SCIENTIFIC NOTE

First reported infestation of a native honeysuckle (Caprifoliaceae) by a native *Rhagoletis* fly (Diptera: Tephritidae) in North America

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Sympatric host plant shifts often have been hypothesised as a critical factor initiating population divergence and speciation in specialised frugivorous Diptera in the genus *Rhagoletis* (Tephritidae) (*e.g.,* Bush 1992; Feder *et al*. 2003). The apple maggot fly, *Rhagoletis pomonella* (Walsh), is a model organism for host race formation via host plant shifts, but its sibling species *Rhagoletis zephyria* Snow has not been implicated as having shifted host plants since its evolution from *R. pomonella* ancestors (Bush 1966; Feder *et al.* 1999). Thus far, *R. zephyria* has been reared only from snowberry, *Symphoricarpos* spp. (Caprifoliaceae), specifically *S. albus* var. *laevigatus* (Fernald) Sidney Fay Blake, *S. albus* var. *albus* (Linnaeus) Sidney Fay Blake, and *S. occidentalis* Hooker (Bush 1966; Gavrilovic *et al.* 2007; Yee and Goughnour 2008), and from introduced Japanese honeysuckle *Lonicera japonica* Thunberg (Caprifoliaceae) in Washington state, U.S.A. (Yee and Goughnour 2008). *Rhagoletis zephyria* populations native to the Pacific Northwest of the U.S.A. attack native *S. albus* var. *laevigatus* (Yee and Goughnour 2008) but are not known to attack native honeysuckles.

During 2021 and 2022 surveys to determine the presence of *Rhagoletis* flies and their parasitoids in various fruit in Washington, berries of native orange honeysuckle, *Lonicera ciliosa* (Pursh) Poiret ex de Candolle (Caprifoliaceae), and snowberries were collected at three sympatric sites in three counties (Table 1): (1) Kittitas County, at Liberty Campground, located in the Interior Douglas-fir ecozone; (2) Lewis County, at the Bevin Lake Rest Area, located in the Interior Redcedar ecozone; and (3) Whatcom County, at Lake Padden, located in the Puget Sound Douglas-fir ecozone (Anonymous 1996). At all sites, honeysuckle and snowberry grew amongst one another or were intertwined, with honeysuckle growing as vines above the snowberry.

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Table 1. Natural infestations of orange honeysuckle (*Lonicera ciliosa*) and snowberry (*Symphoricarpos albus* var. *laevigatus*) by *Rhagoletis zephyria* at sympatric sites in Washington state, U.S.A., in 2021 and 2022.

			Orange Honeysuckle		Snowberry	
Site	**Coordinates; elevation**	**Date collected**	**No. fruit[d]**	**No. puparia**	**No. fruit**	**No. puparia**
Liberty Campground[a]	47.24900, –120.68537; 807 m	19 September 2021	82	2[e]	366	56
Liberty Campground[a]	Same as above	26, 27 September 2022	260	0	829	62
Bevin Lake Rest Area[b]	46.55290, –121.73675; 301 m	28 September 2022	1228	62[f]	685	27
Lake Padden[c]	48.70591, –122.44848; 151 m	2 October 2022	123	0	234	22

[a]Cle Elum, Kittitas County; [b]Packwood, Lewis County; [c]Bellingham, Whatcom County; [d]Each leaf disc had five or six fruit; [e]Both produced morphologically identified adult female *R. zephyria* (Bush 1966; Yee *et al.* 2009); [f]12 representative puparia identified as *R. zephyria* based on polymerase chain reactions method to distinguish *R. zephyria* from *R. pomonella* (Smith *et al.* 2022).

