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SYMPOSIUM

Evidence of Local Adaptation in Plant Virus Effects on Host–Vector Interactions

K. E. Mauck,*,† C. M. De Moraes*,† and M. C. Mescher[1],*,†

*Department of Entomology, Penn State University, University Park, PA 16802, USA; †Department of Environmental Systems Science, ETH Zürich, 8092 Zürich, Switzerland

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[1]E-mail: mescher@usys.ethz.ch

Synopsis Recent research suggests that plant viruses, and other pathogens, frequently alter host–plant phenotypes in ways that facilitate transmission by arthropod vectors. However, many viruses infect multiple hosts, raising questions about whether these pathogens are capable of inducing transmission-facilitating phenotypes in phylogenetically divergent host plants and the extent to which evolutionary history with a given host or plant community influences such effects. To explore these issues, we worked with two newly acquired field isolates of *cucumber mosaic virus* (CMV)—a widespread multi-host plant pathogen transmitted in a non-persistent manner by aphids—and explored effects on the phenotypes of different host plants and on their subsequent interactions with aphid vectors. An isolate collected from cultivated squash fields (KVPG2-CMV) induced in the native squash host (*Cucurbita pepo*) a suite of effects on host–vector interactions suggested by previous work to be conducive to transmission (including reduced host–plant quality for aphids, rapid aphid dispersal from infected to healthy plants, and enhanced aphid attraction to the elevated emission of a volatile blend similar to that of healthy plants). A second isolate (P1-CMV) collected from cultivated pepper (*Capsicum annuum*) induced more neutral effects in its native host (largely exhibiting non-significant trends in the direction of effects seen for KVPG2-CMV in squash). When we attempted cross-host inoculations of these two CMV isolates (KVPG2-CMV in pepper and P1-CMV in squash), P1-CMV was only sporadically able to infect the novel host; KVPG2-CMV infected the novel pepper host with somewhat reduced success compared with its native host and reached virus titers significantly lower than those observed for either strain in its native host. Furthermore, KVPG2-CMV induced changes in the phenotype of the novel host, and consequently in host–vector interactions, dramatically different than those observed in the native host and apparently maladaptive with respect to virus transmission (e.g., host plant quality for aphids was significantly improved in this instance, and aphid dispersal was reduced). Taken together, these findings provide evidence of adaption by CMV to local hosts (including reduced infectivity and replication in novel versus native hosts) and further suggest that such adaptation may extend to effects on host–plant traits mediating interactions with aphid vectors. Thus, these results are consistent with the hypothesis that virus effects on host–vector interactions can be adaptive, and they suggest that multi-host pathogens may exhibit adaptation with respect to these and other effects on host phenotypes, perhaps especially in homogeneous monocultures.

Introduction

Parasites often have profound effects on their hosts, including alteration of host traits that influence parasite transmission (Poulin 2010). Parasite manipulation of host phenotypes has been studied extensively in animal–host systems, and many spectacular examples involving dramatic changes in host behavior, morphology, and physiology have been documented (reviewed in Lefèvre and Thomas 2008; Lefèvre et al. 2009; Poulin 2010; Van Houte et al. 2013). Relatively less research has explicitly investigated the manipulation of plant phenotypes by parasites (Mescher 2012). Furthermore, in both plant– and animal–parasite systems, the manipulation of hosts by vector-borne pathogens is a relatively recent topic of investigation (Lefèvre et al. 2006), though a

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fast-growing body of literature now documents pathogen effects on the frequency and nature of interactions among (primary) hosts and vectors (Lefèvre and Thomas 2008; Mauck et al. 2012; Gutiérrez et al. 2013; Van Houte et al. 2013). Indeed, vector transmission would seem to offer abundant opportunities for manipulation, not only through direct effects on vector behavior (e.g., Stafford et al. 2011; Ingwell et al. 2012), but also through effects on traits of the primary host that influence vector attraction and dispersal, as well as the likelihood of pathogen acquisition by the vector during interactions with the host (Lefèvre et al. 2006; Mauck et al. 2012).

A number of recent studies have examined the effects of vector-borne plant pathogens on host–plant traits that mediate interactions with insect disease vectors (e.g., Mann et al. 2012; Shapiro et al. 2012; Zhang et al. 2012; Salvaudon et al. 2013) and, in general, these studies have reported effects that appear conducive to transmission. Leaving aside the sometimes challenging issue of how to distinguish between adaptive manipulation and fortuitous by-products of pathology in specific cases, we have previously speculated that changes in host traits induced by vector-borne pathogens will typically have neutral to positive effects on pathogen transmission (Mauck et al. 2010, 2012)—on the assumption that selection should rarely be indifferent to effects that impact transmission adversely (Anderson and May 1991). As a consequence of this expectation, we further hypothesized that there should be congruence between a pathogen's mode of transmission—hence, the optimal pattern of host–vector interactions from the pathogen's perspective—and its effects on host traits that influence interactions with vectors, so that pathogens with similar transmission mechanisms might be predicted to have similar effects on relevant suites of host traits. As discussed below, this expectation is consistent with our findings from previous work on *cucumber mosaic virus* (CMV) (Mauck et al. 2010, 2012, 2014), which is also the focus of the current study.

We previously reported a suite of CMV effects on host–plant quality for and attractiveness to aphid vectors that appear conducive to the non-persistent transmission mode of this pathogen and differ in key aspects from effects reported for several viruses exhibiting a different, persistent, mode of transmission (Mauck et al. 2010; Bosque-Pérez and Eigenbrode 2011; Mauck et al. 2012). The key distinction between persistently-transmitted (PT) and non-persistently transmitted (NPT) viruses is that the former form intimates associations with vectors (in some cases colonizing the vector as a secondary host), which then remain infectious over long periods; in contrast, NPT viruses form only transitory associations with the mouthparts of vectors (which typically remain infectious for one to two inoculations following acquisition) and they are acquired and inoculated by vectors during host–plant sampling (probing) prior to the onset of long-term feeding (Ng and Falk 2006; Hogenhout et al. 2008). This primary difference can have important implications for host–vector interactions and transmission. For example, PT viruses transmitted by aphid vectors must typically be ingested during sustained aphid feeding (for hours to days) on the phloem of an infected host plant (thus, these PT viruses have long acquisition access periods). Meanwhile, NPT plant viruses, which are exclusively aphid-transmitted, are acquired rapidly when virions in plant epidermal tissues bind to target sites on the aphid stylet during brief initial feeding probes, and may be lost if vectors stay on the infected host and initiate sustained feeding in the phloem (Martín et al. 1997; Wang and Ghabrial 2002; Ng and Falk 2006).

Consistent with the sustained feeding required for their acquisition, some PT viruses have been found to improve host plant quality for vectors (reviewed in Mauck et al. 2012, see also Zhang et al. 2012; Luan et al. 2012), and to induce changes in host-derived visual and olfactory cues that enhance vector attraction (Bosque-Pérez and Eigenbrode 2011; Mauck et al. 2012). In contrast, we found that infection by CMV strain FNY significantly reduced the quality of squash plants (*Cucurbita pepo* cv. Dixie) for aphid vectors—in part, through dramatic changes in phloem carbohydrate to amino-acid ratios (Mauck et al. 2014)—and caused rapid dispersal of aphids from infected to healthy plants, consistent with the efficient transmission of this NPT virus (Mauck et al. 2010). The expectation that the transmission of NPT viruses is most efficient when aphid vectors probe plant epidermal cells (and assess taste cues) but then disperse to probe a new, susceptible, plant is supported by studies that examined the detailed mechanisms of transmission (Martín et al. 1997) and by others that manipulated aphid behavior (e.g., short vs. long acquisition access periods) and examined effects on transmission (Wang and Ghabrial 2002). Evidence also comes indirectly from observations that NPT viruses are often transmitted most effectively by aphid species more likely to engage in probe-and-disperse behavior, including those that cannot successfully colonize (or perform poorly) on the host plant species from which the virus is acquired (Sigvald 1989; Nanayakkara et al.

2012; Tian et al. 2012). Interestingly, we found that aphids were nevertheless preferentially attracted to the odors of CMV-infected plants, despite their poor quality and reduced palatability, apparently because the virus induces an overall increase in volatile emissions while not changing the composition of the volatile blend (Mauck et al. 2010). The latter observation led us to hypothesize that pathogens that reduce host quality for vectors might often exaggerate pre-existing cues in order to deceptively attract vectors (Mauck et al. 2010), and a somewhat similar effect was recently reported for another, non-viral, plant pathogen (Mann et al. 2012).

