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Abstract

Plant viruses can change hosts in ways that increase vector contacts, virion acquisition, and subsequent vector dispersal to susceptible hosts. Based on this, researchers have proposed that virus-induced phenotypes are the product of adaptations to “manipulate” hosts in ways that increase transmission. Theoretical models of virus spread in crops support this proposition; “manipulative” viruses spread faster and to a greater extent. However, both empirical and theoretical studies on manipulation are disproportionately focused on a few persistently transmitted pathogens, and rarely consider the broader ecological implications of virus infections . To address these knowledge gaps, we documented the effects of different stages of infection by an economically devastating, semi-persistently transmitted crinivirus, *Cucurbit yellow stunting disorder virus* [CYSDV] on *Cucumis melo* (muskmelon) phenotypes, behavior and performance of whitefly vectors (*Bemisia tabaci*) and non-vector aphid competitors (*Aphis gossypii*). Whiteflies were strongly attracted to CYSDV-infected hosts in a symptomatic stage of disease , but not in an asymptomatic stage, and fed more easily on infected plants regardless of symptoms . In contrast, aphids tended to avoid infected hosts, fed for shorter periods of time, and produced fewer offspring on infected hosts . Metabolomics revealed that host manipulations by CYSDV do not rely on virus-induced shifts in leaf primary metabolites or volatiles but may involve changes to phloem architecture and other compounds not measured here. Our study demonstrates a sophisticated host manipulation by CYSDV, whereby infection discourages colonization by a non-vector competitor while inducing a suite of progressively more transmission-conducive changes that encourage virion acquisition by the vector.

Keywords
disease progression, electrical penetration graph, plant virus manipulation, plant volatiles, vector behavior, virus ecology

Declarations

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Availability of data and material UC Riverside partners with Dryad data repository. All data will be made available through Dryad once a manuscript number is assigned to complete the dataset submission. The manuscript number is required to link data to a manuscript in progress. This data statement will be updated should the manuscript be accepted.

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Authors' Contributions KEM and QC conceived the ideas and designed experiments; PS and KEM designed methods for metabolite analysis; QC and PS collected the data; QC led data analysis with input from KEM; KEM led writing of the manuscript and all authors contributed critically to the drafts and gave final approval for publication.

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Consent to participate (N/A)

Consent to publication (N/A)

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Key Message

- Plant viruses may evolve to manipulate hosts in ways that encourage transmission by vectors.
- Manipulation work focuses on a narrow range of viruses and excludes most ecological contexts.
- We studied effects of CYSDV: a virus with an understudied semi-persistent transmission mode.
- We evaluated host phenotypes across disease progression and vector-competitor interactions.
- CYSDV manipulates hosts to increase vector contacts and decrease feeding by non-vector pests.
- Host manipulation by CYSDV occurs through multiple routes and can be a target for management.

Introduction

Virus infections often alter plant phenotypes, with significant consequences for host survival, fitness, and interactions with other organisms (Davis et al. 2015; Eigenbrode et al. 2017; Mauck et al. 2018; González et al. 2020). In the case of arthropod-transmitted plant viruses, such effects can also influence interactions with the mobile vectors. Given the importance of vector-host interactions for transmission, we might expect that selection should favor viruses that change host phenotypes in ways that increase (or at least maintain) vector contacts and feeding behaviors that facilitate virion acquisition (Mauck et al. 2016). In line with this expectation, there are now numerous published reports of viruses altering host phenotypes in ways that should enhance dissemination by vectors (Eigenbrode et al. 2017; Mauck et al. 2018). The bulk of these studies document changes in vector orientation, feeding, and/or dispersal behaviors through choice and no-choice behavioral bioassays (reviewed in (Mauck et al. 2018; Mauck and Chesnais 2020)) and a small number have identified specific host metabolic changes and virus components responsible for eliciting these effects (reviewed in (Mauck et al. 2019; Ziegler-Graff 2020)). Building upon empirical work, several mathematical modeling papers suggest that “manipulative” plant viruses spread more rapidly and to a greater extent, especially in monocultures, relative to those having no effect on host-vector interactions (Roosien et al. 2013; Shaw et al. 2017). Collectively, this body of work provides mounting evidence that virus effects on host phenotypes can influence the probability of subsequent transmission by vectors, and that such effects may be the product of virus adaptations that persist because of the transmission benefits they confer.

