

A putative model of the infection cycle of plum pox virus M strain in Japanese apricot orchards via Asteraceae as an intermediate host.

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Short Report

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Abstract

Plum pox virus (PPV) is one of the most economically important plant viruses. PPV M strain (PPV-M) was first isolated in Japan in 2016. The infection cycle of PPV by vector aphids remains unknown in Japan. Here, we present a seasonal model describing the infection cycle of PPV-M in Japanese apricot orchards. First, by mechanically inoculating twelve herbaceous species belonging to Asteraceae (alternatively Compositae) in orchards with PPV-M, we found that all were susceptible to infection. Of those, *Hemisteptia lyrata* showed high infection rates on the upper leaves. Second, a subsequent survey of multiple aphid species parasitizing *H. lyrata* showed a seasonal infection cycle in which *A. fabae solanella* likely transmitted PPV-M from Japanese apricot leaves to *H. lyrata* in the spring. Then, *A. spiraecola*, an efficient PPV vector known to shift between hosts in summer and autumn, transmitted the disease to healthy Japanese apricot seedlings from PPV-M infected *H. lyrata* in autumn. We propose that PPV-M in Japanese apricot follows a seasonal and multimodal infection cycle involving an intermediate host (Asteraceae) and two aphid species.

Fulltext

Plum pox virus (PPV) belongs to the genus *Potyvirus* in the family *Potyviridae* and is one of the most significant diseases affecting *Prunus* species around the world. In Japan, PPV was first discovered in Japanese apricot (*Prunus mume* Sieb. et Zucc) orchard in Ome City, Tokyo, in April 2009 (Maejima et al., 2010). Subsequent nationwide surveys confirmed PPV-infected Japanese apricot trees in several prefectures. There are nine recognized PPV strains globally: D, M, Rec, EA, W, T, C, CR, and An. PPV-D and PPV-M are the most widespread and economically important PPV strains affecting fruit production in Japan (Maejima et al., 2010; Oishi et al., 2018). To prevent the spread of PPV, it is important to determine and control the infection cycle of the virus within the cultivating areas of Japan.

On infected trees, PPV causes yellow ring spots on leaves and fruits, which greatly impairs economic production. To date, PPV susceptibility of economically significant *Prunus sp.*, as well as weed species that are assumed to be involved in the spread of infection (Llacer, 2006; Morvan & Chastelliere, 1981), have been studied by mechanical inoculation tests. These studies suggest that approximately 28% of weed species that are susceptible to PPV by mechanical inoculation belong to the Asteraceae (alternatively Compositae) family (Llacer, 2006; Milusheva & Rankova, 2002; Morvan & Chastelliere, 1981; Viröček et al., 2004). Additionally, weed species surveys in peach orchards heavily infected with PPV in the Niagara Region of Ontario, Canada, suggest that Asteraceae is a significant reservoir of PPV (Stobbs et al., 2005).

PPV is transmitted by aphids in a non-persistent manner in nature. More than 20 aphid species were reported to be able to transmit PPV (Gildow et al., 2004; Isac et al., 1998; Kunze & Krczal, 1971; Labonne et al., 1995; Levy et al., 2000; Manachini et al., 2004; Németh, 1986). However, PPV transmissibility between hosts differs between aphid species. For example, the PPV transmission rate for *Hyalopecterus pruni* can be relatively low, while *Myzus persicae* can be relatively high depending on environmental and

host-associated factors (Isac et al., 1998; Labonne et al., 1995; Llacer et al., 1992; Basky et al., 1997). PPV can also be transmitted by aphids via an intermediate host. For example, Oosten et al. (1970) showed that *M. persicae* transmitted PPV from peach seedlings to *Lamium amplexicaule* and vice versa in controlled greenhouse experiments. However, it remains to be determined which intermediate host plants are susceptible to PPV and which aphid species transmit PPV between them and *Prunus sp.* in field settings in Japanese apricot orchards.

