Microbial Ecology (2018) 76:453–458
https://doi.org/10.1007/s00248-017-1133-5

INVERTEBRATE MICROBIOLOGY

# Endosymbionts Differentially Alter Exploratory Probing Behavior of a Nonpersistent Plant Virus Vector

G. Angelella[1,2] · V. Nalam[3] · P. Nachappa[3] · J. White[4] · I. Kaplan[1]

Received: 29 September 2017 / Accepted: 18 December 2017 / Published online: 30 December 2017


## Abstract

Insect endosymbionts (hereafter, symbionts) can modify plant virus epidemiology by changing the physiology or behavior of vectors, but their role in nonpersistent virus pathosystems remains uninvestigated. Unlike propagative and circulative viruses, nonpersistent plant virus transmission occurs via transient contamination of mouthparts, making direct interaction between symbiont and virus unlikely. Nonpersistent virus transmission occurs during exploratory intracellular punctures with styletiform mouthparts when vectors assess potential host-plant quality prior to phloem feeding. Therefore, we used an electrical penetration graph (EPG) to evaluate plant probing of the cowpea aphid, *Aphis craccivora* Koch, an important vector of cucurbit viruses, in the presence and absence of two facultative, intracellular symbionts. We tested four isolines of *A. craccivora*: two isolines were from a clone from black locust (*Robinia pseudoacacia* L.), one infected with *Arsenophonus* sp. and one cured, and two derived from a clone from alfalfa (*Medicago sativa* L.), one infected with *Hamiltonella defensa* and one cured. We quantified exploratory intracellular punctures, indicated by a waveform potential drop recorded by the EPG, initiation speed and frequency within the initial 15 min on healthy and watermelon mosaic virus-infected pumpkins. Symbiont associations differentially modified exploratory intracellular puncture frequency by aphids, with *H. defensa*-infected aphids exhibiting depressed probing, and *Arsenophonus*-infected aphids an increased frequency of probing. Further, there was greater overall aphid probing on virus-infected plants, suggesting that viruses manipulate their vectors to enhance acquisition-transmission rates, independent of symbiont infection. These results suggest facultative symbionts differentially affect plant-host exploration behaviors and potentially nonpersistent virus transmission by vectors.

**Keywords** Nonpersistent virus transmission · *Aphis craccivora* · *Arsenophonus* · *Hamiltonella defensa* · Watermelon mosaic virus · Endosymbionts

## Introduction

Facultative endosymbionts (hereafter, symbionts) are common in insect vectors of plant pathogens such as aphids [1] and known to influence a wide array of phenotypes in host organisms including behavior and pathogen transmission (e.g., [2]). Nevertheless, research into symbiont mediation of insect-vectored plant pathogen epidemiology has thus far only involved symbiont interactions with persistently transmitted propagative and/or circulative viruses, in which virus acquisition and transmission can be facilitated by direct interactions between virus and symbiont within an insect's body [3–7]. In contrast, nonpersistent viruses are only briefly transmissible via contaminated mouthparts until the next salivation or plant attack [8], occurring on the order of seconds to minutes [9]. Thus, direct interaction between internally-housed symbionts and nonpersistent viruses is unlikely. However, it is unknown

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s00248-017-1133-5) contains supplementary material, which is available to authorized users.

✉ G. Angelella
ginama@vt.edu

[1] Department Entomology, Purdue University, 901 West State St., Lafayette, IN 47907, USA

[2] Present address: Department Horticulture, Virginia Tech University, 33446 Research Dr., Painter, VA 23420, USA

[3] Department Biology, Indiana University–Purdue University Fort Wayne, 2101 E. Coliseum Blvd., Fort Wayne, IN 46805, USA

[4] Department Entomology, University of Kentucky, S-225 Agricultural Science Center N, Lexington, KY 40546, USA

if symbiont-mediated changes in vector behavior could result in comparable effects on pathogen spread.