The idea that plant viruses can be selected for “manipulating” their hosts to enhance plant-vector contacts has fueled an increasing number of studies across a growing diversity

of pathosystems (Mauck et al. 2018). However, these are strongly biased toward a few taxa with limited diversity of transmission modes. For example, in a survey of virus effects on host phenotypes, we found that viruses having a circulative, persistent transmission mode were overrepresented among both empirical and theoretical studies, and that within this category, the majority of studies focused on viruses from just one family - *Luteoviridae* (Mauck et al. 2018; Mauck and Chesnais 2020). As a result, the study area of 'virus manipulation' lacks information on some of the most important emerging pathogens of concern for agriculture, especially viruses with semi-persistent transmission modes and highly polyphagous vectors (Tzanetakis et al. 2013; Fereres et al. 2016; Maluta et al. 2017; Maluta et al. 2019; Pereira et al. 2019; Ertunc 2020). Beyond limitations on the taxonomic diversity of studied pathosystems, our understanding of virus manipulations and its implications for agriculture is further limited by an emphasis on overly simplified scenarios in empirical work. Even for the most well-studied pathosystems, only a handful of studies, if any, have considered virus manipulation of hosts and vectors in the context of disease progression, host survival, and species interactions among manipulated hosts and other organisms (Mauck and Chesnais 2020).

These omissions limit our ability to discern if virus-induced phenotypes are robust within the very environments in which manipulative virus traits are purported to have evolved. Although time and disease progression are major considerations in plant virus epidemiology, documented instances of putative host manipulation by plant viruses overwhelmingly focus on a single time point. Arbitrary time point selection by a researcher will potentially determine whether a virus-induced phenotype is concluded to be adaptive for the virus (neutral or transmission-enhancing), or detrimental (transmission-limiting). Likewise, virus-induced phenotypes that appear to be conducive to transmission in the laboratory, but which compromise host survival in the context of additional biotic or abiotic stressors, are unlikely to be favored by selection, as the longevity of a host as an inoculum source for virus acquisition by vectors could be significantly reduced. If this is the case in a crop host, it would mean that the phenotype induced by the virus is not likely to be a useful target for management (e.g., through rogueing of infected plants that attract vectors).

In the present study, we focus on these shortcomings and begin to address them in several ways. In response to the relative lack of studies on semi-persistently transmitted viruses compared to viruses with other transmission modes, we decided to focus on an economically important emerging virus that is a major pathogen in cucurbit agroecosystems around the world: the whitefly-transmitted *Cucurbit yellow stunting disorder virus* (CYSDV) (genus *Crinivirus*, family *Closteroviridae*) (Tzanetakis et al. 2013; Wintermantel et al. 2017).

This pathogen is presently the most serious virus threat to muskmelon (*Cucumis melo*) production in the United States, particularly in the Southwest, where approximately 75% of U.S. melon production takes place (Wintermantel et al. 2017). Rapid secondary spread occurs from initial melon infections within a single growing season, with fields often reaching 100% infection by harvest date (Wintermantel et al. 2017). This suggests that host and vector manipulation may play a significant role in the epidemiology of this pathogen.

To explore this while also addressing the need to consider the dynamic nature of virus-induced phenotypes, we evaluated virus effects on host-vector interactions at pre-symptomatic and post-symptomatic time points in disease progression in the primary crop host (*Cucumis melo*). These organismal experiments were complemented by chemical analysis of volatile and non-volatile plant metabolites known to play important roles in host-vector interactions. CYSDV is transmitted in a semi-persistent manner by whiteflies and is acquired from the phloem (Celix et al. 1996; Wintermantel et al. 2017). Therefore, we hypothesized that a transmission-conducive phenotype in CYSDV-infected *C. melo* would include changes that enhance whitefly attraction and facilitate increased uptake of phloem sap followed by eventual dispersal after sufficient feeding to become viruliferous. In prior work, we found evidence that CYSDV-induced changes in *C. melo* stimulate whitefly attraction and settling at a time point where symptoms are strongly apparent (four weeks post-inoculation) and that attenuation of symptoms using defense priming of the immune system disrupts whitefly preferences at this time point (Kenney et al. 2020).

