Raspberry viruses affect the behavior and performance of Amphorophora agathonica in single and mixed infections

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Raspberry viruses affect the behavior and performance of *Amphorophora agathonica* in single and mixed infections

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Abstract

Pathogens may alter their hosts, which consequently increases transmission efficiency by vectors. We examined the effects of *Raspberry leaf mottle virus* [RLMV; *Closterovirus* (Closteroviridae)] and *Raspberry latent virus* [RpLV; *Reovirus* (Reoviridae)], alone and in a co-infection in raspberry, *Rubus idaeus* L. (Rosaceae) cv. Meeker, on the behavior and performance of its vector, *Amphorophora agathonica* Hottes (Hemiptera: Aphididae). Longevity was increased in aphids feeding on all infected-plant treatments compared with healthy plants, but aphid fecundity only increased in the co-infection treatment. In a two-way choice study between infected and healthy plants, aphids showed no difference in preference between plants after 30 min of exposure. After 24 h, aphids significantly preferred to settle on plants infected with RLMV over healthy, but healthy plants over plants infected with RpLV. There were no differences in settling preferences between healthy and co-infected plants. An electrical penetration graph study showed no differences in aphid feeding behavior on plants infected with RLMV and RLMV+RpLV when compared with healthy controls. Our results are consistent with past findings that infected plant's impact vector performance and behavior, but also highlight the need to further investigate greater virus diversity and effects of mixed infections.

Introduction

Vector-borne pathogens have a close relationship with their vector, which is their primary means for transmission to a new host. Thus, it follows that pathogens may alter the host in ways that increase transmission efficiency (Thomas et al., 2005). In plant-pathogen systems, changes may occur through manipulation of the plant (host manipulation) or through manipulation of the vector (vector manipulation). Vector manipulation has been demonstrated in *Rhopalosiphum padi* L., where aphids infected in vitro with *Barley yellow dwarf virus* were subsequently more attracted to healthy wheat plants over infected plants (Ingwell et al., 2012). Host manipulation, also referred to as adaptive manipulation (Poulin, 2000) or behavioral manipulation (Thomas et al., 2005), focuses on how infected plants influence the vector performance (e.g., fecundity or longevity) or vector behavior (e.g., initial attraction and settling preferences).

Mauck et al. (2012) found that changes in vector behavior or performance were related to the mode of virus transmission. Non-persistently transmitted viruses are rapidly acquired from a plant and extended periods of feeding on an infected plant are often associated with decreased rates of virus inoculation (Wang & Ghabrial, 2002). Plants infected with non-persistent viruses are typically equally or more attractive to vectors than healthy plants; however, vectors are more likely to desert infected than healthy plants. Fereres et al. (1999) showed that *Rhopalosiphum maidis* (Fitch) was equally attracted to soybean infected with *Soybean mosaic virus* as to healthy plants, but remained on infected plants shorter than did aphids on

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healthy soybean. In the field, aphids added to plants infected with *Zucchini yellow mosaic virus* (ZYMV) were more likely to emigrate than aphids added to healthy plants (Blua & Perring, 1992). Increased emigration from infected plants in favor of healthy plants has been modeled to increase the rate of pathogen spread (Sisterson, 2008).

Acquisition of semi-persistently and persistently transmitted viruses is increased with longer durations of ingestion by the vector. Semi-persistent viruses are non-circulative and bind to the vector's stylets or foregut (Ng & Falk, 2006; Uzest et al., 2007). Although semi-persistent viruses may be acquired very quickly, acquisition rates of some semi-persistent viruses increase after periods of longer ingestion (Palacios et al., 2002). Persistent viruses are acquired from the phloem of the host plant and are circulated through the vector to the salivary glands, where they may be inoculated into a new host. Persistent viruses may be divided into two categories, those that simply circulate through the host (persistent-circulative) and those that replicate within the insect as well as the plant (persistent-propagative).