To search for potential species of the Asteraceae family that could be important reservoirs of infection to and from Japanese apricot in domestic orchards, we first mechanically inoculated twelve species of Asteraceae native to orchards where PPV occurred with sap from the host plant *Nicotiana benthamiana* infected with PPV-M isolated in Japan (Oishi et al., 2018). Seeds collected from orchards for the twelve Asteraceae species were initially sown in pots in a greenhouse and mechanically inoculated when true leaves emerged. Two to four weeks after inoculation, RT-PCR was performed on the upper and lower (inoculated) leaves of the plants with a primer set for the 3' UTR region (Forward:5'-GTAGTGGTCTCGGTATCTATCATA-3', Reverse:5'-GTCTCTTGACACAAGAACTATAACC-3') of PPV-M (GenBank accession No. LC494687) using a PrimeScript One Step RT-PCR Kit Version 2 (TaKaRa Bio, Kusatsu, Japan) to confirm infection. All Asteraceae species inoculated with PPV in this study were susceptible to PPV and exhibited symptomless infections (Table 1). Since it is difficult to visually confirm asymptomatically infected plants in nature, these plants could be potential undetected reservoirs of PPV in orchards. We sampled the lower (inoculated) leaves of the 12 Asteraceae species twenty times. The RT-PCR results showed infection rates ranging from 15% to 90% in the inoculated leaves (Table 1). Of those, *Bidens pilosa*, *Carpesium divaricatum*, *Hemisteptia lyrata*, and *Senecio vulgaris* showed PPV-M infection rates of more than 75%. Two weeks later, we sampled the upper leaves of all PPV-M positive plants. Two species showed PPV-M infection rates of more than 75% in the upper leaves, *H. lyrata* and *S. vulgaris*. Infection rates in the upper leaves of *B. pilosa* (27%) and *C. divaricatum* (47%) were lower than those of *H. lyrata* or *S. vulgaris* (Table 1) and peak aphid parasitism was also seasonally different, as described later in Fig. 1. *B. pilosa* and *C. divaricatum* produce flower buds in autumn, compared to *H. lyrata* or *S. vulgaris* which bud in the spring (Makino, 1982). The formation of flower buds by reproductive growth through autumn could promote new parasitizing by aphids, which favor soft, young plant tissue (Harris & Maramorosh, 1977). *A. spiraecola* is one of the most effective PPV vectors and is known to undergo host shifting between trees and weeds in autumn (Gildow et al., 2004; Moritsu, 1983). Therefore, *B. pilosa* and *C. divaricatum* hosting *A. spiraecola* may be involved in PPV infection cycle in autumn, despite lower infection rates in the upper leaves of the host. As a result of their potential involvement in the PPV lifecycle, *H. lyrata*, *S. vulgaris*, *B. pilosa*, and *C. divaricatum* were selected for following surveys.

To investigate aphid parasitism from early spring to autumn under field conditions, we placed planters containing one of each intermediate host species (*H. lyrata*, *S. vulgaris*, *C. divaricatum* and *B. pilosa*) into five separate open fields where *Prunus* trees were planted. Every two weeks, parasitizing aphids were captured from the planters and counted. Captured aphids were identified based on the nucleotide sequence of the mitochondrial cytochrome C oxidase 1 gene region using PCR (Folmer et al., 1994). From

our survey, potential PPV vectors were confirmed by comparing identified species to previously reported species known to carry PPV (Gildow et al., 2004; Isac et al., 1998; Kunze & Krczal, 1971; Labonne et al., 1995; Levy et al., 2000; Manachini et al., 2004;

Németh, 1986). We ultimately identified three species of aphids parasitizing the four Asteraceae species in this survey (Fig. 1).

Aphis fabae solanella was first observed on *H. lyrata* in late March. Colonies of this species had expanded to over 40 individuals per plant by mid-April. *A. spiraeicola* was also observed in *H. lyrata* from mid-April to early May, with peak abundance corresponding with *A. fabae solanella*. *M. persicae* and *A. fabae solanella* were long-term parasites persisting from March to May in *S. vulgaris* and *H. lyrata*, respectively (Fig. 1). *M. persicae* and *A. spiraeicola*, are known to be efficient PPV vectors (Gildow et al., 2004). In a previous survey of aphid outbreaks by Kimura et al. (2016) covering a similar area using insect-trapping adhesive plates, *A. fabae solanella* was not captured at all. However, in this study, Asteraceae host plants were used to capture aphids instead of adhesive plates. Therefore, the detection of *A. fabae solanella* may be a result of differences in the capture methods used in this study compared to the previous study (Kimura et al., 2016). Given that peak parasitism in the spring is driven by *A. fabae solanella* and *A. spiraeicola* parasitizing *H. lyrata*, and *M. persicae* parasitizing *S. vulgaris*, from newly germinating symptomatic leaves of Japanese apricot (*P. mume*), we conclude that PPV infected apricot leaves were the probable source of acquisition in the previous year.