To place our findings in a semi-ecological context, we further combine virus-host-vector studies with an exploration of how time and virus infection interact to modify the susceptibility of hosts to a ubiquitous *C. melo* pest that shares the same ecological niche as the whitefly vector: the cotton-melon aphid, *Aphis gossypii* (Hemiptera: Aphididae) (Capinera 2009). Aphids and whiteflies negatively affect hosts by direct removal of resources and through secretion of effector molecules that modify the plant immune system (Kaloshian and Walling 2016; Erb and Reymond 2019). Plants have counter-defenses that mitigate impacts of herbivore feeding by repelling herbivores (antixenosis), reducing herbivore performance, survival, or reproduction (antibiosis), or by enabling tolerance even under moderate levels of herbivory (Núñez-Farfán et al. 2007; Mitchell et al. 2016). Virus infection can fundamentally change the expression of these traits as a component of vector manipulation strategies. But under real-world conditions, this may not always be beneficial for the virus if transmission-conducive host phenotypes are also more attractive to, or more easily exploited by, non-vector herbivores (Belliure et al. 2010; He et al. 2012; Nachappa et al. 2013; Kersch-Becker and Thaler 2014; Su et al. 2016; Peñaflor et al. 2016; Ángeles-López et al. 2017). This could

ultimately be detrimental for virus fitness if vectors encounter more competition on infected hosts or if novel susceptibility phenotypes accelerate host decline. Non-vectors that initially benefit from virus-induced changes can also modify plants over time in ways that counteract virus manipulations of the same pathways (Ángeles-López et al. 2017). Thus, exploring broader "off-target" effects of putative manipulations can provide insight into the adaptive significance of virus effects on host phenotypes; a necessary step before proceeding with mechanistic studies to identify genetic variations associated with manipulative effects (Mauck et al. 2019) or studies to disrupt virus manipulation in crops (Bak et al. 2019).

Given the overlap among whiteflies and the cotton-melon aphid in cues used for host selection, feeding locations, resources consumed, and defensive pathways altered (Zarate et al. 2007; Rodriguez et al. 2014; Mugford et al. 2016; Xu et al. 2019; Cui et al. 2019), we consider it an essential step to determine if there is also overlap in responses to putative host manipulations by CYSDV. We explored these possible off-target effects in tandem with on-target putative manipulations across two time points in disease progression (pre-symptomatic and symptomatic) relative to sham-inoculated non-infected hosts in the same phenological stages. Behavior and performance assays for both insects are considered in the context of symptom expression, primary metabolites, leaf color, and odor cues. Exploring the spectrum of changes that drive insect selection among CYSDV-infected and non-infected hosts has revealed the extent to which CYSDV may manipulate its own transmission in the field, as well as new pathways to target for disrupting vector attraction.

Materials and methods

Organisms

Whiteflies (*Bemisia tabaci* MEAM1 biotype, formerly biotype B; Hemiptera: Aleyrodidae) were collected in 2006 from cotton at the Maricopa Agricultural Center, AZ, USA (Himler et al. 2011). *Aphis gossypii* used in our experiments were established from aphids collected from squash about a decade ago near Reedley, CA, USA. Melons (*Cucumis melo* cv. "Iroquois") served as the host in all experiments and were used to maintain the aphid colony. We used cowpea plants (*Vigna unguiculata* cv. "CT Pinkeye Purple Hull") to maintain the whitefly colonies. We sowed seeds individually in starter trays, then transplanted seedlings into 10*10*10 cm pots filled with UC Soil Mix 2 (Matkin and Chandler 1957) and approximately 4g of Osmocote slow-release 14-14-14 fertilizer with micronutrients. Melons and cowpeas were maintained in an insect-free growth chamber (23 ± 1 °C, 60 ± 5% RH, and 16L:8D photoperiod) until ready for use in colonies.

