition, *P. avium* and *P. salicina*, previously recorded as hosts (Yee and Norrbom 2017), were also infested. In the current study, these natural hosts are distinguished from “unconfirmed” hosts in which fruit were not from the field but were infested in the laboratory. According to the International Standards for Phytosanitary Measures 37 (FAO 2016), a natural host is “a plant species or cultivar that has been scientifically found to be infested by the target fruit fly species under natural conditions and able to sustain its development to viable adults.”

The four newly recorded *Crataegus* hosts have red or orange fruit that ripen in September to October, similar to *Crataegus monogyna* Jacquin (English hawthorn), a species frequently attacked in late summer and fall (Tracewski *et al.* 1987). Other *Crataegus* species with these physical and phenology traits thus may also be attacked. In addition, the four newly recorded hosts all have in common relatively large fruit (compared with *C. douglasii*: 10 mm diameter; 0.65 g), an additional trait that may make them attractive to *R. pomonella*.

Seven *Prunus* species in the subgenus *Prunus*, called “plums”, have been recorded as natural hosts for *R. pomonella* across North America (Yee and Norrbom 2017). However, varieties or subspecies of infested plums are rarely mentioned (Yee and Goughnour 2008). For example, unidentified varieties of plum and prunes were listed as *R. pomonella* hosts in Oregon in the mid-1980s (AliNiazee and Brunner 1986). Here, Italian, French Petite, and Friar Black varieties of *P. domestica*, as well as *P. domestica* subsp. *syriaca*, are identified as potentially highly susceptible plums in addition to *P. salicina* – suggesting most forms of plums are susceptible to attack in Washington.

*Prunus avium* (sweet cherry), in the subgenus *Cerasus*, is reported as a natural host of *R. pomonella* in the PNW for the first time (in 2017), despite many years when cherry collections were negative for the fly. Specifically, *R. pomonella* was detected from *P. avium* collections made in July to August over 10 years (2008–2017) neither in Roslyn at sites documented to have *R. pomonella* (W.L.Y., unpublished) nor at other western Washington sites in earlier studies (Yee and Goughnour 2008). This is unlike the situation in Utah, where *P. avium*, as well as *P. mahaleb* L. (mahaleb cherry) and *P. cerasus* (tart cherry), were reported hosts for *R. pomonella* in 1985–1986 surveys (Allred and Jorgensen 1993). In addition, *P. cerasus* was reported as a host for the fly in Wisconsin (Shervis *et al.* 1970). This, combined with the fact that plums in the PNW were recorded as hosts in the mid-1980s (AliNiazee and Brunner 1986), suggests that cherries are less likely than plums to be attacked by *R. pomonella* in the PNW. The positive *P. avium* tree in the Cherry Grove site in Vancouver in 2017 was ~5 m from positive *P. salicina* and ~50 m from infested *M. domestica*, possible sources of the infestation.

In addition to natural *Prunus* hosts, *P. armeniaca* (30%) x *P. salicina* (70%) (both subgenus *Prunus*), known by the common name Dapple Dandy pluot (Anonymous 2019), is a newly recorded host for *R. pomonella* in the laboratory. Both of its parental fruit are natural hosts for *R. pomonella* (Lienk 1970; Yee and Goughnour 2008). Based on its infestation rate relative to that of *M. domestica*, Dapple Dandy pluot appears highly suitable for larvae development. Whether other *P. armeniaca* x *P. salicina* hybrids with different per cent parentages are similarly suitable for *R. pomonella* will require further testing.

Another newly recorded host for *R. pomonella* in the laboratory is *V. vinifera* (Red Globe grape). While *V. vinifera* is a natural host for Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) (e.g., Roditakis *et al.* 2008), there is no record of it being a natural host for *R. pomonella*. Thus, the Red Globe variety of *V. vinifera* was probably attacked because other fruit were absent. In addition, its large size (25–27 mm in diameter) and smooth surface apparently made it acceptable for ovipositing flies.

More field research than is presented in the current work is needed to determine whether tropical or subtropical fruit could be hosts for *R. pomonella* in nature. Future studies include exposing more fruit to flies in the field, as well as using fruit documented to be untreated with insecticides. However, of the 37 tropical and subtropical fruit exposed to *R. pomonella* in the field, only *M. indica* produced puparia, raising the possibility that most of the tested fruit are unsuitable for the fly. The presence of natural apple fruit in trees could have deterred *R. pomonella*’s use of the fruit hung in trees, but because fly populations in test trees were high, at least some flies probably encountered the tropical and subtropical fruit. If so, flies did not oviposit in most of these fruit, eggs did not hatch, or larvae could not complete development in them.