In both years, orange honeysuckle berries and snowberries were brought to, and held in, the laboratory at 20–23 °C with 30–40% relative humidity. Berries were laid on hardware cloth in rubber tubs for larvae to emerge and pupariate. To determine infestation rates by fly larvae, numbers of puparia and of honeysuckle and snowberry berries were counted. In 2021, puparia were collected and stored in moist sand inside plastic cups held at ~20 °C for 10 days, chilled at ~3 °C for ~6 months, and then transferred to ~23 °C for adult fly eclosion. Flies that eclosed were identified morphologically using wing band pattern, wing shape, and aculeus length (Bush 1966; Yee *et al.* 2009). In 2022, 12 representative puparia from honeysuckle from the Bevin Lake Rest Area site were tested using a conventional polymerase chain reaction (PCR) method designed to distinguish between *R. zephyria* and *R. pomonella* using a partial ribosomal nontranscribed spacer (NTS) region (Smith *et al.* 2022). The NTS PCR amplicons obtained from honeysuckle puparia were compared with those of Washington-origin *R. pomonella* and hybrid *R. pomonella* × *R. zephyria* puparia that were generated in the laboratory in 2019 (methods in Yee and Goughnour 2011) and frozen at –80 °C until analysis in 2022. Orange honeysuckle was identified morphologically based on a combination of habit, stem, leaf, and fruit characters in Lyons and Merilees (1995) and Bell and Dempster (2012).

Laboratory rearing indicated *R. zephyria* infested both orange honeysuckle and snowberries at sympatric sites in 2021 and 2022 (Table 1). Honeysuckle was positive for puparia at the Liberty Campground and Bevin Lake Rest Area sites, whereas snowberry was positive at all three sites. From the 2021 Liberty Campground collection, two adult female flies eclosed from the orange honeysuckle collection and were identified as *R. zephyria* (Figure 1), as were flies that eclosed from the snowberry collection. All 12 honeysuckle pupae from

the Bevin Lake Rest Area site in 2022 possessed the base pair (bp) fragment diagnostic for *R. zephyria*, whereas hybrids generated in the laboratory to serve as controls possessed both the *R. zephyria* and unique bp fragment diagnostic for *R. pomonella* (Figure 2). Voucher fly and plant specimens are maintained at Washington State Department of Agriculture, Yakima, Washington.

Figure 1. Dorsal (top) and lateral (bottom) view of a female *Rhagoletis zephyria* reared from *Lonicera ciliosa* fruit collected at Liberty Campground near Liberty, Washington, U.S.A. on 19 September 2021.