Building on our initial observations with CMV-FNY, we recently undertook an analysis of existing literature to explore whether the observed patterns might hold more generally for PT and NPT pathogens (Mauck et al. 2012). Our findings were broadly consistent with predictions regarding adaptive effects of PT and NPT viruses on plant–vector interactions (including host–plant quality for vectors, vector propensity to settle on or colonize plants, and vector attraction to plant-derived visual or olfactory cues), which were developed based on knowledge of transmission mechanisms, vector biology, and theoretical models of virus spread (discussed in Mauck et al. 2012). However, the strength of the conclusions that can currently be drawn is limited to some extent by the relative scarcity of studies examining NPT viruses, which are understudied relative to their ecological and economic importance (NPT viruses constitute ~42% of known insect-transmitted viruses [Hogenhout et al. 2008], and their impacts on human agriculture are exacerbated by their rapid mode of transmission, which facilitates disease spread despite efforts to suppress vector populations with insecticides [Roberts et al. 1993; Perring et al. 1999]). Furthermore, the predicted trends are not universal, as virus effects that do not fit the predicted patterns have been reported in some systems (Kersch-Becker and Thaler 2013; Casteel et al. 2014). However, recent work on well-studied virus–host–vector systems provides additional support for the hypothesized patterns of effects by elucidating specific, vector-relevant, biochemical changes in host plants in response to virus infection (or the transgenic expression of wild type or mutant virus genes) (e.g., Luan et al. 2012; Zhang et al. 2012; Westwood et al. 2013; Mauck et al. 2014) and by further developing expectations on the temporal dynamics of vector attraction to infected and healthy plants (Medina-Ortega et al. 2009; Ingwell et al. 2012; Rajabaskar et al. 2014).

In the current study, we expand on our previous work with CMV by examining the effects of two newly collected field CMV isolates, one from squash (*C. pepo*) and one from pepper (*Capsicum annuum*), on host phenotypes and host–vector interactions in their native hosts (the species from which the virus was isolated) as well as the effects of the squash isolate when transferred to a novel host (pepper, which is a susceptible host for this isolate but from a different plant family than the native host). Many of the empirical studies discussed above, as well as others included in the analyses of Mauck et al. (2012) have focused on single virus–host–vector combinations and/or worked primarily with laboratory-maintained cultures—for which we often know little about the evolutionary history and which may not accurately reflect host adaptation under natural conditions. Relatively little is thus known about natural variation in effects on host–vector interactions among virus strains or across virus–host plant combinations, despite the fact that many plant viruses are multi-host pathogens capable of infecting a variety of plant species (and even families) and of being transmitted by more than one vector species. To better understand how virus-induced changes in host phenotype may alter transmission dynamics in real-world settings, it will be useful to compare effects of multiple virus genotypes across phylogenetically divergent hosts using uniform methods. In addition to producing such a comparison for two newly isolated strains of CMV, the current study provides a test predictions arising from the hypothesis that the observed effects of CMV on host–vector interactions are adaptive for the pathogen. For example, if such effects do reflect pathogen adaptation, we might expect to observe transmission-conducive effects of both strains in their native hosts but to see a disruption of these effects in the novel host—previous studies have reported evidence of local adaptation by multi-host viruses with regard to fitness-related traits including infectivity and virus titer (Sacristán et al. 2005; Malpica et al. 2006; Agudelo-Romero et al. 2008). If, however, the effects of the virus on host–vector interactions arise as by-products of other aspects of pathology there is no reason to expect this pattern of outcomes, and we might rather predict uniform effects across strains and hosts—consistent with this possibility, an early study on a strain of CMV maintained on *Nicotiana glutinosa* reported reduced host quality for the aphid *Myzus persicae* on the closely related host, *Nicotiana tobacum*, as well as hosts in other plant families such as *Zinnia elegans* (Asteraceae) and *Gomphrena glutinosa* (Amaranthaceae)

(Lowe and Strong 1963); however, this study (like many others to date) provided little information regarding the origin or culture history of the virus isolate employed, limiting our ability to draw inferences about potential virus adaptation for transmission-relevant effects on host phenotypes.

Methods

Viruses, plants, and insects

KVPG2-CMV was collected from a field of cultivated *C. pepo* growing in Kampsville, IL, USA, in September of 2009. Cultivated *C. pepo* was locally abundant in the region of collection, with wild *C. pepo* ssp. *texana* also available as a host plant throughout the region. Virus identity was verified through DAS-ELISA and we tested the sample to ensure that there was no co-infection by ZYMV or WMV, which are common co-occurring NPT cucurbit viruses. P1-CMV was collected from a cultivated pepper (*C. annuum*) field in Wisconsin's ''Central Sands'' vegetable growing region in summer 2008 by Dr Shahideh Nouri and Dr Russell Groves, and virus identity was verified by PCR amplification and sequencing of the 2b and CP CMV genes (Nouri 2012). Peppers are a common, locally abundant crop in this region.

Cultivated squash (*C. pepo* cv. Dixie, Willhite Seeds Inc.) and cultivated peppers (*C. annuum* cv. California Wonder, Johnny's Seeds Inc.) were grown in 12 cm^3 square pots in ProMix potting soil (autoclaved to destroy soil phytopathogens) containing 5 g of slow-release fertilizer (Osmocote 14-14-14 N-P-K) and trace micronutrients (Scott's Micromax Micronutrients). All plants were grown in an insect-free, walk-in growth chamber with a 16:8 light:dark photoperiod maintained with banks of fluorescent and incandescent bulbs set to 23°C during the day and 21°C at night. When *C. pepo* plants were at the cotyledon stage, they were inoculated with 5 cm^2 of frozen stock tissue infected with KVPG2-CMV (stored at −80°C). Pepper plants were inoculated with either KVPG2-CMV or P1-CMV when they had grown their first set of true leaves. All inoculations occurred from common stocks of young, highly symptomatic tissue preserved at −80°C, which was generated from one mechanical inoculation event for each virus (each virus was mechanically inoculated in the native host only two to three times following isolation from the field). Squash was the stock host for KVPG2-CMV and pepper the host for P1-CMV. To perform inoculations, frozen tissue was ground on a cold surface then combined with 15 ml of chilled 0.1 M potassium phosphate buffer. Carborundum powder was added and ∼100 μl of the solution was applied to the surface of each squash cotyledon or pepper true leaf and spread using a small cotton swab. Plants designated for the healthy treatment were mock-inoculated in the same manner, but using healthy tissue from either squash or pepper to provide a control for the effects of the mechanical inoculation and the influence of plant-derived factors. Both isolates successfully infected their native host with near ∼100% success rates. KVPG2-CMV was also able to infect the novel host pepper (with a success rate of 60–70%). And P1-CMV exhibited only sporadic inoculation success in squash.

Aphis gossypii was collected from a *C. pepo* plant in State College, PA, USA, and raised in the laboratory on *C. pepo* cv. Dixie. *Myzus persicae* was obtained from the Penn State Plant Pathology Department and was raised on *Brassica rapa* (cultivated turnips, cv. Purple Top White Globe). These hosts were chosen because we have previously found them to be capable of supporting large populations and stimulating winged offspring production (large numbers of alate aphids were needed for our behavioral experiments). Clones of each species were propagated to multiple colonies to generate sufficient alates for experiments, and colonies were maintained under a 16:8 photoperiod at 25°C. Cages were cleaned and colonies re-established on new host plants every 7–10 days.

Assessment of plant quality for aphid vectors

Aphid population growth on different virus–host combinations was assessed relative to healthy, mock-inoculated, hosts as a measure of plant quality. For each assay, standard age cohorts of aphids were created by transferring adult aphids from the main colony to two to three infected and mock-inoculated plants of the particular virus–host combination being examined and allowing them to reproduce for 36 h. Adults were then removed and first instar aphids were carefully transferred to the test plants by excising leaf tissue around the area on which they were feeding, and placing it gently on a leaf of the receiving plant. This method ensures almost 100% survival of nymphs with minimal disruption, since they are not handled during the transfer and are allowed to withdraw their stylets from the cuttings and disperse to test plants on their own. Ten nymphs were transferred to each test plant (10–12 plants per treatment), and all plants were housed in mesh cages in a growth chamber (settings as above for plant culture). Aphids were allowed to reproduce for 10 days

(roughly two generations). Each virus–host combination was performed as a separate experiment with the appropriate mock-inoculated healthy control, and we assessed aphid growth for every viable vector–host combination (both aphid species on squash, *M. persicae* only on pepper). Test plants were 2–3 weeks post-inoculation (squash) or 4 weeks post-inoculation (peppers) at the start of the experiments. Data were analyzed for each virus–host–vector combination by two-sample *T*-tests with log transformation to normalize residuals if necessary (Minitab v. 14).