The isolate of CYSDV used in experiments was originally collected from muskmelons in the Imperial Valley in 2006 Bill Wintermantel (USDA-ARS, Salinas) who initiated a pure culture and maintained the virus on *C. melo (Wintermantel et al. 2009)*. We maintained CYSDV in Iroquois melons growing in bugdorms in a climate-controlled greenhouse with supplementary LED lighting (25 ± 1 °C, 60 ± 5% RH, and 16L:8D photoperiod). We performed transmissions by allowing whiteflies to feed for 48-h on CYSDV-infected melon plants (acquisition access period) and then by transferring 25-30 whiteflies to plants in the first true leaf phenological stage (two-week-old plants) for a three-day inoculation access period. We then gently removed whiteflies with an aspirator. Symptom development consisting of yellowing of leaf margins and interveinal discoloration was observable after ~21 days post-inoculation (dpi) and virus infection was also confirmed using double-antibody sandwich enzyme-linked immunosorbent assay with polyclonal CYSDV antibodies (BIOREBA CYSDV complete kit 960, Art No. 162372). We treated sham-inoculated (*i.e*., non-infected) plants similarly using non-viruliferous whiteflies. All bioassays described below were carried out on plants at two- or four-weeks post-inoculation or post sham-inoculation (wpi) in a greenhouse under controlled conditions (25 ± 1 °C, 60 ± 5% RH, and 16L:8D photoperiod). Comparisons of 2 wpi and 4 wpi plants (and sham controls) necessitated performing inoculations of these cohorts separately (i.e., plants in the 4 wpi cohort, followed 2 weeks later by plants in the 2 wpi cohort). To minimize any confounding factors, 2 wpi and 4 wpi plants received CYSDV from the same source culture and were grown on the same bench in the same greenhouse using identical culture methods.

Whitefly and aphid preference tests

We assessed whitefly preferences through assays allowing access to all cues (volatile, visual, and contact) and assays allowing only volatile cues. For the all-cue preference assays, groups of 30 non-viruliferous whiteflies were allowed to select among four treatments consisting of two CYSDV-infected melon plants (one 2 wpi and the other 4 wpi) and two sham-inoculated melon plants (one 2 weeks post sham-inoculation and the other 4 weeks post sham-inoculation). Presence of a whitefly on the surface of one of these treatments (settling) was considered a choice and whitefly positions among treatments were evaluated at 1, 2, and 24 hours after release. Assays were conducted as in Kenney et al. (2020) and are described in detail in the Electronic Supplementary Materials (ESM). We performed assays permitting access to only volatile cues in an opaque arena described in detail in Supplementary Figure 2. We performed 16 replications of dual choice tests between 4 wpi CYSDV-infected plants and their corresponding sham-inoculated plants. We chose to focus on this treatment pair because CYSDV-infected plants at 4 wpi were the only treatment to elicit whitefly attraction in the full-cue access tests. Whiteflies on each mesh-

covered hole were counted at 5, 10, 15, and 20 minutes, then averaged across all time points (as in Mauck et al. 2010) and converted to percentages of the total whiteflies that had entered the arena.

To determine if aphid non-vectors respond to infection presence and severity in a similar way as whitefly vectors, we carried out dual choice tests examining aphid settling preferences between healthy and infected plants within each disease progression time point. For each test, a pair of CYSDV-infected and sham-inoculated melon plants (either both 2 wpi or 4wpi) were selected and the third leaf of the vine was presented to the aphids in a dual choice arena (Supplementary Figure 3). We released 20 adult aphids (either alates or apterous) into each arena from a tube screwed to the bottom of the Petri dish. Aphids were allowed to settle on the exposed abaxial leaf surfaces. We counted the number of aphids settled on each leaf at 1, 2 and 24h to assess initial preferences (1-2 hours) and final preferences (24 hours). In total, 20-22 pairs of infected and sham-inoculated melon plants were used per infection x disease progression factor combination.

Whitefly and aphid feeding behavior

We used the DC-EPG system as previously described by (Tjallingii 1988) to investigate the effects of CYSDV infection in melons on feeding behavior of the vector *B. tabaci* and non-vector *A. gossypii*. To create electrical circuits that each included a plant and an insect, we tethered each insect by attaching a thin wire, 2.5 µm platinum (Wollaston process wire; Sigmund Cohn Corp., Mt. Vernon, New York, USA) for *B. tabaci* (Chesnais and Mauck 2018; Milenovic et al. 2019) and 12.5 µm gold for *A. gossypii* (Peng and Walker 2018), to the pronotum using conductive water-based silver glue. To facilitate the tethering process, non-viruliferous female whiteflies were immobilized for 30-45 seconds at -20 °C in a freezer and placed on a Petri dish lid that was set on top of an ice pack, under a dissecting microscope. For *A. gossypii,* individuals were immobilized at the edge of a pipette tip using a vacuum pump and then attached by a gold wire to the dorsum. After a 30-minute starvation period, we positioned each whitefly or aphid on the abaxial face of the leaf (the preferred feeding location) and inserted a second electrode into the soil of each potted plant to close the electrical circuit. We recorded from eight insects simultaneously over an eight-hour period of the photophase using a Giga-8 DC-EPG amplifier. Each insect-plant system was housed inside a Faraday cage located in a climate-controlled room held at 24 ± 1 °C. We used the PROBE 3.5 software (EPG Systems, www.epgsystems.eu) to acquire and analyze EPG waveforms and relevant EPG variables were calculated with EPG-Calc 6.1 software (Giordanengo 2014). We chose variables based on different EPG waveforms (described by (Janssen et al. 1989) for whiteflies and described by (Tjallingii and Hogen Esch 1993) for aphids) corresponding to behaviors relevant to virus transmission (for whiteflies) and