Aphid host-plant acceptance and initiation of feeding involve a consistent order of behaviors, wherein virus inoculation occurs during particular stages. Nonpersistent viruses are transmitted by aphids during exploratory "taste" probes with their needle-like mouthparts in epithelial or mesothelial plant tissue, resulting in contact with gustatory receptors that indicate host-plant identity and quality [10–12]. This behavior precedes host-plant acceptance, whereupon an aphid locates the vascular bundle and sieve elements and begins phloem ingestion [13]. Importantly, nonpersistent virus acquisition and transmission do not occur during phloem ingestion [8]; rather, this process only occurs in the initial stages of plant probing [9, 14, 15]. As a result, analysis of feeding-behavioral mediation of nonpersistent virus transmission should focus on this critical early period when an aphid's stylet first contacts the epidermal cells of a potential host plant.

We examined whether symbionts of a nonpersistent virus vector, *Aphis craccivora* Koch (the cowpea aphid), modify behaviors related to virus acquisition and transmission. The cowpea aphid, *A. craccivora*, is well-suited to explore whether symbiont infection affects plant probing behaviors involved in virus transmission. Our prior research studying the epidemiology of cucurbit crop diseases in the Midwestern U.S. revealed that *A. craccivora* is strongly correlated with nonpersistent virus infection [16, 17]. This aphid species is capable of nonpersistent transmission of the watermelon mosaic virus, papaya ringspot virus type-W, cucumber mosaic virus, and zucchini yellow mosaic virus [18–20], all of which are common and damaging in Midwestern cucurbits [21]. However, *A. craccivora* landing and transmitting viruses in crops represent a mosaic of individuals varying in symbiont presence and identity. Molecular analyses of winged *A. craccivora* flying into pumpkin fields concluded that most aphids likely originated from source populations on either alfalfa (*Medicago sativa* L.) or black locust (*Robinia pseudoacacia* L.) [Angelella et al., *in review*]. Previous surveys and experimental work show that *A. craccivora* collected from these two host-plants possess distinct communities of intracellular bacterial symbionts [22, 23]. Most notably, alfalfa-origin *A. craccivora* often contain *Hamiltonella defensa*, whereas locust-origin *A. craccivora* usually possess *Arsenophonus* sp. [22–24]. These are mutually exclusive associations, i.e., we have no record of locust-collected *A. craccivora* testing positive for *H. defensa* and vice versa. Host-plant symbionts appear to mediate aphid dietary breadth, but their contribution to other components of *A. craccivora* ecology and behavior are unknown [25]. Using behavioral assays with an electrical penetration graph (EPG), we tested the impact of symbiont presence and identity on aphid probing behaviors previously shown to mediate virus transmission.

## Methods

### Aphid Colonies

We obtained colonies from J. White's lab at the University of Kentucky, propagated from one locust-origin clone (LE) and one alfalfa-origin clone (AC) [24]. Two isolines (aphids with the same genetic background) of each clone were maintained exclusively through asexual reproduction: LE infected with *Arsenophonus* (*Ars*+), LE cured (*Ars*-), AC infected with *Hamiltonella defensa* (*Ham*+), and AC cured (*Ham*-). The same *Arsenophonus* strain is found throughout *A. craccivora* colonies worldwide [22, 26] (excepting one unique strain found in a single *A. craccivora* colony on alfalfa [22]), and nearly identical *H. defensa* strains and APSE4 phages are found throughout colonies in the USA [27]. The integrity of cured and symbiont-associated isolines were verified with PCR (see [24]). To mitigate natal plant preferences, we maintained isolines on a universally-accepted host, fava bean (*Vicia fava* L.). Test aphids were age-synchronized by isolating 4th instar nymphs on fava bean leaf discs and testing only apterous adults found 2 days later.

### Virus Inoculations

We mechanically inoculated *Watermelon mosaic virus* (WMV) in pumpkin (*Cucurbita pepo* L.) with freeze-dried plant tissue (ATCC, Manassas, VA) during the two-leaf cotyledon stage following protocol described by Eigenbrode et al. [28], by rubbing a cotyledon sprinkled with carborundum powder with a cotton swab moistened in a mixture of 0.1 M phosphate buffer and ground WMV-inoculated leaf tissue (American Type Culture Collection, Manassas, VA), rinsing with distilled water, and subsequently placing the plant in the dark for a minimum of 8 h. In a two-year field survey of commercial pumpkin fields, WMV was the most prevalent among an assemblage of four cucurbit viruses [16]. "Healthy" control pumpkins were sham-inoculated using only carborundum powder and 0.1 M phosphate buffer solution (pH 7). Virus inoculations were verified with diagnostic WMV ELISA with a threshold for positive samples at an optical density (OD) value $\geq 0.2$ and $\leq 0.1$ OD for negative samples (Agdia® Inc., Elkhart, IN). Pumpkins were maintained in a growth chamber under 16:8 LD, 25% r.h. and 23 °C. We propagated "Mystic Plus" cultivar pumpkins with powdery mildew resistance (Harris® Seeds, Rochester, NY) in autoclaved soil (Hummert International, Earth City, MO) and 6″ pots (Hummert International, Earth City, MO) with 1 tsp. Osmocote® (The Scotts Company, Maryville, OH), using plants 2–3 weeks post-inoculation.