Because increased ingestion time corresponds to increased rates of virus acquisition for semi-persistently and persistently transmitted viruses, host manipulation predicts that plants infected with these viruses will have greater attraction and settling rates of aphids over those on healthy plants. In addition, performance outcomes, such as longevity or fecundity, may be increased on plants infected with semi-persistent and persistent viruses (Mauck et al., 2012). *Myzus persicae* (Sulzer) and *Aphis fabae* Scopoli were more attracted to sugar-beet leaves infected with the semi-persistent *Beet yellows virus* (BYV) than to healthy leaves. Both species also reproduced more quickly and had greater longevity on BYV plants than healthy ones (Baker, 1960). Plants infected with the persistent *Potato leaf roll virus* (PLRV) are consistently more attractive to vectors than healthy plants and are associated with increased growth rates, longevity, and fecundity (Castle & Berger, 1993; Castle et al., 1998; Eigenbrode et al., 2002; Srinivasan et al., 2006). The adaptive value for the virus of increased settling and improved performance by the vector may be due to greater crowding on host plants, which results in increased vector migration to new potential hosts (Gildow, 1980, 1983; Zhang et al., 2000).

Mauck et al. (2012) found strong support among the published literature for their hypothesis that virus transmission type predicts the direction of changes in vector performance or behavior. However, they identified several short-comings of this conclusion, among which are included a relatively low diversity in the virus families examined, few studies that focused on both vector performance and behavioral changes, and little attention to naturally occurring (non-agricultural) systems or mixed viral infections. In addition, studies of semi-persistent and persistent-propagative viruses are under-represented in the literature.

The objectives of this study were to examine the effects of singly infected and mixed-infected host plants on the performance (longevity and fecundity), attraction, settling, and feeding behaviors of an aphid vector. We electronically monitored the feeding behavior of aphids on healthy and infected plants to determine whether changes in feeding behavior may explain differences in aphid attraction and settling behavior. The model system that we used was two viruses that co-infect red raspberry, *Rubus idaeus* L. cv. Meeker (Rosaceae). *Raspberry leaf mottle virus* (RLMV) [*Closterovirus* (Closteroviridae)] is a semi-persistently transmitted member of the family Closteroviridae. *Raspberry latent virus* (RpLV) [*Reovirus* (Reoviridae)] is a persistent-propagatively transmitted virus in the family Reoviridae. Both viruses are transmitted by the aphid *Amphorophora agathonica* Hottes (Hemiptera: Aphididae). We hypothesized that: (1) single and mixed infections of RLMV and RpLV would improve the performance of *A. agathonica*; (2) aphids would show increased attraction toward and settling on infected plants over healthy plants; and (3) aphids would feed differently on healthy vs. infected plants, and in such a manner as to improve the probability of transmission.

Materials and methods

Plants and insects

The raspberry cultivar Meeker was obtained as plugs from North American Plants (McMinnville, OR, USA). Plugs were planted individually in 10-cm pots with 2.16 g l^{-1} of 21-2-11 N-P-K fertilizer (Apex, Boise, ID, USA). Plants were grown in a greenhouse at a light and temperature regime of L16 (21 °C):D8 (6 °C) until sizeable for grafting.

Virus source plants were single-infected RLMV and RpLV plants that had previously been collected from Meeker plantings in production fields in Whatcom County, WA, USA (48°95′N, 122°46′W). A co-infected plant (RLMV+RpLV) had previously been generated by graft inoculation. The new Meeker plants were grafted with the single- or mixed-infection plant treatments, and healthy controls were mock inoculated. Two months after grafting, plants were tested using RT-PCR to ensure the grafts were successful.

Ten adult *A. agathonica* were collected from a commercial raspberry field in Whatcom County, WA, USA, in September 2012 and maintained as a single colony on Meeker plants in a growth chamber (Percival Scientific, Perry, IA,

USA). Aphids were reared at 21 °C and a L16:D8 photoperiod. Plants were replaced weekly to maintain high quality.

Aphid performance on infected plants

An aphid cohort was obtained by isolating reproductively mature adults on a Meeker leaf in a Petri dish. After 24 h, the nymph cohort was caged individually on a fully expanded leaf each on either a healthy plant, a plant infected with RLMV or RpLV, or a plant co-infected with RLMV+RpLV. Clip cages were made from 15-ml plastic tubes cut into 2-cm lengths and attached to the leaf with a rubber coated washer and metal clip. The metal clip was supported on a wooden stake to reduce stress to the leaf petiole. Aphids were checked daily for the presence of nymphs until they died. When nymphs were present, they were counted and removed from the cage. Aphids were moved to a newly expanded leaf when leaf quality declined. Experiments were performed with one aphid per plant as a replicate. Seven replicates were tested per treatment per trial, and three trials were conducted with new plants. Differences in the pre-reproductive period (days from birth to first nymph born), fecundity, and longevity between treatments were compared using a linear mixed model with trial as a random factor (Proc GLIMMIX). Tukey's honestly significant difference was used to correct for multiple comparisons. All analyses were conducted using SAS (version 9.2.3; SAS Institute, Cary, NC, USA).