nutrition (both insects): stylet pathways in plant tissues except phloem and xylem; salivation in phloem; and passive phloem sap ingestion.

Plant quality assessments

To determine whether CYSDV-infection affects whitefly performance, adult whiteflies were collected and released into two clip-cages (~50 females per cage) on the third and fourth leaves of each melon plants (either CYSDV-infected or sham-inoculated after two or four wpi). Three days after infestation, the number of live females and the number of eggs laid per female were determined by counting individual eggs under a stereomicroscope. Whitefly oviposition is dependent on females maintaining access to sufficient nutrients and we used oviposition as a proxy for performance in this study (Xu et al. 2019).

To determine the effect of CYSDV-infection on aphid performance, we evaluated population growth on infected and healthy plants. Preliminary experiments indicated that leaf four of 4 wpi CYSDV-infected plants (that used in all other assays) frequently underwent senescence in response to establishment of *A. gossypii* colonies. Therefore, we opted to evaluate population growth across the time period in which plants are transitioning from 2 wpi to 4 wpi (from day 18 post-inoculation to day 29 post-inoculation). To standardize cohorts of aphids for experiments, we infested four young melon plants with 15 apterous and 10 alate adults and allowed offspring production for 36 hours. We used the resulting offspring cohort two days later for experiments (2nd-3rd instar). To infest plants, a small section of leaf with five aphids present was excised and placed on the 3rd leaf from the base, which was enclosed in a drawstring mesh cage that allowed access from either side (petiole and leaf tip). Aphids were allowed to reproduce for eleven days (approximately 2 generations) after which we counted the number present on the infested leaves. Two replicate experiments were performed with 6-8 replications of each treatment within each experiment.

Quantification of primary metabolites and volatile emissions

Quantification of leaf primary metabolites. To determine whether CYSDV infection and disease progression modify primary metabolism, we quantified sugars and amino acids in leaf tissue. We collected approximately 12-15 small (7.5mm diameter) leaf discs from in between major veins, weighed the tissue, and flash froze it in liquid nitrogen before storing at -80 °C. We sampled the same leaf position used in preference tests, performance tests, and EPG recordings (third leaf for the earlier time point, fourth leaf for the later time point), as well as the seventh leaf, which was asymptomatic in both 2 wpi and 4 wpi treatments. Both lower and upper leaves from 11 CYSDV-infected plants (4wpi), 15 sham-inoculated plants (4wpi), 16 CYSDV-infected plants (2wpi), and 16 sham-inoculated plants (2wpi) were

sampled. Leaf discs were removed from one side of the leaf for consistency, and the tip of the leaf was removed for semi-quantitative ELISA (Kenney et al. 2020). Extraction and derivatization of leaf metabolites was performed as previously described (Mauck et al. 2014, 2015) (details in Supplemental Materials). The GC-MS system used to identify and quantify metabolites consisted of a Thermo Scientific Trace 1310 gas chromatograph coupled with an AI 1310 autosampler and a TSQ Duo triple quadrupole mass spectrometer. Data acquisition and processing were controlled by Chromeleon 7 software (GC-MS parameters and quantifications in Table 1).