Aphid emigration tests

Preference tests that permitted access to contact cues were performed using winged morphs of both aphid species. For each test, 36 wandering aphids were collected from the main colony by gently tapping aphids walking on the surface of the colony cage into glass petri dishes (ensuring that no damage to mouthparts occurred during collection). Aphids were starved for 1.5 h prior to each test and briefly chilled at 6°C to arrest flight during experiment set up. Thirty-six chilled aphids were transferred using a moist paintbrush to a 3-cm diameter piece of Whatman filter paper within a cold room at 4–6°C. Aphids were mobile and able to walk slowly, but were unable to disperse by flying from the filter paper while in the cold room. Once aphids were collected on the paper disc, it was immediately placed on either an infected or mock-inoculated release plant at one end of a $35 \times 35 \times 60$ cm mesh cage (in a room at 24°C). A choice plant was placed on the other side of the cage to provide a target for immigration. To focus on patterns of emigration relevant to virus spread, an infected release plant was always paired with a mock-inoculated (healthy) choice plant, and vice versa. As aphids warmed, they dispersed by walking onto the release plant where they were exposed to contact cues (aphids are stimulated to probe on surfaces with which they make tarsal contact). As initial observations indicated that most aphids dispersed from the disc by 60 min (but that many still remained after 30 min), we recorded distributions at 60 min, 120 min, and 24 h post release. Squash used in these experiments were 3 weeks post-inoculation ±3 days, and peppers were 4 weeks post-inoculation ±3 days. Tests were performed in a windowless room with diffuse artificial lighting directly overhead to discourage aphid response to positional light cues, and plants were moved from direct sunlight to their positions in the cage immediately prior to the start of the tests. Lights were turned off at night and back on in the morning to maintain the same photoperiod that plants and aphids had experienced prior to the start of tests. The number of tests that could be performed on 1 day was limited by the number of alates available, but tests were always at least performed as pairs, with one test having a healthy release plant and the other having an infected release plant (sample sizes for each release plant type within each virus–host–vector combination are therefore always equal). We performed four to nine tests for each release plant status (infected or healthy) within each virus–host–vector combination (our sample sizes are consistent with previous research employing this type of test, e.g., Eigenbrode et al. 2002; Srinivasan et al. 2006; Mauck et al. 2010).

Tests of aphid responses to plant odors

Volatile-based choice tests permitted aphids access only to odor cues without contact or visual cues. Tests were performed as described previously (Mauck et al. 2010) using a static-air arena similar to that employed in previous work (e.g., Eigenbrode et al. 2002; Srinivasan et al. 2006; Ngumbi et al. 2007; Medina-Ortega et al. 2009). For each test, 24 alate *A. gossypii* were starved for 1.5 h and briefly chilled to stifle movement, then placed on a starting platform within a plexiglass arena, the top of which was covered with dual layers of opaque screening that obscured visual cues but allowed volatile cues to permeate through to the arena enclosure. One healthy and one virus-infected leaf within each virus–host combination were positioned on top of foam ''washer'' supports placed on top of the opaque screening (to raise the leaves slightly and prevent stylet probing through the mesh) and secured with an inverted glass funnel. The amount of leaf area placed above the arena was equal for both treatments and the leaves chosen for use from each choice plant were matched based on position (age). Squash plants were 3 weeks post-inoculation and pepper plants were 4 weeks post-inoculation. Visual cues around the arena were obscured by an opaque paper screen, and the number of aphids present below each leaf was recorded every 15 min for 75 min (subsamples). The total number of aphids responding over the entire time period was determined and divided by 5 (the number of time points) to obtain an average number of responders for each treatment within each individual test replicate (the sample) (as in Mauck et al. 2010). We used *A. gossypii* for all volatile-based tests since preliminary transmission tests showed it to be the most

efficient vector for both virus genotypes, and since we have typically seen a stronger response to volatile cues in general with *A. gossypii* relative to *M. persicae*. Pre-tests were performed with the assay to ensure that aphids were attracted to volatiles of both hosts (squash or pepper vs. an artificial leaf providing no volatile cues). All tests were performed between 1 and 4 pm on identical windowsills when the weather was sunny and calm, and we typically performed two to three simultaneous replicate tests per day (each with a separate set of plants) over a period of 3–4 days. Positions of plants were varied to avoid always placing one treatment on one side of the arena. For each virus–host combination, nine to ten total replicates were performed and results were analyzed using two-sample *T*-tests (Minitab v. 14). Sample sizes are consistent with previously published studies including similar experiments (e.g., Eigenbrode et al. 2002; Medina-Ortega et al. 2009; Mauck et al. 2010; Rajabaskar et al. 2013).

Volatile collection and analysis

Volatiles were sampled from individual plants using a push-pull sampling system set up in a greenhouse with natural and artificial lighting (16:8 photoperiod) ($N = 6$ plants per inoculation treatment per virus–host combination). Squash plants (3 weeks post-inoculation) were enclosed in 9 l glass chambers fitted above a guillotine base, which closed around the stem of the plant, and pepper plants (4 weeks post-inoculation) were enclosed in similar 3 l glass domes. Different chamber sizes were used to accommodate different plant sizes and ensure that plants were not crushed or crowded when in the chambers (which would cause stress). Clean, charcoal-filtered air was pumped into the chambers at a rate of 5 l/min for the squash, and 2.5 l/min for peppers. Headspace was sampled from the chambers at a rate of 1 l/min through ports fitted with adsorbent volatile traps. Traps contained 45 mg of Super-Q adsorbent (Altech). To prevent the loss of small molecular weight compounds due to continuous air flow across the adsorbent, headspace was collected on multiple filters over the course of the day (three filters for each squash plant and two filters for each pepper plant) and final volatile amounts for each time point were summed to obtain a total emission for each volatile over the course of the entire day. Due to logistical constraints (available number of ports for simultaneous sampling), we performed each virus–host combination collection as a separate experiment. P1-CMV-infected peppers were sampled in March 2011, KVPG2-CMV-infected peppers were sampled in August 2011, and KVPG2-CMV-infected squash were sampled in the month of November 2011. Because of this there are small differences in the volatile blends of control pepper plants (volatile emissions vary with light intensity, temperature, and other factors—Niinemets et al. 2004). However, since our goal was to compare each infection treatment to its simultaneously sampled control, this separation of collection times does not significantly impacts our results.

Volatiles were eluted from Super-Q traps using 150 μl of high purity dichloromethane (Burdick and Jackson) and combined with 5 μl of internal standard mixture containing 80 ng/μl of nonyl acetate and 40 ng/μl of n-octane (Sigma). Quantification was performed using an Agilent 6890 gas chromatograph fitted with a flame ionization detector and an Agilent HP-1 column (15 m × 0.25 mm, 0.25 μm film thickness). Helium was used as a carrier gas at a flow rate through the column of 0.7 ml/min. One microliter of sample was injected into the inlet set to splitless mode and held at 250°C. The oven was held at 35°C for 0.5 min and then temperature was increased at a rate of 8°C/min up to 220°C with a post run hold at 275°C for 2 min. Chromatograms were recorded and processed using Agilent's Chemstation software (2003). Retention indices were calculated for each compound relative to a standard mix of alkanes (Sigma). Samples, selected standards, and the alkane mixture were also run on an Agilent 6890 GC fitted with an Agilent 5973 *Network* Mass Selective Detector in electron ionization mode fitted with an HP-1MS column (30 m × 0.25 mm, 0.25 μm film thickness). The same temperature program was used with the source set to 230°C, the transfer line set to 280°C, and scanning from mass 30 to 550. Tentative identifications were made based on comparison of retention times, retention indices, and mass spectra with select pure standards (when available) run on the same instruments and with the NIST spectral library and published retention indices (NIST Webbook, www.pherobase.com). Total volatiles were calculated for each replicate plant and the effect of infection status on total volatile emissions was analyzed using the Mann–Whitney test (Minitab v. 14). The contribution of each volatile component to the total blend was also calculated for each replicate plant by dividing the total emission for each compound by the total volatiles emitted. Proportions were then compared for infected and healthy plants within each virus–host combination using non-parametric *T*-tests in order to determine whether the relative ratios of different compounds varied due to infection. As standard collection methods have been

found to consistently induce the release of a stress volatile in Solanaceous plants—the homoterpene (3E,7E)-4,8,12-trimethyl-1-3-7-11-tri-decatetraene (TMTT) (Takabayashi et al. 1994)—this compound was analyzed separately for all pepper collections (Supplementary Fig. S1).