### Electrical Penetration Graphs

Electrical penetration graph (EPG) systems allow for the quantification and comparison of nuanced feeding behaviors of miniscule organisms such as aphids. They work by creating an electrical circuit between an aphid and the plant, which closes when they make contact. The electrical wavelengths flowing through the aphid-plant circuit vary predictably by behavior [29] and can then be used to infer and quantify activities which would not otherwise be visible. We used a GIGA 8 complete system (EPG Systems, Waginengen, Netherlands) [13]. We glued a 20 μm gold wire to the dorsum of an adult *A. craccivora* using silver glue carefully applied with the tip of an insect pin, while aphids were restrained on a stand-mounted pipette tip attached to a vacuum. The other end of the gold wire was connected to an EPG "aphid" probe. The wired "plant" electrodes were placed in the soil, and the "aphid" probes adjusted to allow for sufficient contact between the aphid and the plant surface. Aphid behaviors were recorded via an output wire connected to a computer running the Stylet+ software (EPG Systems, Wageningen, Netherlands).

Aphids were initially recorded for 8 h on plants, ample time for host acceptance and phloem feeding to occur [29]. No phloem feeding or sieve element location activities were recorded, suggesting pumpkin is not a preferred host plant for any of the *A. craccivora* isolines. This corresponds to observations that while *A. craccivora* frequently landed within Midwestern pumpkin fields [16], they were not observed colonizing the plants [G. Angelella, *personal observation*]. Subsequently, we limited analyses to 1 hour on plants, which is a reasonable time frame within which for nonpersistent virus transmission to occur [9]. Parameters recorded to characterize shallow intracellular probes during plant exploration included time to first potential drop (first.pd) and frequency of potential drops within the first 15 min (pd.15) [29]. Measurements began from the moment aphids made contact with the leaf. We replicated this design 15 times on each virus treatment for all four colonies ($n = 15$ plants × 4 colonies × 2 WMV-inoculated/control categories) with new aphids used for each observation. Assays were conducted during the fall of 2014.

### Data Analysis

Individual trials during which aphids disconnected, lost contact with the leaf, or died were excluded from analyses ($n = 75$), and first.pd analysis included only trials in which aphids initiated probing ($n = 70$) (Table S1). Statistical analyses were conducted using R Statistical Software v. 3.2.4 [30]. First.pd and pd.15 were tested for normality and models for homogeneity of variances prior to analysis. We conducted analyses with symbiont nested within host-plant origin. The first.pd data were square-root transformed and analyzed with a three-way ANOVA, whereas pd.15 count data were highly skewed and thus analyzed with Poisson regression. We reanalyzed pd.15 data after removing one locust-origin outlier (defined as Cook's distance > 1). Mean separations were analyzed with Tukey-Kramer Honest Significant Difference tests.

## Results and Discussion

Virus and symbiont presence both affected the frequency of aphid exploratory intracellular punctures in certain isolines (Table 1), but they did not affect the speed of probe initiation (Table S2) (treatment means and standard deviation described in Table S3). Generally, aphids showed a propensity toward more intracellular punctures on virus-infected plants (Table 1, Fig. 1). This trend was consistent across aphid treatments with the sole exception of locust-origin aphids infected with *Arsenophonus*, which displayed decreased intracellular puncture frequency on virus-infected plants (Table S3). This could indicate that aphids are able to perceive virus infection-derived cues during early stages of plant colonization and "taste testing." Similar virus-mediated changes in vector feeding behavior via manipulation of plant olfactory and gustatory cues are well documented as a mechanism enhancing acquisition and transmission efficiency (e.g., [31–33]).