RpLV effects on aphid performance

To clarify whether changes in aphid performance on RpLV plants were due to virus-induced changes on the plants or to replication of the virus within the aphid, we examined differences in healthy vs. infected aphid performance on clean plants. Clonal aphid nymph pairs were obtained by isolating adults individually in a 24-well plate, where each well contained moistened filter paper and a Meeker leaf disk. Nymphs born within a 24-h period to the same adult were considered to be genetically identical pairs. After 24 h, one of the two aphids in each pair was caged to a healthy plant, while the other aphid was caged on a plant with RpLV. Aphids fed for 5 days, which is long enough for successful acquisition of RpLV by at least 80% of the aphids (Quito-Avila et al., 2012). The aphids were then caged individually on the same healthy plant, with each plant used for only one clonal pair of aphids. Data collected were the same as for the performance on infected plants (above). After each aphid died, we attempted to extract RNA to test for successful acquisition of RpLV, but unfortunately the RNA quality degraded too quickly after death to obtain reliable results of the precise RpLV acquisition rate in this study. Each aphid pair was a replicate, with eight replicates tested per trial, and three trials conducted. Differences in the pre-reproductive period, fecundity, and longevity between treatments were compared using a paired t-test (Proc MIXED).

Aphid attraction and settling

The assay design was modified from Srinivasan et al. (2006) and Castle et al. (1998). Two treatment plants, one healthy and one infected (with RLMV, RpLV, or RLMV+RpLV) were placed on opposite sides of the test arena, which consisted of a 14-cm Petri plate placed on a stage (Figure 1). The youngest fully expanded leaflet from the test plant was inserted into the Petri plate and held into place using Parafilm®. All possible exits from the arena were sealed off using parafilm.

Fifteen late-instar nymphs and adult *A. agathonica* were held in a small Petri plate for 1 h prior to the beginning of the assay. After 1 h, the aphids were added to the edge of the test arena, equidistant from the two test leaves. Aphids were free to walk on and to probe the test leaves. The aphids on each leaf were counted at 30 min and 24 h after introduction.

The study was replicated 16 times per infected-plant treatment, using a new plant pair for each replicate. Because there was low correlation between aphid choice at 30 min and 24 h, each time point was analyzed separately. The fraction of aphids selecting each leaf was analyzed using a generalized linear mixed model with a binomial distribution (Proc GLIMMIX, SAS 9.2.3).

Feeding behavior

Aphid feeding behaviors on healthy plants and plants infected with RLMV and RLMV+RpLV were recorded using an AC-DC electrical penetration graph monitor (Backus & Bennett, 2009) using a 10^9-Ω input impedance. Plants infected with RpLV alone were not tested because this single infection is rarely observed under field

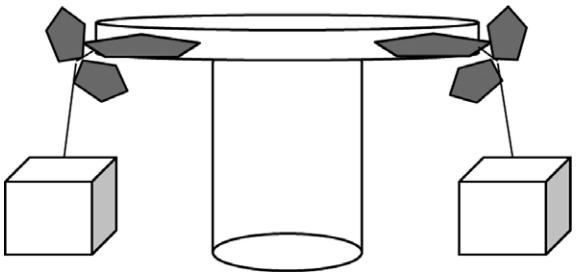

Figure 1 Experimental setup for the attraction and settling assay. A Petri dish arena was set on top of a stage and the terminal leaflet of the two test plants was inserted. Leaves remained attached to the test plant. Aphids were added to a central location. Assay design was modified from Srinivasan et al. (2006) and Castle et al. (1998).

conditions. Young adult aphids were starved for 0.5 h, during which time they were connected to an electrode using 25.4-μm gold wire (sold as '0.001 in'; Sigmund Cohn, Mt. Vernon, NY, USA) ca. 12 mm long. Direct current of 40 mV was applied to the plant through a copper electrode inserted into the soil at the base of the plant. When the aphid fed on the plant, the circuit was completed and the voltage change was measured using a DI-710 analog-to-digital board and Windaq Lite v. 3.38 software (Dataq Instruments, Akron, OH, USA). Three insect–plant combinations were tested each day in a randomized complete block design, with one of each treatment tested per day. Recordings began in the afternoon each day (15:00 to 17:00 hours) and continued for 24 h. The treatments were replicated 21 times.