Measurement of virus titers

KVPG2-CMV inoculum was prepared and mechanically inoculated into 10 squash plants in the cotyledon stage and 10 pepper plants with their first true leaves just emerged, as described above. Inoculations alternated between host plant species and took ~15 min to perform, ensuring that the inoculum was fully viable throughout the inoculations. Pepper plants were similarly inoculated with P1-CMV and grown under the same conditions as the KVPG2-CMV plants. After 3 weeks of growth (as described above), samples of tissue were taken from the most recent fully expanded leaf of each symptomatic plant (10 squash and 6 peppers for KVPG2-CMV, 7 peppers for P1-CMV) by using a 6 mm diameter cork borer to punch 10 discs from random points throughout the entire leaf. Discs were quickly weighed, placed in Eppendorf tubes with three stainless steel balls (4 mm diameter), and flash frozen in liquid nitrogen. The cork borer was cleaned and dried in between samples. Samples were ground to a fine powder in liquid nitrogen-cooled cyroblocks using a Geno/Grinder for 1 min at 1100 shakes per minute. Virus titer was determined using the Agdia DAS-ELISA kit for CMV (covers all strains). The amount of buffer added to each sample was standardized to the nearest microliter based on the weight of sample collected, and ELISA was performed for each sample in duplicate according to the manufacturer's instructions and absorbance values averaged across the two wells to obtain a final value. Absorbance above background (buffer) levels was measured at 490 nm on a Spectramax 190 Spectrophotometer by designating blank wells and using software (Spectramax Pro) to automatically subtract background absorbance from values for sample wells. Positive controls from Agdia were included to verify that the test functioned correctly. Data were analyzed using a non-parametric ANOVA (Kruskal–Wallis test) with multiple comparisons using the Mann–Whitney test (Minitab v. 14).

Results

Plant quality and aphid emigration assays

When the squash isolate, KVPG2-CMV, infected the native squash host (*C. pepo*), plant quality was reduced relative to healthy hosts for both *A. gossypii* and *M. persicae* (Fig. 1A and C) (*A. gossypii* $T=-4.51$, $df=13$, $P=0.001$; *M. persicae* $T=-3.79$, $df=13$, $P=0.002$). Alate *A. gossypii*, for which squash is a preferred host plant, responded to this reduced quality with an increased rate of emigration from infected plants (Fig. 1B) (significant infection treatment × time effect in repeated measures ANOVA $F=6.80$, $df=2$, $P=0.003$; 120 min $T=-2.35$, $df=13$, $P=0.035$; 24 h $T=-4.89$, $df=11$, $P<0.0001$) and dispersal to healthy plants (mean aphids per choice plant at 24 h: healthy mock = 9, infected = 1.22, $T=-4.74$, $df=9$, $P=0.001$). *Myzus persicae*, for which squash is not an optimal host, did not disperse at different rates from infected versus healthy plants, but had very high overall rates of dispersal from squash (around 5 aphids remaining on release plants after 24 h, relative to around 25 *A. gossypii* remaining on healthy release plants) (Fig. 1D) (significant time point effect in repeated measures ANOVA $F=1993.01$, $df=2$, $P=0.001$).

In contrast to these results, the KVPG2-CMV isolate increased host quality when infecting the novel host pepper (*C. annuum*). *Myzus persicae* populations were significantly higher on KVPG2-CMV-infected pepper relative to healthy pepper (Fig. 2A) ($T=3.03$, $df=18$, $P=0.007$). Additionally, in emigration tests, *M. persicae* preferred to remain on KVPG2-CMV-infected pepper plants rather than disperse to healthy, mock-inoculated pepper hosts (Fig. 2B) (significant infection status effect in repeated measures ANOVA $F=135.19$, $df=1$, $P=0.007$). Of alates that did disperse, those that left healthy plants showed a trend toward preferring infected plants (mean aphids per choice plant at 24 h: healthy mock = 1.33, infected = 3.5, $T=1.86$, $df=7$, $P=0.106$). Tests with *A. gossypii*, which does not prefer pepper as a host, mirrored those done with *M. persicae* on the non-preferred squash host: *A. gossypii* alates did not disperse at different rates from infected versus healthy plants, but exhibited high overall rates of dispersal from pepper plants regardless of infection status (Fig. 2C) (significant time point effect in repeated measures ANOVA $F=35.30$, $df=2$, $P=0.028$).

The pepper isolate, P1-CMV, was tested in the native host only, since it was only rarely able to successfully infect squash hosts—only two plants showed symptoms out of ~75 inoculated in several separate trials. Populations of *M. persicae* were not significantly different on P1-CMV-infected versus healthy mock-inoculated pepper hosts, but showed a trend toward reduced population growth on

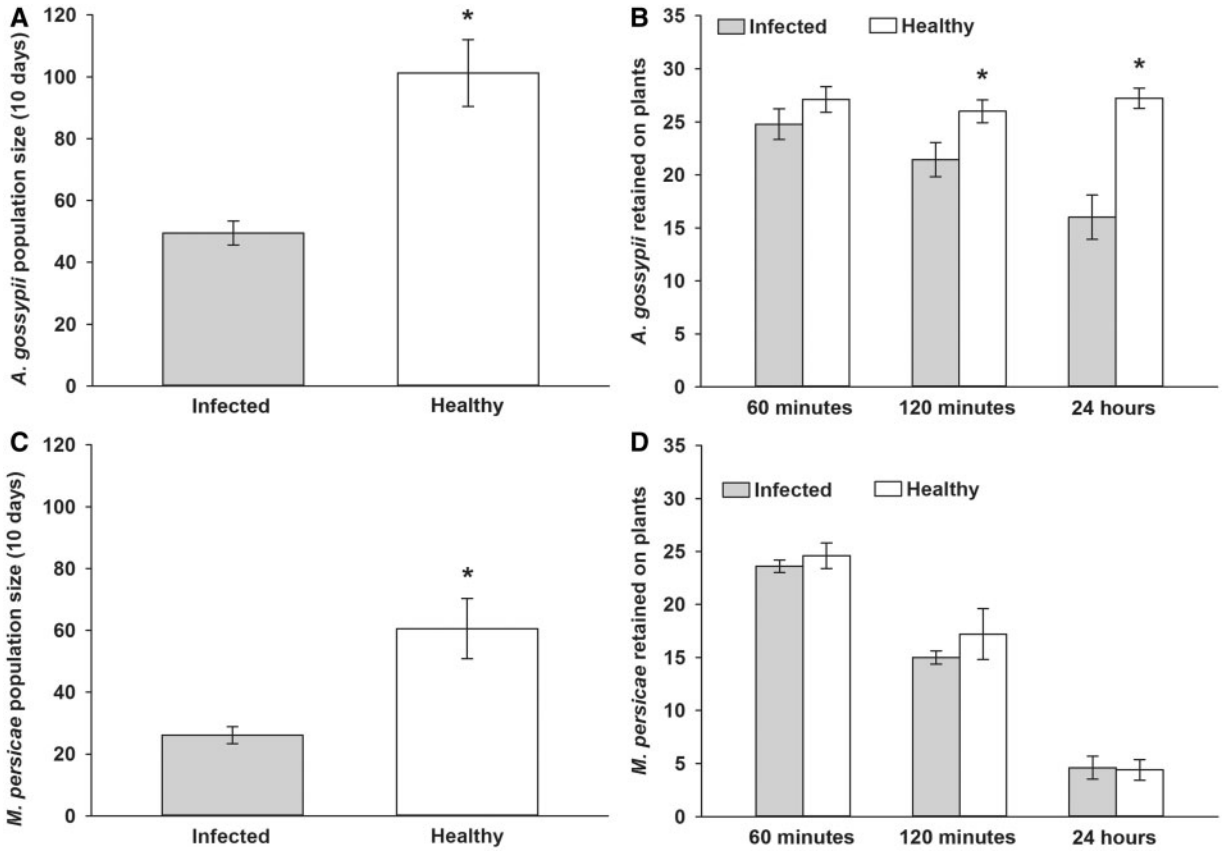

Fig. 1 Quality and palatability of KVPG2-CMV-infected squash to aphid vectors. (**A**) *Aphis gossypii* population size on infected and healthy (mock-inoculated) plants after 10 days ($N = 12$). (**B**) Retention of *A. gossypii* on infected and healthy release plants over three time points in emigration experiments ($N = 9$ tests per release plant type). (**C**) *Myzus persicae* population size on infected and healthy plants after 10 days ($N = 9$). (**D**) Retention of *M. persicae* on infected and healthy release plants over three time points in emigration experiments ($N = 5$ tests per release plant type). In all graphs, bars show mean ± SE and * indicates significance at $P < 0.05$.

infected hosts (Fig. 3A) ($T = -1.59$, df = 15, $P = 0.133$). Similarly, in emigration tests, dispersal from release plants was low overall, with no significant difference between infected and healthy plants, but again trends toward lower retention of aphids on infected plants (Fig. 3B) (marginal effect of infection status on aphid dispersal $F = 9.68$, df = 1, $P = 0.09$).