In the laboratory tests of tropical and subtropical fruit, *M. acuminata* x *balbisiana* (Blue Java banana) was noted as a newly recorded host and the only such fruit infested. Unlike in *M. domestica* trees, where

55 *M. acuminata* x *balbisiana* were hung, the lack of alternative fruit in the laboratory may have forced the flies to oviposit in it. The skin of *M. acuminata* x *balbisiana* is ~1 mm thick, versus ~2–3 mm for *M. acuminata* (Cavendish banana): this perhaps allowed easier ovipositor penetration by flies, especially when the fruit were ripe (as for *Bactrocera invadens* Drew, Tsuru & White; Cugala *et al.* 2013).

In conclusion, findings indicate *R. pomonella* consistently infests *C. douglasii* and feral *M. domestica* in central Washington, with *C. douglasii* being more frequently infested in sites where the fly is not managed. Most *Crataegus* and most types of *Prunus* in the subgenus *Prunus* in southwestern Washington may be suitable hosts for *R. pomonella*. Additional assessments are needed to determine the suitability of tropical and subtropical fruit. Despite the need for more work, the current results provide a basis for further research and hypotheses concerning host use by *R. pomonella* and its potential impact on both U.S. and tropical and subtropical fruit markets.

## ACKNOWLEDGMENTS

We thank Dana Jones and Peter Chapman (USDA-ARS) for field assistance, Doug Stienbarger and Justin O’Dea (Washington State University Clark County Extension) for providing space and logistical support to conduct the work, Nicanor Liquido (USDA-APHIS-PPQ-S&T-CPHST, PERAL, Honolulu, HI, U.S.), Bradley Sinclair (Canadian National Collection of Insects and Ottawa Plant Laboratory, Ottawa, Ontario, Canada), and two anonymous referees for reviews that improved the manuscript, and the U.S. Department of Agriculture – Foreign Agricultural Service for partial funding.

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**Appendix 1.** Fly-managed (insecticide-treated) and unmanaged sites in central Washington State, U.S., surveyed for *Rhagoletis pomonella* infestation in *Crataegus douglasii* and feral *Malus domestica*, 2010–2017, and temperate fruit sites in southwestern Washington State, U.S., surveyed for *Rhagoletis pomonella* infestation, 2015–2018.

Site name	Coordinates, elevation	Ecosystem <sup>a</sup>
<u>Fly-managed (insecticide-treated) <i>Crataegus douglasii</i> and feral <i>Malus domestica</i> sites sampled in 2013 and 2014</u>		
Union Gap	46°33'49"N, 120°27'57"W, 301 m	Sagebrush
West Valley	46°32'25"N, 120°49'17"W, 636 m	Bunchgrass
Ellensburg	46°59'05"N, 120°32'27"W, 460 m	Bunchgrass
<u>Unmanaged <i>Crataegus douglasii</i> and feral <i>Malus domestica</i> sites sampled in 2010–2017</u>		
Klickitat Co.	45°56'14"N, 121°07'02"W, 271 m	Ponderosa Pine
Nile	46°49'47"N, 120°56'43" W, 621 m	Ponderosa Pine
Cle Elum	47°11'49"N, 120°55'08"W, 586 m	Ponderosa Pine
Roslyn	47°13'10"N, 120°59'19"W, 669 m	Ponderosa Pine
Goldendale	45°49'31"N, 120°48'54"W, 499 m	Ponderosa Pine
Brooks Memorial Park	45°56'60"N, 120°39'59"W, 797 m	Ponderosa Pine
Wenas	46°50'11"N, 120°43'11"W, 683 m	Bunchgrass
<u>Temperate fruit sites sampled in 2015–2018</u>		
Devine	45°37'56"N, 122°37'05"W, 55 m	Coast Forest
Woodland	45°56'24"N, 122°40'21"W, 52 m	Coast Forest
Woodland (town)	45°54'12"N, 122°44'49"W, 9 m	Coast Forest
Heritage Orchard (H.O.) (0.4 ha plot)	45°40'38"N, 122°39'04"W, 73 m	Coast Forest
Terrace Garden (T.G.)	45°40'31"N, 122°38'59"W, 82 m	Coast Forest
Leverage Park	45°39'03"N, 122°39'27"W, 41 m	Coast Forest
Salmon Creek	45°42'44"N, 122°40'41"W, 6 m	Coast Forest
Cherry Grove	45°40'33"N, 122°38'54"W, 75 m	Coast Forest
Vancouver Orchard	45°38'05"N, 122°33'18"W, 95 m	Coast Forest
H.O., Upper Field	45°40'29"N, 122°38'47"W, 95 m	Coast Forest

<sup>a</sup>Ecosystem classification based on Lyons and Merilees (1995).