Data were exported to The Observer (Noldus Information Technology, Wageningen, The Netherlands) and scored for the number and duration of pathway behaviors (C, salivation and other behaviors occurring in the plant epidermis and mesophyll), potential drops (PD, intracellular punctures), xylem ingestion (G), phloem salivation (E1), and phloem ingestion (E2). Insects that did not make any probes during the 24-h recording period were discarded from further analysis. Thus, the total numbers of insects analyzed in each treatment were: healthy, n = 17; RLMV, n = 19; and RLMV+RpLV, n = 17. Calculation of sequential and non-sequential response variables was done using a new SAS program designed to calculate nearly 100 response variables matching those produced in the Excel program of Sarria et al. (2009) (TA Ebert, University of Florida, pers. comm.). We selected a subset of the response variables calculated for further analysis. The variables analyzed were: time to the first probe, time to the first E1 (representing first phloem recognition event), duration of non-probing, C, E1, E2, and G behaviors, and number of C, E1, E2, G, and PD behaviors. Any insect that did not perform a certain behavior was excluded from analysis of that behavior. Variables were compared among treatments using iteratively optimized generalized linear models (Proc GLIMMIX). Following the protocol of Littell et al. (2006), non-normally distributed response variables were modeled using the identity link function and a Kenward-Rogers degree of freedom adjustment to account for unequal sample sizes between treatments (Littell et al., 2006).

Results

Performance

All three infected-plant treatments (RLMV, RpLV, and RLMV+RpLV) significantly increased the longevity of aphids ($F_{3,73} = 7.47$, $P<0.001$; Figure 2A). Aphid fecundity was increased on the mixed-infection treatment only ($F_{3,70} = 2.95$, $P = 0.038$; Figure 2A). There were no differences in the pre-reproductive development time among aphids feeding on healthy and infected plants ($F_{3,73} = 2.46$, $P = 0.07$; Figure 2A). Aphids infected with RpLV but developing on healthy plants did not show any changes in their development time, fecundity (total no. nymphs), or longevity compared with healthy aphids ($P>0.1$; Figure 2B) suggesting that multiplication of RpLV within aphids does not directly affect their fitness.

Attraction and settling

Thirty minutes after addition to the two-way choice arena, aphids did not show a significant attraction to either RLMV, RpLV, or RLMV+RpLV plants over healthy ones (RLMV: $F_{1,15} = 1.87$, $P = 0.2$; RpLV: $F_{1,15} = 0.75$, $P = 0.4$; RLMV+RpLV: $F_{1,15} = 0.05$, $P = 0.8$; Figure 3A). After 24 h, aphids significantly preferred RLMV plants to healthy plants ($F_{1,15} = 4.54$, $P = 0.05$). However, the opposite was true when aphids were given a choice between RpLV and healthy plants: they significantly preferred to settle on healthy plants after 24 h ($F_{1,15} = 4.89$, $P = 0.04$). There was no difference in the proportion of aphids that settled on either treatment when exposed to RLMV+RpLV and healthy plants ($F_{1,15}<0.01$, $P = 0.9$; Figure 3B).

Electrical penetration graph monitoring

There were no significant differences in any of the calculated response variables relating to pathway behaviors, phloem salivation, phloem ingestion, or xylem ingestion between aphids feeding on healthy plants and plants infected with either RLMV or RLMV+RpLV (Table 1).

Discussion

Based on our results, RLMV, RpLV, or RLMV+RpLV-infected plants enhanced the performance of *A. agathonica* compared with healthy plants. The pre-reproductive development rate was not changed, but aphids on all three infected-plant treatments had increased longevity. In the co-infection treatment, aphids also had increased fecundity over aphids feeding on healthy plants. Reasons for improved performance may relate to the amino acid composition and concentration of the phloem sap of the infected plants. *Barley yellow dwarf virus*-infected wheat had decreased amino acid concentration that correlated with poor performance by the aphid *Sitobion avenae* (Fabricius) (Fiebig et al., 2004). Raspberry plants co-infected with RLMV and *Black raspberry necrosis virus* (BRNV) showed an overall increase in amino acid concentrations; however, the closely related vector *Amphorophora idaei* Börner had longer developmental times on infected plants