Volatile analysis, odor-based choice tests and virus titers

KVPG2-CMV infection in the native squash host resulted in enhanced attraction of *A. gossypii* alates in odor-based choice tests that excluded contact and visual cues (Fig. 4A) ($T = 2.41$, df = 10, $P = 0.037$). Analysis of volatile emissions over a 12-h period demonstrated that infected hosts produced overall larger quantities of volatiles per unit of leaf tissue (Fig. 4B) (Mann–Whitney test $W = 52$, $P = 0.0453$, $N = 6$/treatment). When each volatile is converted to a proportion of the total amount released for each plant, and compared between infected and healthy plants, it is evident that there is little change in the relative ratios. Only two compounds differ significantly between infected and healthy plants in terms of the relative percentage of the total blend (Fig. 4C). These were ethyl acetophenone isomer 2 ($H = 5.77$, df = 1, $P = 0.016$) and an unknown compound ($H = 3.69$, df = 1, $P = 0.022$)—designated R and T, respectively, in Fig. 4C. Two other compounds exhibited insignificant trends: ethylbenzaldehyde isomer 1 ($H = 3.71$, df = 1, $P = 0.054$) and another unknown compound ($H = 3.58$, df = 1, $P = 0.059$)—designated H and A, respectively, in Fig. 4C.

KVPG2-CMV infection in the novel pepper host did not result in enhanced attraction of alate *A. gossypii* in odor-based choice tests (Fig. 5A) ($T = 1.53$, df = 11, $P = 0.154$). Infection also did not influence the total volatile emissions relative to healthy mock-inoculated plants (Fig. 5B) (Mann–Whitney $W = 44$, $P = 0.471$, $N = 6$/treatment). However, infection did have a significant influence on the ratio of

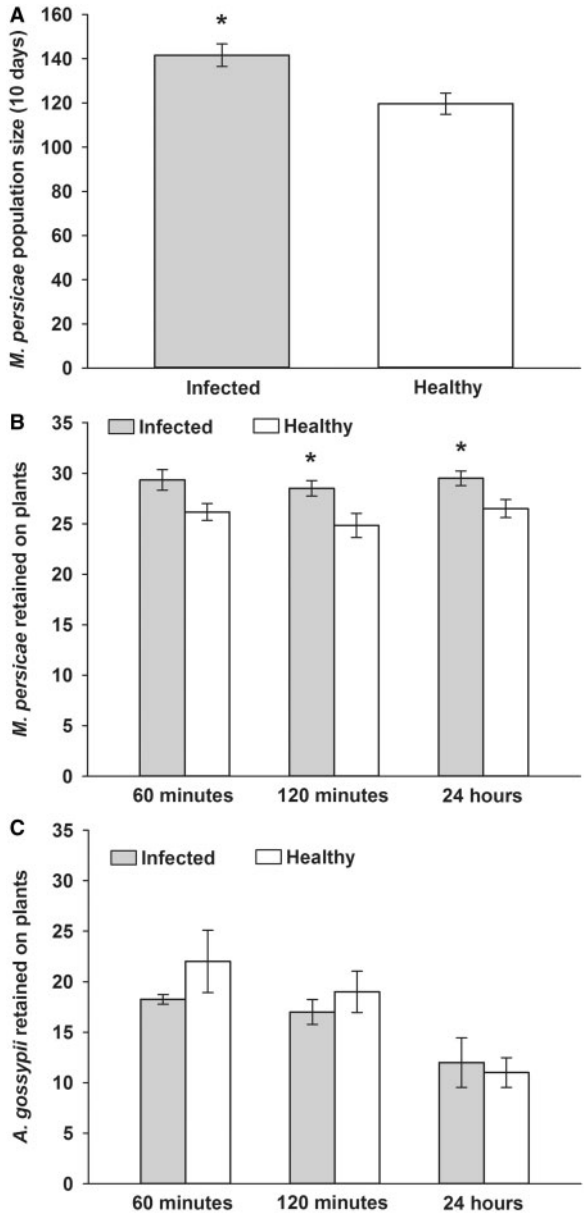

Fig. 2 Quality and palatability of KVPG2-CMV-infected pepper to aphid vectors. (**A**) *Myzus persicae* population size on infected and healthy (mock-inoculated) plants after 10 days ($N = 12$ infected, $N = 10$ healthy). (**B**) Retention of *M. persicae* on infected and healthy release plants over three time points in emigration experiments ($N = 9$ tests per release plant type). (**C**) Retention of *A. gossypii* on infected and healthy release plants over three time points in emigration experiments ($N = 4$ tests per release plant type). In all graphs, bars show mean ± SE and * indicates significance at $P < 0.05$.

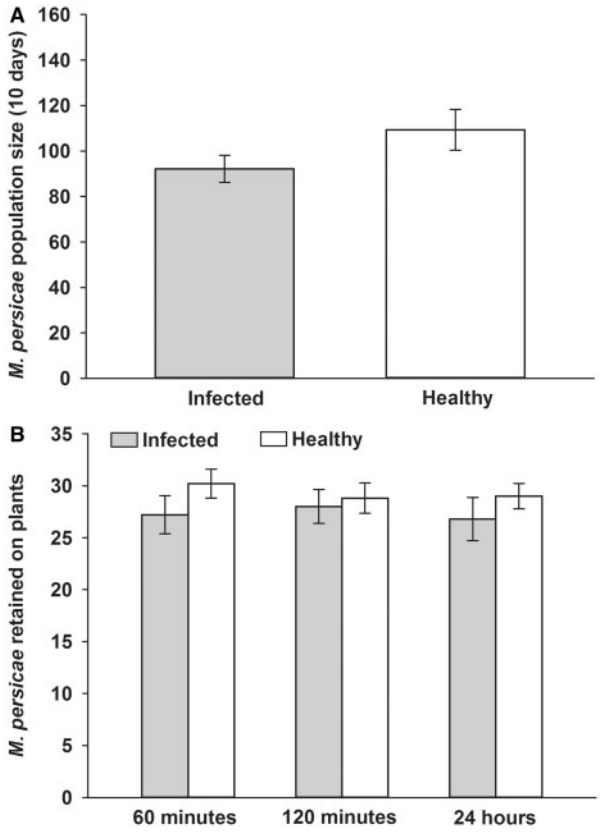

Fig. 3 Quality and palatability of P1-CMV-infected pepper to *M. persicae*. (**A**) *Myzus persicae* population size on infected and healthy (mock-inoculated) plants after 10 days ($N = 10$). (**B**) Retention of *M. persicae* on infected and healthy release plants over three time points in emigration experiments ($N = 5$ tests per release plant type). In all graphs, bars show mean ± SE and * indicates significance at $P < 0.05$.

compounds within the blend (Fig. 5C). The proportion of the total blend differed significantly between infected and healthy plants for seven compounds (designated by the following numbers in Fig. 5C): (1) ethylbenzene [$H = 8.93$, df = 1, $P = 0.003$], (2) styrene [$H = 4.41$, df = 1, $P = 0.036$], (7) unknown [$H = 8.37$, df = 1, $P = 0.004$], (11) linalool [$H = 8.34$, df = 1, $P = 0.004$], (12) unknown terpene [$H = 7.44$, df = 1, $P = 0.006$], (14) naphthalene [$H = 5.04$, df = 1, $P = 0.025$], and (19) indole [$H = 8.34$, df = 1, $P = 0.004$]). Trends ($P < 0.10$) were observed for seven more compounds: (3) alpha-pinene ($H = 2.85$, df = 1, $P = 0.092$), (5) 2-ethyl hexanal ($H = 3.16$, df = 1, $P = 0.076$), (9) limonene ($H = 3.11$, df = 1, $P = 0.076$), (10) *E*-beta ocimene ($H = 3.71$, df = 1, $P = 0.054$), (16) decanal ($H = 3.11$, df = 1, $P = 0.078$), (20) unknown ($H = 3.19$, df = 1, $P = 0.074$), and (21) trans-alpha bergamotene ($H = 3.11$, df = 1, $P = 0.078$). These differences were not consistently toward one infection treatment or the other—some compounds being a higher relative percentage of the infected blend, and others a higher relative percentage of the healthy blend (Fig. 5C). TMTT emissions did not differ between treatments, but were substantially more variable among replicates in the infected plant treatment