Effects of symbionts on feeding behavior varied depending on symbiont type (Table 1). Specifically, we documented

**Table 1** Generalized linear model fitted with Poisson distribution, analyzing the effects of symbiont association and virus treatment (WMV inoculated or mock inoculated) on the number of intracellular probes made by *Aphis craccivora* on pumpkin leaf tissue. Symbiont is nested within *A. craccivora* host-plant origin (locust- or alfalfa-origin). Intracellular probes measured during the initial 15 min of an EPG analysis. One locust-origin outlier was removed prior to analysis[a]

| | Estimate | SE | $z$ | $P$ |
|---|---|---|---|---|
| *Intercept | 1.00 | 0.18 | 5.50 | < 0.001 |
| Origin | 0.41 | 0.25 | 1.67 | 0.095 |
| *Virus | 0.49 | 0.24 | 2.02 | 0.043 |
| **Origin x virus | − 0.96 | 0.36 | − 2.69 | 0.007 |
| Locust-origin:symb | 0.39 | 0.20 | 1.94 | 0.052 |
| *Alfalfa-origin:symb | 0.51 | 0.24 | 2.14 | 0.033 |
| **(Locust-origin:symb) x virus | 1.03 | 0.31 | 3.29 | 0.001 |
| (Alfalfa-origin:symb) x virus | 0.09 | 0.31 | 0.29 | 0.77 |
| RSD = 234.45 | | | | |
| df = 66 | | | | |

*Indicates significance ($\alpha = 0.05$); **Indicates significance ($\alpha = 0.001$)

[a] The results of two interaction effects changed following the removal of locust-origin outlier. Prior to outlier removal: origin x virus $\beta = -0.42$, SE = 0.33, $Z = -1.28$, $P = 0.2$; (locust-origin:symb) x virus $\beta = 0.48$, SE = 0.28, $Z = 0.75$, $P = 0.081$; RSD = 275.10, df = 67

a host-plant origin by symbiont interaction whereby locust-origin *A. craccivora* infected with *Arsenophonus* probed less frequently than those cured of symbionts (Fig. 1a), and alfalfa-origin *A. craccivora* infected with *H. defensa* probed more frequently than cured aphids (Fig. 1b). Upon direct comparison, alfalfa-origin aphids in their "normal" state (i.e., with symbionts) probe WMV-infected plants more than twice as frequently as locust aphids (Table S3). From an applied perspective, this suggests that alfalfa-derived *A. craccivora* might be more likely to acquire virus from infected plants compared with those originating from locust trees. However, this pattern in our results is entirely driven by symbiont presence. When cured, the opposite pattern occurs; namely, locust-origin aphids probe over twice as frequently as alfalfa-origin aphids.

The mechanism(s) which might drive these symbiont-mediated behavioral patterns are unknown. One possibility supported by previous research in other systems is that symbionts could directly affect plant-insect interactions by interfering with or counteracting herbivore-induced plant defenses [34–41]. For example, enterobacterial symbionts present in oral secretions can interfere with herbivore-induced defenses [37]. In whiteflies, *H. defensa* can also suppress jasmonic acid (JA) and JA-related defenses via salivary elicitors [39]. Salivary elicitors could provide a conduit for plant-endosymbiont interactions if *Arsenophonus* and *H. defensa* and associated products are not restricted within specialized aphid host cells [42]. Thus far, both symbionts have been identified within *A. craccivora* host cells [26, 27], but *H. defensa* was also detected in hemolymph [27]. *Arsenophonus* has been isolated from the salivary glands of whiteflies [4], but not aphids, to our knowledge. It would therefore be of interest to examine the composition of *A. craccivora* saliva with and without symbiont infections to determine whether symbiont-derived products or the bacteria themselves occur therein. Regardless, the timescale over which plant-symbiont interactions would need to induce behavioral changes in this study was < 15 min, raising the question of whether interactions could occur quickly enough to explain this outcome. Prior research with aphids shows mixed results regarding the feasibility of such an interaction occurring within this timeframe (e.g., [43–45]).