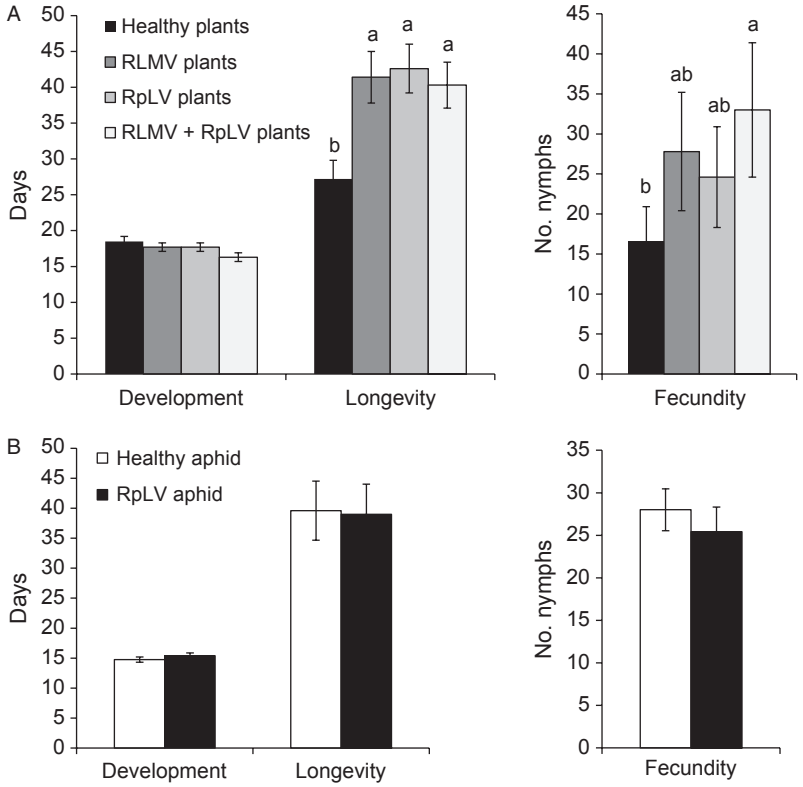

Figure 2 Mean (± SE) development, longevity, and fecundity of (A) healthy *Amphorophora agathonica* aphids feeding on healthy, *Raspberry leaf mottle virus* (RLMV) infected, *Raspberry latent virus* (RpLV) infected, or RLMV+RpLV-infected raspberry plants, and (B) healthy aphids or aphids infected with RpLV. Means capped with different letters are significantly different (Tukey's HSD test: $P<0.05$). Aphid performance variables are defined in the Materials and methods section.

over the healthy raspberry controls (McMenemy et al., 2012). The authors hypothesized that the high levels of the amino acid glutamate in RLMV+BRNV-infected plants may have reduced plant suitability (Chen et al., 1997; McMenemy et al., 2012). Because McMenemy et al. (2012) did not identify the amino acid composition of plants infected singly with RLMV, we cannot directly compare our data. However, it is clear that different combinations of co-infections (RLMV+BRNV vs. RLMV+RpLV) have different performance outcomes for aphids in raspberry.

Increased performance on plants infected with RpLV appears to be due to changes in the plant, rather than a fitness effect of RpLV replication within *A. agathonica*. The extent to which persistent-propagative transmitted plant viruses affect vector fitness is unknown. The persistent-propagative *Tomato spotted wilt virus* (Bunyaviridae) was shown to have no effect on the fitness of its thrips vector *Frankliniella occidentalis* (Pergande) (Wijkamp et al., 1996). However, *Tobacco curly shoot virus* (Geminiviridae) increased *B. tabaci* longevity by 18-fold (Jiu et al., 2007; Hogenhout et al., 2008). Few examples of fitness effects of persistent-propagative viruses may be recorded because these viruses evolved in insects and moved secondarily to plants (Nault, 1997; Power, 2000). Our data show no evidence of a loss or gain of aphid fitness as a result of RpLV propagation; this neutral effect may in itself be adaptive to RpLV because there is no negative effect related to virus replication within the insect. Alternatively, because the acquisition rate of RpLV by the aphids in this study is unknown, the results observed may be caused by poor RpLV infection rather than lack of an effect of RpLV replication.