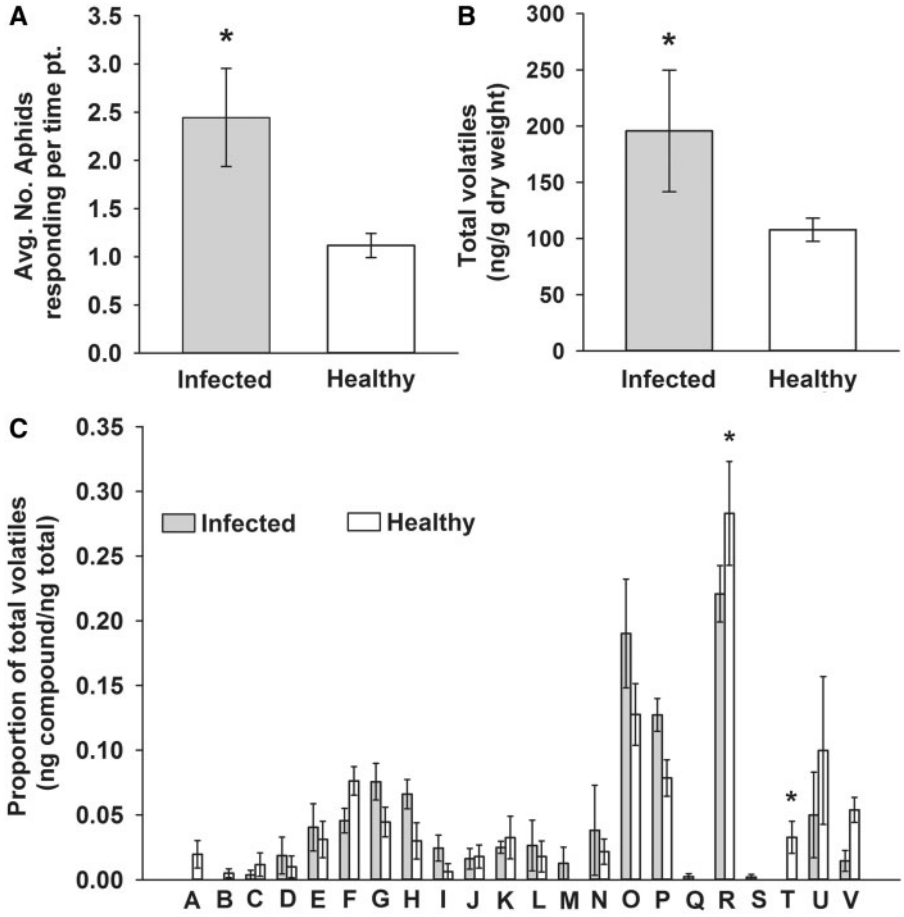

Fig. 4 Volatile emissions from KVPG2-CMV-infected squash and vector preferences. (**A**) Settling preferences of *A. gossypii* in arena-based choice tests that presented only odor cues ($N = 9$ tests) (mean ± SE). (**B**) Total volatile emissions from KVPG2-CMV-infected and healthy (mock-inoculated) squash plants over a 12-h daylight period ($N = 6$) (mean ± SE). (**C**) Volatile composition of the total blend. Each bar shows the contribution of a single compound (indicated by letters) to the total blend (average proportion across all samples within a treatment ± SE). * indicates significant differences at $P < 0.05$. A = unknown, B = unknown, C = limonene, D = *E*-β-ocimene, E = unknown, F = nonanal, G = linalool, H = ethylbenzaldehyde isomer 1, I = ethylbenzaldehyde isomer 2, J = unknown, K = 1,4-benzenedicarboxaldehyde, L = unknown aromatic compound, M = unknown monoterpene, N = ethyl acetophenone isomer 1, O = unknown, P = ethyl acetophenone isomer 2, Q = acetylacetophenone isomer 1, R = acetylacetophenone isomer 2, S = α-humulene, T = unknown, U = caryophyllene oxide, V = (3E,7E)-4,8,12-trimethyl-1-3-7-11-tri-decatetraene.

(Supplementary Fig. S1A [Mann–Whitney $W = 32$, $P = 0.298$]).

P1-CMV infection in the native pepper host did not result in enhanced attraction of alate *A. gossypii* in odor-based choice-tests (Fig. 6A) ($T = -1.61$, df = 17, $P = 0.126$) and did not significantly increase volatile emissions (Fig. 6B) (Mann–Whitney $W = 40$, $P = 0.936$, $N = 6$/treatment). However, in contrast to infection of pepper with KVPG2-CMV, P1-CMV infection did not strongly alter the ratio of compounds in the blend (Fig. 6C). Instead, similar to the squash isolate in its native host, the pepper isolate induced only minor changes, with the relative percentage one compound differing between infected and healthy plants: unknown 2 ($H = 3.97$, df = 1, $P = 0.046$)—designated K in Fig. 6C. One other compound exhibited an insignificant trend: indole ($H = 3.69$, df = 1, $P = 0.055$)—designated P in Fig. 6C. TMTT emissions did not differ between treatments, but similar to the KVPG2-CMV-infected peppers, the P1-CMV-infected peppers showed more variation among replicates in emission levels (Supplementary Fig. S1B [Mann–Whitney $W = 45$, $P = 0.379$]).

Titers of each isolate differed significantly among the different virus–host combinations (Fig. 7) (non-parametric ANOVA [Kruskal–Wallis] with isolate-host combination as the factor: $H = 19.23$, df = 2, $P < 0.0001$). The lowest titer level was observed for the squash isolate, KVPG2-CMV, infecting the novel host pepper (KVPG2-CMV-pepper vs. KVPG2-CMV-squash $W = 21$, $P = 0.001$; KVPG2-CMV-pepper vs. P1-CMV-pepper $W = 21$, $P = 0.003$).

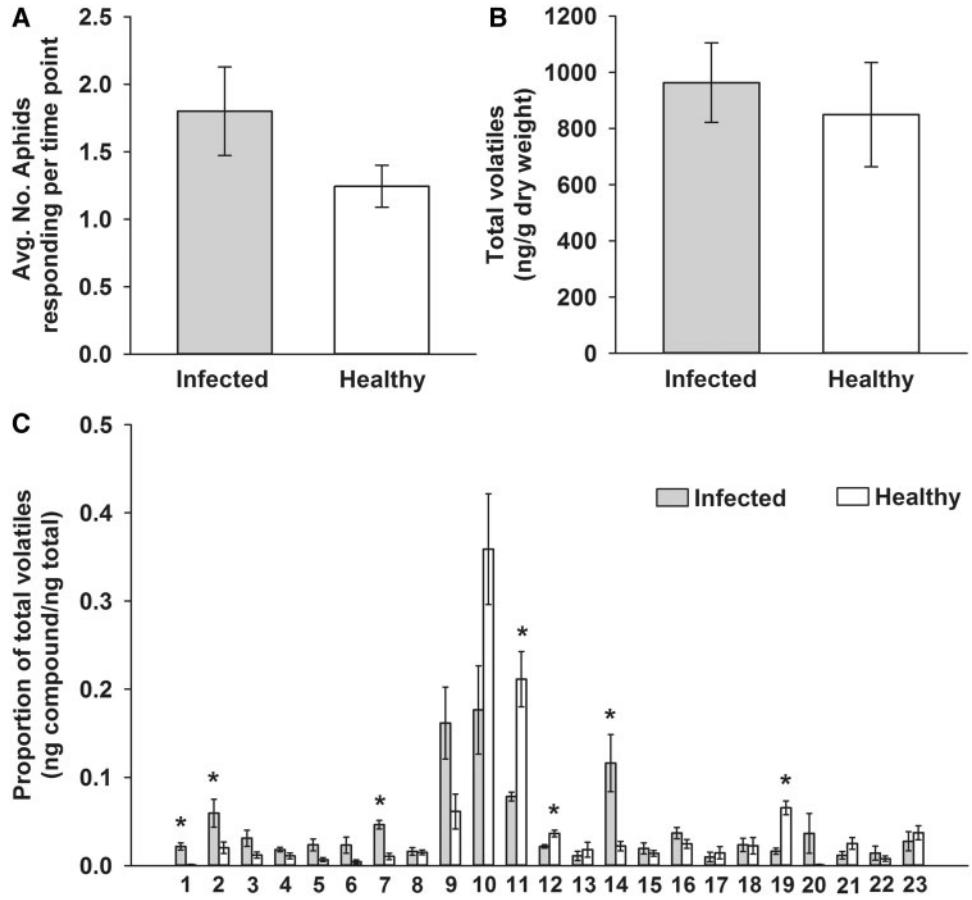

Fig. 5 Volatile emissions from KVPG2-CMV-infected pepper and vector preferences. (**A**) Settling preferences of *A. gossypii* in arena-based choice tests that presented only odor cues ($N = 9$ tests) (mean ± SE). (**B**) Total volatile emissions from KVPG2-CMV-infected and healthy (mock-inoculated) pepper plants over a 12-h daylight period ($N = 6$) (mean ± SE). (**C**) Volatile composition of the total blend. Each bar shows the contribution of a single compound (indicated as numbers) to the total blend (average proportion across all samples within a treatment ± SE). * indicates significant differences at $P < 0.05$. 1 = ethylbenzene, 2 = styrene, 3 = α-pinene, 4 = unknown, 5 = 2-ethyl hexanal, 6 = β-pinene, 7 = unknown, 8 = myrcene, 9 = limonene, 10 = *E*-β-ocimene, 11 = linalool, 12 = unknown terpene, 13 = unknown, 14 = naphthalene, 15 = unknown, 16 = decanal, 17 = unknown, 18 = benzothiazole, 19 = indole, 20 = unknown, 21 = *trans*-α-bergamotene, 22 = unknown, 23 = unknown.