Because intracellular puncture frequency is associated with nonpersistent virus acquisition [9, 14, 15], we expect that nonviruliferous aphids exhibiting increased puncture frequencies on infected plants could translate into increased virus acquisition rates and vice versa. It is feasible, then, that differential intracellular puncture frequency could impact the relative abundance of viruliferous aphids within a given area, and the subsequent likelihood of healthy plants encountering a viruliferous aphid and being inoculated. However, we have examined only one of a suite of factors affecting nonpersistent virus epidemiology. For example, aphid dispersal behavior, response to visual, gustatory and olfactory cues, and pathogen acquisition/inoculation rates are all important elements of nonpersistent virus epidemiology [46]. Moving forward, we suggest exploring the potential of symbionts to modulate other aspects of nonpersistent virus dynamics as well.

This is the first experimental attempt to assess facultative endosymbiont impacts in insect-vectored nonpersistent virus epidemiology. From the perspective of the virus, increased aphid probing is highly adaptive and facilitates movement to new uninfected plants. We show that nonpersistent virus infection in a plant enhances aphid probing frequency, but that this response is differentially affected by aphid symbiont infection. Overall, results indicate a behavioral basis by which nonpersistent virus transmission and/or acquisition efficiency by vectors may be influenced by symbiont association, and suggest that the potential roles of symbionts in nonpersistent virus epidemiology merit further attention.

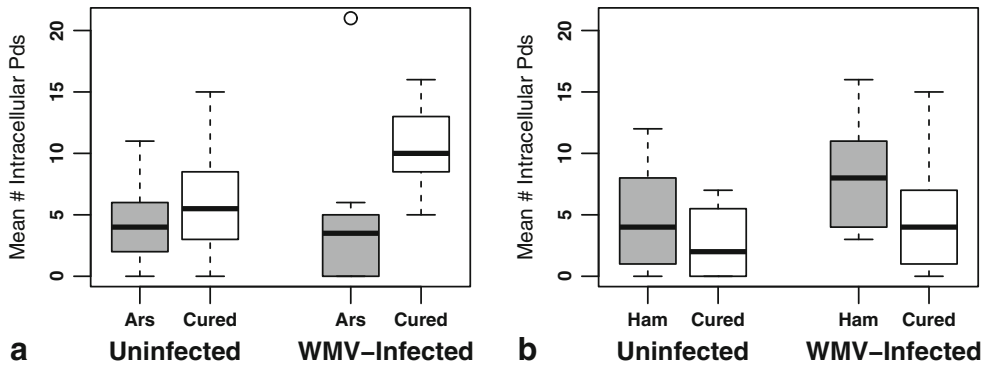


**Fig. 1** The interaction effect between aphid symbiont association and watermelon mosaic virus (WMV) infection in pumpkin (*Cucurbita pepo*) on the frequency of exploratory intracellular punctures, quantified within the initial 15 min of electrical penetration graph analysis. *Aphis craccivora* isolines (aphids with the same genetic background) were compared with and without symbiont infection. The dataset included one outlier, which is circled. **a** Locust-origin (*Robinia pseudoacia*) isolines with and without *Arsenophonus* infection ("Ars," "Cured," respectively). **b** Alfalfa-origin (*Medicago sativa*) isolines with and without *Hamiltonella defensa* infection ("Ham," "Cured," respectively). Pds = potential drops, indicating intracellular stylet punctures

**Acknowledgements** We would like to thank Allison Dehnel for shipping *Aphis craccivora* from J. White's lab at the University of Kentucky so often to initiate colonies at Purdue for use in this study. We would also like to thank an anonymous reviewer for helpful comments on an earlier version of this article. Funding provided by United States Department of Agriculture-National Institute of Food and Agriculture Predoctoral Fellowship grant 107483. Aphid colonies at the University of Kentucky were maintained on funds from United States Department of Agriculture-National Institute of Food and Agriculture grant 2014-67013-21576.