The vast majority of prior studies support the hypothesis that aphid attraction and settling is enhanced on hosts infected with semi-persistent and persistent viruses (Mauck et al., 2012). Our data showed no evidence for greater initial attraction by aphids after 30 min of exposure on infected plants. The mechanism driving differential attraction is in large part due to changes in the volatile profiles of infected plants. Infected plants typically do not elicit novel compounds, but rather produce exaggerated amounts of attractive compounds already produced by the host plant (Mauck et al., 2010). McMenemy et al. (2012) found increased attraction by

A. idaei to RLMV+BRNV-infected raspberry that could be partly attributed to enhanced amounts of (*Z*)-3-hexenyl acetate. Again, the co-infection combination of RLMV+BRNV used in that study resulted in different behavioral outcomes than we observed with RLMV and RpLV alone and in combination. Further study on the mechanisms of these virus interactions would be valuable for understanding how mixed infections affect vector performance behavior.

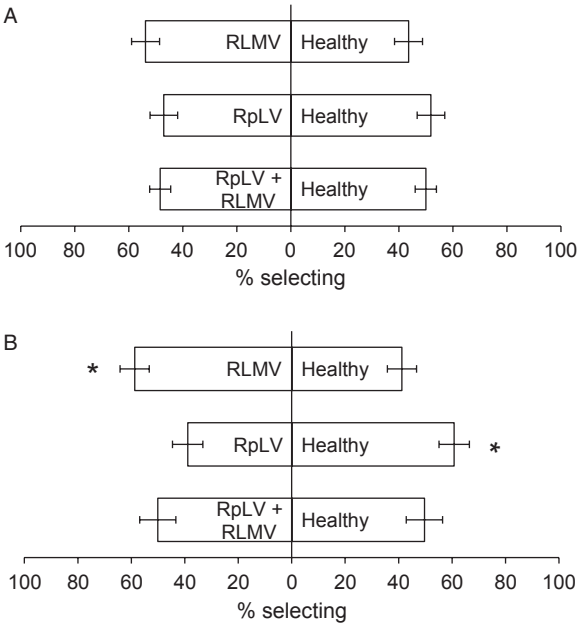

Figure 3 Distribution of *Amphorophora agathonica* aphids (mean % ± SE) over differently treated raspberry leaves in a two-choice assay at (A) 30 min and (B) 24 h after introduction to the arena. Leaves were from healthy, *Raspberry leaf mottle virus* (RLMV) infected, *Raspberry latent virus* (RpLV) infected, or RLMV+RpLV-infected plants. An asterisk indicates a significant difference between the fractions of aphids choosing for either option (GLMM with binomial distribution: $P<0.05$).

After the attraction phase, an increased or neutral preference for settling by aphid vectors is typically observed among plants infected with semi-persistent and persistently transmitted viruses (Mauck et al., 2012). True to this pattern, *A. agathonica* preferred RLMV over healthy plants at 24 h. Increased settling has been observed for another Closterovirus; BYV-infected sugar-beets were preferred by four species of aphids in the greenhouse (Baker, 1960; Macias & Mink, 1969). *Amphorophora agathonica* preferred to settle on healthy over RpLV-infected plants, placing this experiment with a small minority of studies that failed to find a positive or neutral settling preference for a persistent virus (Power, 1996; Mauck et al., 2012). To our knowledge, RpLV is both the first *Reovirus* and the first double-stranded RNA virus to be studied for changes in vector behavior. Incorporating a greater diversity of viruses into research on behavioral preferences of vectors will help to discern whether RpLV is an outlier or whether virus family and genome type play a large role in the behavioral effects.

We hypothesized that differences in the attraction and settling behaviors of aphids on infected plants may be explained by plant changes that affect feeding behavior. However, no feeding differences were observed between RLMV and RLMV+RpLV as compared with healthy plants. Electrical penetration graph studies conducted with

Table 1 Mean (± SE) selected electrical penetration graph response variables of *Amphorophora agathonica* aphids on healthy, *Raspberry leaf mottle virus* (RLMV) infected, or RLMV+*Raspberry latent virus* (RpLV) infected raspberry leaves, as durations or counts per insect. Means were compared using a mixed model (Proc GLIMMIX)