The same virus in the native squash host reached a much higher titer, and the pepper isolate, P1-CMV, also reached a higher titer in the native pepper host relative to the novel virus–host combination and the squash isolate in its native host (KVPG2-CMV-squash vs. P1-CMV-pepper $W = 55$, $P = 0.0008$).

Discussion

Evidence of local adaptation in multi-host plant pathogens has previously been reported for fitness relevant traits including infectivity and virus accumulation (Sacristán et al. 2005; Malpica et al. 2006; Agudelo-Romero et al. 2008; Lalić et al. 2011), and it has been suggested that such adaptation may play an important role in facilitating the ecological success of such pathogens (Malpica et al. 2006). The current study provides evidence that such local-host adaptation occurs in CMV and that it can also influence host–plant traits that mediate interactions with insect vectors. We found that a CMV strain isolated from pepper (P1-CMV) was only sporadically able to infect squash plants, while a newly isolated strain from squash (KVPG2-CMV) successfully infects pepper but reaches titers significantly lower than those observed for this strain in its native host. Furthermore, in the novel pepper host KVPG2-CMV induced changes in host–plant traits relevant to host–vector interactions that appear maladaptive with respect to transmission, while host–plant phenotypes were more conducive to transmission when each strain infected its native host.

KVPG2-CMV induced a suite of changes in traits of its native squash host similar to those that we

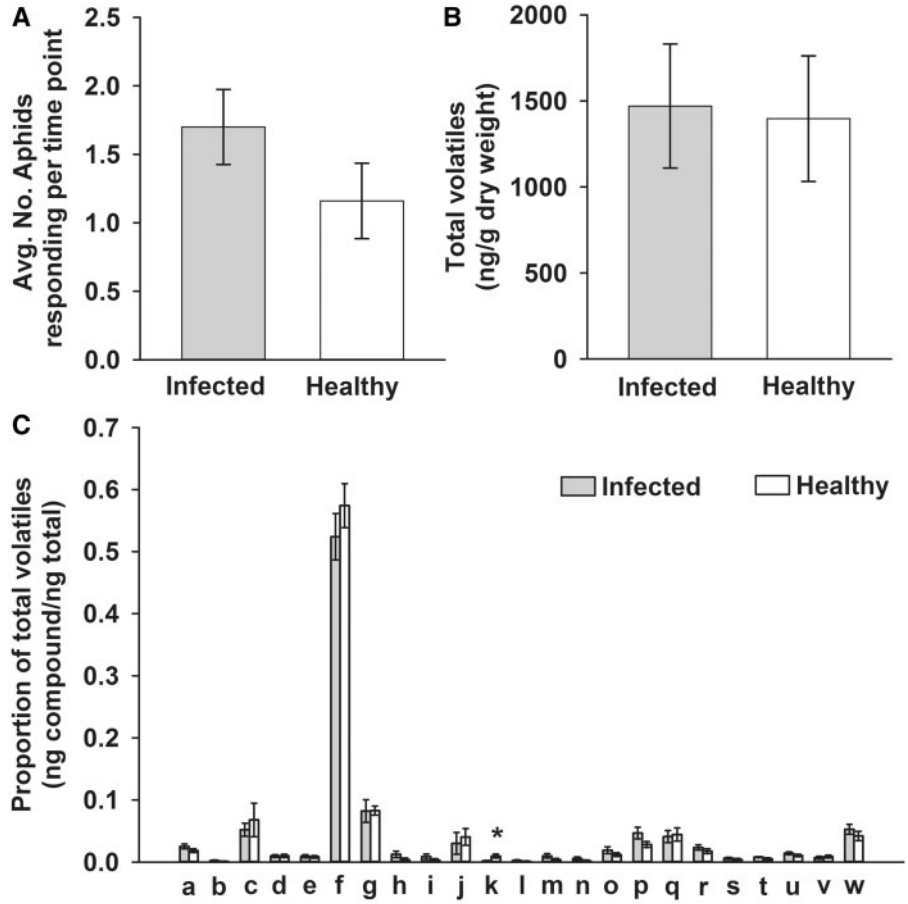

Fig. 6 Volatile emissions from P1-CMV-infected pepper and vector preferences. (**A**) Settling preferences of *A. gossypii* in arena-based choice tests that presented only odor cues ($N = 10$ tests) (mean ± SE). (**B**) Total volatile emissions from P1-CMV-infected and healthy (mock-inoculated) pepper plants over a 12-h daylight period ($N = 6$) (mean ± SE). (**C**) Volatile composition of the total blend. Each bar shows the contribution of a single compound (indicated as lowercase letters) to the total blend (average proportion across all samples within a treatment ± SE). * indicates significant differences at $P < 0.05$. a = myrcene, b = limonene, c = *E*-β-ocimene, d = *cis*-linalool oxide, e = *trans*-linalool oxide, f = linalool, g = unknown, h = ethyl benzaldehyde isomer 1, i = ethyl benzaldehyde isomer 2, j = *Z*-3-hexenyl butyrate, k = unknown, l = unknown, m = unknown, n = ethyl acetophenone, o = 3,7-dimethyl-2,6-octadienal, p = indole, q = β-elemene, r = *trans*-α-bergamotene, s = β-farnesene, t = unknown sesquiterpene 1, u = unknown sesquiterpene 2, v = Valencene, w = unknown.

previously reported for another isolate (CMV-FNY) in squash (Mauck et al. 2010). Host plant quality was significantly reduced both for the aphid *A. gossypii*, a strong colonizer that prefers squash as a host, and for *M. persicae*, a weak colonizer that prefers (and performs better on) Brassicaceae and Solanaceae. In addition, infection by KVPG2-CMV elicited a significant increase in rates of dispersal (following initial exposure to host plants) by *A. gossypii*. No similar effect of infection on dispersal rates was observed for *M. persicae*; however, this aphid exhibited very high rates of dispersal from squash plants regardless of whether the plants were infected by CMV-KVPG2, likely reflecting the poor quality of this plant as a host for this aphid. Given the strong expectation that NPT viruses benefit from rapid aphid dispersal following initial probes (Martín et al. 1997; Wang and Ghabrial 2002), these findings are consistent with effective transmission of KVPG2-CMV from squash by both strong and weak aphid colonizers. They are also consistent with effects of CMV-FNY (another isolate to which squash is highly susceptible) on carbohydrate to amino-acid ratios in the phloem, and in leaf tissues where aphids initially probe and acquire gustatory cues (Mauck et al. 2014). Carmo-Sousa et al. (2014) recently obtained complementary results showing that infection of melon plants by an isolate of CMV originally collected from melon crops has a deterrent effect on feeding by *A. gossypii*, as assessed through electrical penetration graphing.

KVPG2-CMV infection also elicited elevated volatile emissions from squash, with relatively minor effects on the composition of the blend, consistent with our previous findings for CMV-FNY infecting

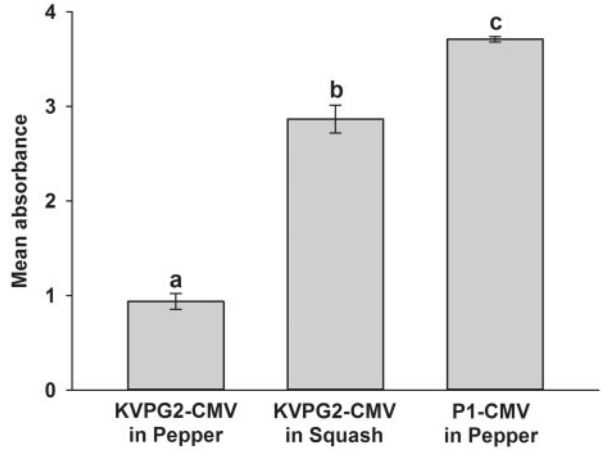

Fig. 7 Virus titers in different virus–host combinations. Bars represent the mean absorbance after accounting for buffer controls (background) ± SE. Letters indicate significant differences ($P < 0.05$) among different virus–host combinations in post-hoc comparisons performed following a non-parametric ANOVA (Kruskal–Wallis test). $N = 10$ squash and 6 peppers for KVPG2-CMV, 7 peppers for P1-CMV.

squash (Mauck et al. 2010). And we also observed increased aphid attraction to the odors of infected plants in olfactometer assays, again consistent with our previous results (Mauck et al. 2010). Preferential vector attraction to infected hosts and, perhaps equally or more important, the avoidance of vector discrimination against those hosts can facilitate transmission (Sisterson 2008; Roosien et al. 2013), and this is likely to be particularly true for NPT viruses, which must be re-acquired by individual vectors after each inoculation event (Ng and Falk 2006).