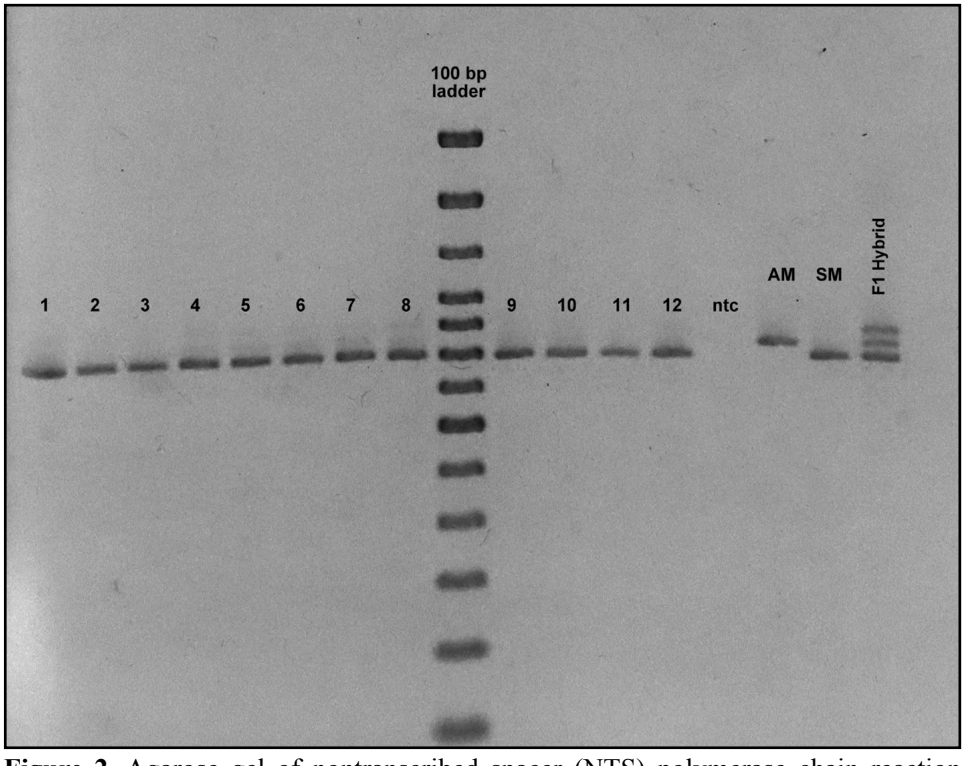

Figure 2. Agarose gel of nontranscribed spacer (NTS) polymerase chain reaction (PCR) products of puparia collected from honeysuckle (*Lonicera ciliosa*; samples 1–12), non-template control (ntc), *Rhagoletis zephyria* (SM, 790 bp), apple maggot (*R. pomonella*; AM, 840 bp), and *R. zephyria* x *R. pomonella* hybrid puparia (F_1 hybrid). Agarose gel (1.5%) run for 2 hours at 155 V. Stained with EtBr.

Our finding is the first reported infestation of a native honeysuckle by a native *Rhagoletis* fly in North America. Orange honeysuckle is native to western North American forests from northern California northwards to British Columbia in Canada (Washington Native Plant Society 2022). In eastern North America, invasive honeysuckles from Japan, Korea, China, and/or central Asia (*Lonicera morrowii* Asa Gray and hybrids *Lonicera* × *bella* [*L. morrowii* × *L. tatarica* Linnaeus] and *Lonicera* × *amoena* [*L. tatarica* × *Lonicera korolkowii* Stapf]) are attacked by *R. zephyria* that hybridised with the blueberry maggot *Rhagoletis mendax* Curran (a sibling species of *R. pomonella*) and called the '*Lonicera* fly' (Schwarz *et al.* 2005).

Here, we show that the fly attacking orange honeysuckle is genetically not a hybrid of *R. zephyria* and *R. pomonella* based on PCR amplification of the NTS region (Smith *et al.* 2022). In Europe, *Lonicera xylosteum* Linnaeus has long been known to be a development host of European cherry fruit fly *Rhagoletis cerasi* (Linnaeus) (Boller *et al.* 1998). Also, two sister taxa related to *R. cerasi* in central Asia, *Rhagoletis flavicincta* Enderlein and *Rhagoletis almatensis* Rohdendorf, both attack the honeysuckle *L. stenantha* Pojarkova (Hulbert 2018). Thus, honeysuckle is used as a host by a clade of flies near the base of the genus *Rhagoletis* (Hulbert 2018). Consequently, there is a past history and perhaps a degree of associated ancestral standing variation in *Rhagoletis* for using the plant

as a host. Moreover, as snowberry is a member of the honeysuckle family (Caprifoliaceae), it may be expected that there is a large degree of similarity in host fruit volatiles, nutrition, and secondary compounds between the two plants, furthering the potential for a host shift.

Whether our finding in Washington state represents a host shift by *R. zephyria* onto orange honeysuckle or incidental infestations due to deteriorating snowberry condition in September when honeysuckle fruit is optimal for fly attack remains to be determined. Across sites, snowberry was more heavily infested than orange honeysuckle, suggesting incidental infestations occurred, but more data are needed to verify this supposition. However, infestation of honeysuckle at two disparate sites over two seasons suggest these may not be isolated events. If incidental infestations occur frequently enough, a form of *R. zephyria* that shifts onto and specialises on orange honeysuckle could evolve. Also, *Rhagoletis* had not been reared from native *Lonicera* previously (Yee and Goughnour 2008), consistent with a recent host shift. To address this possibility further, the fruiting phenology of orange honeysuckle *versus* snowberry, which appeared to be earlier for snowberry at the Bevin Lake Rest Area site based on relative fruit condition, and the presence of *R. zephyria* infestations in honeysuckle in the absence of snowberries need to be determined.

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