Parameter	Healthy	RLMV	RLMV+RpLV	F	d.f.	P
Time to first probe (min)[1]	226.1 ± 294.9	205.9 ± 228.9	137.5 ± 161.6	0.29	2,50	0.75
Time to first E1 (min)[1]	296.5 ± 63.7	392.6 ± 79.8	403.9 ± 86.8	0.87	2,50	0.42
Duration of NP (min)	701.7 ± 186.9	550.3 ± 138.7	587.9 ± 156.6	0.35	2,50	0.70
Duration of C (min)	284.2 ± 30.6	352.7 ± 28.9	315.9 ± 30.6	1.33	2,50	0.27
Duration of E1 (min)	45.7 ± 14.3	38.4 ± 11.4	46.3 ± 14.5	0.20	2,50	0.82
Duration of E2 (min)	872.1 ± 467.5	665.3 ± 316.9	911.5 ± 488.6	0.30	2,46	0.74
Duration of G (min)	76.8 ± 24.8	85.1 ± 38.9	89.3 ± 28.9	0.07	2,7	0.94
No. C events	19.2 ± 3.1	21.1 ± 3.2	19.0 ± 3.1	0.16	2,50	0.85
No. PD events	257.7 ± 27.7	282.6 ± 26.2	263.2 ± 27.7	0.24	2,50	0.79
No. E1 events	10.9 ± 2.5	8.9 ± 1.9	7.4 ± 1.7	1.06	2,50	0.35
No. E2 events	16.0 ± 4.7	8.9 ± 2.2	9.6 ± 2.8	1.80	2,46	0.18
No. G events	2.3 ± 0.8	3.4 ± 2.5	2.7 ± 0.9	0.53	2,7	0.61

NP, non-probing (stylets withdrawn from plant); C, pathway behaviors; E1, phloem salivation; E2, phloem ingestion; G, xylem ingestion; PD, potential drops (intracellular punctures).

[1]'Time to' refers to time from the beginning of the recording to the start of the event.

Aphis gossypii Glover feeding on plants infected with ZYMV found more probes but fewer phloem contacts within those probes compared with healthy controls (Blua & Perring, 1992). Plants infected with PLRV were found to enhance *M. persicae* feeding at the epidermis and mesophyll levels because there were decreased incidences of stylet penetration difficulties (waveform F) and fewer short test probes (Alvarez et al., 2007). Both these studies found that feeding differences occurred only when symptoms were obvious. Therefore, the feeding differences observed in those studies may be due to structural changes occurring in the leaves as a result of advanced infection. RLMV and RpLV produce no obvious visual symptoms in Meeker, and this may explain why feeding behaviors were not different from those on healthy plants.

Plants co-infected with multiple viruses are known to undergo competition or synergistic interactions. In many cases, one virus increases its titers when co-infecting a plant over the titers observed when infecting the plant alone (Wintermantel et al., 2008; Quito-Avila & Martin, 2012). RpLV and RLMV do not appear to exhibit a synergistic or antagonistic interaction during co-infection because titer levels of both viruses remain at similar levels compared with titers when singly infected (Quito-Avila, 2011). Interestingly, the mixed virus combination RLMV+RpLV showed no significant differences in settling behaviors in two-way choice tests, despite a positive preference for RLMV alone and a negative preference for RpLV alone. Too few mixed virus systems have been studied for aphid preference to hypothesize whether the co-infected plant was equally attractive to healthy plants because (1) the effect of both virus infections 'cancelled' each other out or (2) there were novel changes occurring due to the co-infection. Regardless, our data show that RpLV gains a competitive advantage when it is found in combination with RLMV. In a single infection of RpLV, aphids had a preference for healthy plants, which would ultimately decrease the likelihood that RpLV would be acquired. However, deterrent effects of RpLV infection appear to be mitigated by co-infection with RLMV, with aphids showing no significant preference for healthy or co-infected plants. Although transmission of RpLV may be enhanced with co-infection, RLMV experiences a competitive disadvantage when in combination with RpLV. Aphids were more likely to settle on singly infected RLMV plants but they were only equally likely to settle on the RLMV+RpLV-infected as on healthy plants.

We have shown that infections of raspberries with RLMV, RpLV, or RLMV+RpLV increase *A. agathonica* performance relative to healthy plants. Yet, despite a positive performance change for aphids on all infected-plant treatments, aphids were less likely to settle on RpLV-infected plants. Our results illustrate the need for research on a wider diversity of plant virus families and how they are similar and different in their ecological effects on plants and vectors. In addition, how viruses affect each other within the plant may help shed light on the frequency of single- vs. co-infections in the field. Continued understanding of the complex relationships between virus infection and vector transmission will illuminate the evolutionary forces at play, as well as improve the understanding of virus epidemiology and disease management.

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