P1-CMV exhibited much weaker effects on host traits influencing interactions with aphid vectors than those observed for KVPG2-CMV (and previously for CMV-FNY) in squash. Indeed we observed no significant effects on any of the traits examined, although most showed non-significant trends in the directions observed for the squash isolates (i.e., toward reduced aphid performance, increased aphid dispersal, elevated volatile emissions, and enhanced aphid attraction). Furthermore, there were few changes in the composition of the volatile blend in P1-CMV-infected plants. The observations clearly do not provide evidence of manipulation of host–plant phenotypes by P1-CMV; however, they are consistent with our expectation that well-adapted pathogens should exhibit neutral to positive effects (from the pathogen's point of view) on host–vector interactions, as selection is likely to act against effects that have significant adverse effects on transmission (Mauck et al. 2012).

Also consistent with that expectation, the squash-adapted isolate KVPG2-CMV elicited effects on the phenotype of the novel host pepper that would appear detrimental to transmission by aphid vectors, which, as discussed above, is thought to be favored by rapid aphid dispersal and disfavored by the initiation of long-term feeding. Infection by this isolate enhanced population growth of *M. persicae*, a strong colonizer of pepper. And this aphid also exhibited reduced dispersal from infected relative to healthy plants. (The other aphid species examined, *A. gossypii*, performed very poorly on pepper—our strain could not survive on this host plant for more than 4 days—and exhibited high rates of dispersal from this host regardless of infection status, similar to the pattern observed for *M. persicae* on squash.) KVPG2-CMV also induced dramatic changes in the composition of the volatile blend emitted by infected pepper plants relative to healthy controls. While this altered blend might be expected to provide a salient cue for vectors, we observed no effect on aphid preferences; however, we would not necessarily expect to see such an effect in this instance, as the aphids employed in our assays are naïve with respect to this novel cue and have no experiential or evolutionary context in which to associate it with corresponding effects on host–plant quality. Furthermore, interpreting the ecological significance of the altered volatile emissions is complicated by the positive effects of KVPG2-CMV on the quality of pepper host plants for aphids, as the ability of CMV to elevate volatile emissions without inducing strong changes in blend composition has been hypothesized to function adaptively by mediating the ''deceptive'' attraction of vectors to infected hosts despite their poor quality (Mauck et al. 2010). In any event, the most salient observation drawn from the overall suite of effects observed for KVPG2-CMV is the dramatic departure from the pattern of effects observed for this strain in its native squash host and those observed for the pepper isolate P1-CMV, each of which appears significantly more conducive to efficient transmission by aphid vectors.

Taken together, the findings discussed above provide evidence of local adaption of CMV to hosts and suggest that such adaptation may extend to effects on host–plant traits mediating interactions with aphid vectors. We observed significantly reduced virus titers for KVPG2-CMV in the novel host pepper compared with those observed for either strain in its native host—virus accumulation is correlated with transmission success for CMV and other NPT viruses (Froissart et al. 2010)—and the superior performance of P1-CMV in pepper indicates that this

plant is not a universally poor host for CMV. Furthermore, in the course of preparing plants for these experiments, we also documented infectivity differences: each isolate infected nearly 100% of plants in its native host, whereas KVPG2-CMV typically infected only 60–70% of inoculated pepper plants, and P1-CMV successfully infected squash plants only sporadically. And, as discussed, the effects of KVPG2-CMV on host–plant traits mediating interactions with aphids were found to differ dramatically from those observed for either isolate in its native host, and in ways that would appear detrimental to transmission of the virus by the vector.

Regardless of whether CMV and other viruses may conclusively be said to manipulate host vector interactions, our results demonstrate that evolutionary history influences aspects of the infected host's phenotype that directly influence aphid behaviors relevant to transmission. This work thus builds on previous studies demonstrating that a host's physiological phenotype can influence its reservoir potential (e.g., Malmstrom et al. 2005; Borer et al. 2009). For example, ''quick return'' phenotypes (annual plants) tend to serve as better reservoirs for multi-host PT viruses than ''slow return'' phenotypes (long-lived perennials) because they are relatively less well defended and encourage vector feeding and reproduction (necessary for acquisition and spread of PT viruses) (Cronin et al. 2010). Our current findings suggest that virus effects on quality and palatability within a host species may further influence reservoir potential, so that hosts in which the virus induces a transmission-facilitating (beneficial) phenotype are more likely to serve as inoculum sources than hosts in which a non-beneficial phenotype is induced (e.g., Westwood et al. 2013). Under this scenario, selection may favor virus genotypes capable of inducing beneficial alterations to the phenotype of susceptible hosts that are common in the landscape at the expense of inducing such phenotypes in all possible hosts, an unavoidable consequence of the antagonistic pleiotropy thought to mediate virus specialization on frequently encountered hosts (Elena et al. 2009). Whether or not local adaptation among generalist viruses occurs in this way may be determined by the frequency of encounters with similar or disparate hosts, with a heterogeneous host environment tending to disfavor specialization, and a homogeneous host environment tending to favor local adaptation (Sacristán et al. 2004; Elena et al. 2009; Bedhomme et al. 2012). This may be one reason why we observed host-specific effects on phenotype using viruses obtained near the end of the season from cultivated monocultures of their native hosts. In the future, it would be interesting to explore the effects of viruses isolated from agricultural and natural environments when infecting both cultivated and wild host plants.

Local biotic interactions may also modify selection pressure on viruses, including possible selection for or against manipulative genotypes. For instance, the NPT virus PVY is a common pathogen of potato crops that can reach high titers without producing visible symptoms (Draper et al. 2002), and horizontal spread is thought to be driven mostly by non-colonizing aphids that are likely to probe and disperse from a non-host plant (Sigvald 1989; Kirchner et al. 2011; Boquel et al. 2012) as well as vertical spread through infected tubers (Crosslin et al. 2006). So, it is not surprising that most researchers have found neutral effects (Castle and Berger 1993; Eigenbrode et al. 2002; Srinivasan and Alvarez 2007) to occasional positive effects (Srinivasan and Alvarez 2007; Kersch-Becker and Thaler 2013) of PVY on potato-colonizing aphid vector performance. Non-colonizing vectors would respond primarily to host identity regardless of infection status (as seen in our results), which would not favor manipulative variants over non-manipulative variants. The presence of predators or parasitoids in a local system may also modify selection pressure by alleviating non-dispersal of colonizing vectors from infected plants (reviewed in Finke 2012). Aphid parasitoid attack, in particular, increases spread of NPT viruses by colonizers through non-consumptive interactions (disturbance and induction of aphid alarm pheromone emission) that stimulate the probe-and-wander behavior necessary for NPT virus acquisition and inoculation (Hodge et al. 2011; Jeger et al. 2011; Dáder et al. 2012). Thus, although we see a strong pattern of NPT viruses reducing host palatability and quality for vectors and encouraging dispersal after acquisition (reviewed in Mauck et al. 2012, see also Carmo-Sousa et al. 2014; Westwood et al. 2013) or at least having neutral effects on these parameters (Salvaudon et al. 2013), there are clearly exceptions to this pattern among the as-yet studied NPT viruses (Salvaudon et al. 2013; Kersch-Becker and Thaler 2013), and these exceptions may be due to other, consistently present biotic factors that neutralize selection for viral variants that reduce host palatability to or quality for vectors. As seen from our data crossing KVPG2-CMV into a novel pepper host, exceptions to the pattern may also be due to a lack of previous interaction with or adaptation to a potential host plant. Future work should consider virus history with a given host when exploring virus-induced host

phenotypes, mechanisms of phenotype induction, and implications for virus transmission in different types of plant communities.

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Supplementary data

Supplementary Data available at *ICB* online.

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