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

## References

1. Oliver KM, Degnan PH, Burke GR, Moran NA (2010) Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. Annu Rev Entomol 55:247–266
2. Ezenwa VO, Gerardo NM, Inouye DW, Medina M, Xavier JB (2012) Animal behavior and the microbiome. Science 338:198–199
3. Gottlieb Y, Zchori-Fein E, Mozes-Daube N, Kontsedalov S, Skaljac M, Brumin M, Sobol I, Czosnek H, Vavre F, Fleury F (2010) The transmission efficiency of tomato yellow leaf curl virus by the whitefly *Bemisia tabaci* is correlated with the presence of a specific symbiotic bacterium species. J Virol 84:9310–9317
4. Rana VS, Singh ST, Priya NG, Kumar J, Rajagopal R (2012) *Arsenophonus* GroEL interacts with CLCuV and is localized in midgut and salivary gland of whitefly *B. tabaci*. PLoS One 7: e42168
5. Su Q, Oliver KM, Pan H, Jiao X, Liu B, Xie W, Wang S, Wu Q, Xu B, White JA, Zhou X, Zhang Y (2013) Facultative symbiont *Hamiltonella* confers benefits to *Bemisia tabaci* (Hemiptera: Aleyrodidae), an invasive agricultural pest worldwide. Environ Entomol 42:1265–1271
6. Kliot A, Cilia M, Czosnek H, Ghanim M (2014) Implication of the bacterial endosymbiont *Rickettsia* spp. in interactions of the whitefly *Bemisia tabaci* with *Tomato yellow leaf curl virus*. J Virol 88: 5652–5660
7. Pinheiro PV, Kliot A, Ghanim M, Cilia M (2015) Is there a role for symbiotic bacteria in plant virus transmission by insects? Curr Opin Insect Sci 8:69–78
8. Fereres A, Collar J (2001) In: Harris KF, Smith OP, Duffus JE (eds) Analysis of noncirculative transmission by electrical penetration graphs. Virus-insect-plant interactions. Academic Press, San Diego
9. Powell G (1991) Cell membrane punctures during epidermal penetrations by aphids: consequences for the transmission of two potyviruses. Ann Appl Biol 119:313–321
10. Wensler RJD, Filshie BK (1969) Gustatory sense organs in the food canal of aphids. J Morphol 129:473–492
11. Tjallingii W (1995) Regulation of phloem sap feeding by aphids. Regulatory mechanisms in insect feeding. Springer, New York
12. Martin B, Collar JL, Tjallingii WF, Fereres A (1997) Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. J Gen Virol 78:2701–2705
13. Tjallingii WF, Hogen Esch T (1993) Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. Physiol Entomol 18:317–328
14. Powell G, Pirone T, Hardie J (1995) Aphid stylet activities during potyvirus acquisition from plants and an *in vitro* system that correlate with subsequent transmission. Eur J Plant Pathol 101:411–420
15. Fereres A, Moreno A (2009) Behavioural aspects influencing plant virus transmission by homopteran insects. Virus Res 141:158–168
16. Angelella G, Egel D, Holland J, Nemacheck J, Williams C, Kaplan I (2015) Differential life history trait associations of aphids with nonpersistent viruses in cucurbits. Environ Entomol 44:562–573
17. Angelella GM, Holland JD, Kaplan I (2016) Landscape composition is more important than local management for crop virus–insect vector interactions. Agric Ecosyst Environ 233:253–261
18. Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJE (1996 onwards) Plant viruses online: descriptions and lists from the VIDE database. vol. Version: 16th January 1997
19. Dallwitz MJ (1980) A general system for coding taxonomic descriptions. Taxon 29:41–46
20. Dallwitz MJ, Paine TA, Zurcher EJ (1993) User's guide to the DELTA system: a general system for processing taxonomic descriptions. CSIRO Division of Entomology, Canberra
21. Paulsrud B (2005) Workshop summary. Midwest pest management strategic plan for processing & jack-o-lantern pumpkins: Illinois, Indiana, Iowa and Missouri. University of Illinois Extension, Urbana, Illinois, pp. 109
22. Brady C, Asplen M, Desneux N, Heimpel G, Hopper K, Linnen C, Oliver K, Wulff J, White J (2014) Worldwide populations of the aphid *Aphis craccivora* are infected with diverse facultative bacterial symbionts. Microb Ecol 38:1–10
23. Brady CM, White JA (2013) Cowpea aphid (*Aphis craccivora*) associated with different host plants has different facultative endosymbionts. Ecol Entomol 38:433–437
24. Wagner SM, Martinez AJ, Ruan YM, Kim KL, Lenhart PA, Dehnel AC, Oliver KM, White JA (2015) Facultative endosymbionts mediate dietary breadth in a polyphagous herbivore. Funct Ecol 29: 1402–1410
25. White JA, McCord JS, Jackson KA, Dehnel AC, Lenhart PA (2017) Differential aphid toxicity to ladybeetles is not a function of host plant or facultative bacterial symbionts. Funct Ecol 31:334–339
26. Jousselin E, Cœur d'Acier A, Vanlerberghe-Masutti F, Duron O (2013) Evolution and diversity of *Arsenophonus* endosymbionts in aphids. Mol Ecol 22:260–270
27. Dykstra HR, Weldon SR, Martinez AJ, White JA, Hopper KR, Heimpel GE, Asplen MK, Oliver KM (2014) Factors limiting the spread of the protective symbiont *Hamiltonella defensa* in the aphid *Aphis craccivora*. Appl Environ Microbiol 80:5818–5827
28. Eigenbrode SD, Ding H, Shiel P, Berger PH (2002) Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). Proceedings of the Royal Society of London Series B. Biol Sci 269:455–460
29. Prado E, Tjallingii WF (1994) Aphid activities during sieve element punctures. Entomol Exp Appl 72:157–165
30. R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
31. Ingwell LL, Eigenbrode SD, Bosque-Perez NA (2012) Plant viruses alter insect behavior to enhance their spread. Sci Rep 578:1–6
32. Mauck KE, Bosque-Perez NA, Eigenbrode SD, De Moraes CM, Mescher MC (2012) Transmission mechanisms shape pathogen effects on host-vector interactions: evidence from plant viruses. Funct Ecol 26:1162–1175
33. Mauck KE, De Moraes CM, Mescher MC (2010) Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. Proc Natl Acad Sci 107:3600–3605
34. Visôtto L, Oliveira M, Guedes R, Ribon A, Good-God P (2009) Contribution of gut bacteria to digestion and development of the velvetbean caterpillar, *Anticarsia gemmatalis*. J Insect Physiol 55: 185–191
35. Barr KL, Hearne LB, Briesacher S, Clark TL, Davis GE (2010) Microbial symbionts in insects influence down-regulation of defense genes in maize. PLoS One 5:e11339