In autumn, peak parasitism for *A. spiraeicola* was observed in *C. divaricatum* and *B. pilosa* from mid-September to early October (Fig. 1). In nature, we expect that the two plant species would act as a poor reservoir of PPV because of the low infection rate in upper leaves (Table 1). Rather, *C. divaricatum* and *B. pilosa* are likely parasitic hosts for *A. spiraeicola* in autumn, improving aphid over-wintering ability. When *A. spiraeicola* undergoes host shifting between trees and weeds in autumn, it is hypothesized that alate aphids acquire PPV from *H. lyrata* or *S. vulgaris* could land on healthy apricot trees and transmit the virus. Therefore, *A. spiraeicola* may be a possible PPV vector from infected *H. lyrata* and/or *S. vulgaris* to Japanese apricot trees.

To demonstrate which aphid species transmit PPV to *H. lyrata* and/or *S. vulgaris* from spring to summer, we conducted a transmission test using three species of aphids that were found to be established on the intermediate host plants by field surveys. Three aphid species (*A. fabae solanella*, *A. spiraeicola* and *M. persicae*) were captured alive from the planters used in the field surveys and maintained on their respective host plants in a greenhouse or incubator continually.

First, aphids were transferred to a plastic case using a thin paintbrush and fasted at 4°C for 2 hours. Thirty fasted aphids were placed on the leaves of the PPV-M-infected *P. mume* and allowed to feed for 5 min to acquire PPV. Aphids were then transferred to a healthy plant (*H. lyrata* or *S. vulgaris*) and allowed to feed overnight. After aphids were excluded by spraying insecticide, inoculated plants were moved to the greenhouse. Four weeks later, the plants were tested for infection by RT-PCR using the aforementioned primer set of the 3'UTR region.

The results showed that *A. fabae solanella* infected 71% (10/14) of *H. lyrata* host plants (Table 2). This transmission rate was similar to that obtained by mechanical inoculation. Since *A. fabae solanella* does not colonize *Prunus* sp., unlike *A. spiraecola* and *M. persicae* (Jörg & Lampel, 1996), *A. fabae solanella* temporarily lands on *Prunus* sp. and could acquire PPV by short-term feeding period. Inoculations to *H. lyrata* by *A. spiraecola* and *S. vulgaris* by *M. persicae* were unsuccessful (Table 2). We propose that the vector that transmits PPV to *H. lyrata* in spring could be *A. fabae solanella* in a non-persistent manner.

To confirm this hypothesis, we conducted a transmission test from PPV-M-infected *H. lyrata* to *P. mume* seedlings using *A. spiraecola* as the vector. *M. persica* host plants were used as a positive control for the experiment. The results showed that *A. spiraecola* infected 78% (11/14) of *P. mume* seedlings with PPV (Table 3). When *A. spiraecola* undergoes host shifting between trees and weeds in autumn, it is hypothesized that alate aphids that acquired PPV from weeds could land on healthy *P. mume* trees and transmit the virus in a nonpersistent manner. Previous studies have indicated that herbaceous plants are unlikely reservoirs for the virus because of low infection rates and strong selection for PPV strains adapted to weeds that do not infect their original woody hosts (Calvo et al., 2014; Carbonell et al., 2013; Salvador et al., 2008). However, our results show that PPV-infected *H. lyrata* maintained infectivity to woody host seedlings for several months.

In the study, we estimated the seasonal infection cycle of PPV-M using Asteraceae herbaceous plants as intermediate hosts under field conditions. We propose a model in which *A. fabae solanella* likely overwinters on two host plants in nature, *Cirsium* spp. and *S. nigrum* (Jörg & Lampel, 1996), and relocates to and initially acquires the virus from PPV-infected *P. mume* leaves in the spring, as shown in Fig. 2. Then, PPV-positive aphids fly to *H. lyrata* and form colonies. At that time, the aphids transmit PPV to *H. lyrata*, suggesting that *H. lyrata* maintains PPV infections throughout the summer and serves as a reservoir for the virus. In this study, we confirmed that *H. lyrata* can act as a potential reservoir, but it remains to be confirmed in real-world scenarios. In conclusion, we propose alate *A. spiraecola* relocating from *C. divaricatum* and *B. pilosa* transmit PPV to healthy *P. mume* trees in autumn in a nonpersistent manner via PPV positive *H. lyrata* as an intermediate host (Fig. 2). Therefore, future surveys for PPV-infected weeds in Japanese apricot orchards should focus on *H. lyrata* as a potential PPV reservoir.