36. Boone CK, Keefover-Ring K, Mapes AC, Adams AS, Bohlmann J, Raffa KF (2013) Bacteria associated with a tree-killing insect reduce concentrations of plant defense compounds. J Chem Ecol 39: 1003–1006
37. Chung SH, Rosa C, Scully ED, Peiffer M, Tooker JF, Hoover K, Luthe DS, Felton GW (2013) Herbivore exploits orally secreted bacteria to suppress plant defenses. Proc Natl Acad Sci 110: 15728–15733
38. Zhu F, Poelman EH, Dicke M (2014) Insect herbivore-associated organisms affect plant responses to herbivory. New Phytol 204: 315–321
39. Su Q, Xie W, Wang S, Wu Q, Liu B, Fang Y, Xu B, Zhang Y (2014) The endosymbiont *Hamiltonella* increases the growth rate of its host *Bemisia tabaci* during periods of nutritional stress. PLoS One 9:e89002
40. Staudacher H, Schimmel BC, Lamers MM, Wybouw N, Groot AT, Kant MR (2017) Independent effects of a herbivore's bacterial symbionts on its performance and induced plant defences. Int J Mol Sci 18:182
41. Wang J, Peiffer M, Hoover K, Rosa C, Zeng R, Felton GW (2017) *Helicoverpa zea* gut-associated bacteria indirectly induce defenses in tomato by triggering a salivary elicitor(s). New Phytol 214:1294–1306
42. Hansen AK, Moran NA (2014) The impact of microbial symbionts on host plant utilization by herbivorous insects. Mol Ecol 23:1473–1496
43. Moran PJ, Thompson GA (2001) Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defense pathways. Plant Physiol 125:1074–1085
44. Morkunas I, Gabryś B (2011) Phytohormonal signaling in plant responses to aphid feeding. Acta Physiol Plant 33:2057–2073
45. Argandona V, Chaman M, Cardemil L, Munoz O, Zuniga G, Corcuera L (2001) Ethylene production and peroxidase activity in aphid-infested barley. J Chem Ecol 27:53–68
46. Katis NI, Tsitsipis JA, Stevens M, Powell G (2007) Transmission of plant viruses. In: van Emden HF, Harrington R (eds) Aphids as crop pests. CABI, Cambridge