Declarations

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Author Contributions

S.T. is the principal researcher of this study. S.T., S.I., M.O. and T.N. designed the work. S.I. and M.O. performed the assays and analyzed the results. S.I. wrote the first version of the manuscript. S.T., M.O.

and T.N. contributed to the manuscript writing, editing and critical reading. All authors have read and agreed to the published version of the manuscript.

Compliance with Ethical Standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1. Infection rates of inoculated and upper leaves of Asteraceae (alternatively Compositae) herbaceous species inoculated by plum pox virus M strain isolated in Japan.

Plant species	No. of infected plants/No. of inoculated plants (% transmission)	
	inoculated leaves	upper leaves
<i>Artemisia indica</i> var. <i>maximowiczii</i>	11/20 (55)	4/11 (36)
<i>Aster microcephalus</i> var. <i>ovatus</i>	/20 (20)	0/4 (0)
<i>Aster scaber</i>	3/20 (15)	1/3 (33)
<i>Bidens frondosa</i>	4/20 (20)	nt
<i>Bidens pilosa</i>	15/20 (75)	4/15 (27)
<i>Carpesium divaricatum</i>	15/20 (75)	7/15 (47)
<i>Carpesium triste</i>	11/20 (55)	8/11 (73)
<i>Crassocephalum crepidioides</i>	9/20 (45)	0/9 (0)
<i>Galinsoga quadriradiata</i>	9/20 (45)	1/9 (11)
<i>Hemisteptia lyrata</i>	18/20 (90)	14/18 (78)
<i>Senecio vulgaris</i>	16/20 (80)	12/16 (75)
<i>Youngia japonica</i>	14/20 (70)	8/14 (57)

Inoculated leaves No. of infected plants /No. of inoculated plants

Upper leaves No. of positive plants by RT-PCR /No. of infected plants

nt not tested

Table 2. Plum pox virus M strain transmission rate by aphids colonized on Asteraceae herbaceous.

Source plants	Aphid species	Inoculated plants	No. of infected plants */No. of inoculated plants (% transmission)		
			Expt.1	Expt.2	mean (%)
<i>Prunus mume</i>	<i>Aphis fabae solanella</i>	<i>Hemisteptia lyrata</i>	6/8 (75)	4/6 (66)	71
	<i>Aphis spiraecola</i>	<i>Hemisteptia lyrata</i>	0/8 (0)	nt	0
	<i>Myzus persica</i>	<i>Senecio vulgaris</i>	0/8 (0)	nt	0

nt: not tested.

* Tested by RT-PCR

Table 3. Plum pox virus M strain transmission rate by aphids to *Prunus mume* from infected *Hemisteptia lyrata*

Source plants	Aphid species	Inoculated plants	No. of infected plants */No. of inoculated plants (% transmission)		
			Expt.1	Expt.2	mean (%)
<i>Hemisteptia lyrata</i>	<i>Aphis spiraecola</i>	<i>Prunus mume</i>	5/8 (63)	6/6 (100)	78
	<i>Myzus persica</i>		4/4 (100)	nt	100

nt: not tested.

* Tested by RT-PCR

Figures

Plant species	Aphid species	Month																			
		Spring										Summer								Autumn	
		Mar.	Apr.			May.			Jun.			Jul.			Aug.		Sep.	Oct.			
<i>Hemisteptia lyrata</i>	<i>Aphis fabae solanella</i>																				
	<i>Aphis spiraecola</i>																				
<i>Senecio vulgaris</i>	<i>Aphis fabae solanella</i>																				
	<i>Myzus persicae</i>																				
<i>Carpesium divaricatum</i>	<i>Aphis spiraecola</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns										
<i>Bidens pilosa</i>	<i>Aphis spiraecola</i>	ns	ns																		

Number of PPV vector aphid colonized Asteraceae herbaceous plants

■ : over 20 aphids,
 ■ : 10~20 aphids,
 □ : ~10 aphids,
 □ : 0

ns : not surveyed