Volatile collection and quantification by gas chromatography and mass spectrometry. For volatile collections, we focused on assaying sham-inoculated and CYSDV-infected plants at four weeks post-inoculation, as infection at this time point elicited whitefly attraction in assays permitting access to all cues, but infection at two weeks post-inoculation did not. Eight CYSDV-infected plants and 6 sham-inoculated plants were used. Volatile collections were performed using a push-pull volatile sampling system, with 2 L per minute of charcoal-filtered clean air pushed into 7.5 L jars enclosing symptomatic portions of plants, and corresponding plant portions on sham-inoculated plants. We cleaned jars and teflon guillotine bases with zero-residue ammonia-based soap, distilled water, and rinses of acetone and hexanes, respectively. Volatiles were sampled by pulling headspace air across a trap containing 40mg of Hayesep-Q adsorbent (Mesh 80-100, Hayes Separations, Inc.) at a rate of 1 L per minute. Collections were performed during the photophase (11:00-17:00). We eluted volatiles from traps with 150uL of dichloromethane (Acros Organics 326600025) spiked with 600 ng of nonyl acetate (Sigma Aldrich W278807-SAMPLE) and 300 ng of n-octane (Sigma Aldrich 74820-5ML) as internal standards. Blank collections were also performed to account for any trace background contaminants. We used the GC-MS system described above for volatile identification and quantification (settings in Table 2).

Statistical analyses

Data on whitefly settling preference were analyzed using approximate Friedman tests on responding whiteflies. When a significant effect was detected, a pairwise comparison using Wilcoxon signed rank test (*P*-value adjustment with “holm” method) at the 0.05 significance level was used to test for differences between treatments. The whitefly settling rates varied irregularly with the leaf color (percentage of yellow), and we therefore analysed the data with a generalised additive model (GAM; “mgcv” package (Wood 2017)) with “yellow” as a smoothed predictor. The error distribution and model fit were checked with the gam.check function. Data on whitefly volatile-based preference were analyzed using a paired t-test. Data on aphid settling preferences were analyzed using Wilcoxon signed rank test. We used

generalized linear models (GLM) with a likelihood ratio and Chi-square test to assess whether there was an effect of plant infection status on both *B. tabaci* and *A. gossypii* feeding behaviors. We included the CYSDV infection status ("virus") and weeks post-inoculation ("week") as main factors and also studied their interaction ("virus:week"). Data on feeding behavior (probing and phloem sap ingestion phases) was not normally distributed, accordingly we carried out a GLM using a Gamma (link = "inverse") distribution. When a significant effect of one of the main factors was detected or when an interaction between factors was significant, a pairwise comparison using Estimated Marginal means (package R: "emmeans") (*P*-value adjustment with Tukey method) at the 0.05 significance level was used to test for differences between treatments.

Data on whitefly (and aphid) performance were not normally distributed (count data), and accordingly, were analyzed using a generalized linear model (GLM) with errors modeled using a Poisson distribution. A quasi-likelihood function was used to correct for overdispersion, and Log was specified as the link function in the model. We included "plant infection", "session" and "clip-cage" as main factors and also studied their interaction. The fit of all generalized linear models was checked by inspecting residuals and QQ plots. For carbohydrate metabolites, we analyzed compounds separately by leaf position using general linear models, with "plant infection" and "week post inoculation" as factors and post-hoc Tukey tests for significant main effects. Most compounds required log transformation to meet normality assumptions of the model. Mean values are reported with the standard errors of the means (SEM) and sample sizes in ensuing figures. To test if the different factors "plant infection", "week post inoculation" and "leaf position" explain a significant proportion in amino acid composition and quantity variations, we used a Redundancy Analysis (RDA) following the procedure described in Hervé et al. (2020) (see ESM for full details). To test if the infection explains plant volatiles emissions, we used a PPLS-DA procedure as described in (Hervé et al. 2018) (see ESM for full details). Plant volatile blends were log transformed before PPLS-DA and the significance of the treatment was assessed using a permutation analysis (999 repetitions) implemented in the MVA.test from the RVAideMemoire package. As a follow-up, we used a decision-tree-based method 'Random Forest' (RF) for variable selection to detect the most important compounds that account for significant differences (see ESM for full details). We used out-of-bag (OOB) error rates as the importance score for variable selection implemented as backward elimination in the package varSelRF. Performance of the RF models was evaluated by the misclassification error rate. All statistical analyses were performed using Minitab v. 14 or R software (version 4.0.2) (R Core Team 2020).

Results

Whitefly and aphid preference tests

Responding whiteflies preferentially settled on 4wpi CYSDV-infected melon leaves after 1h, 2h and 24h (Approximate Friedman tests, $P < 0.001$) (Fig. 1). To a lesser extent, whiteflies also preferred to settle on the 4wpi sham-inoculated leaves over 2 wpi sham-inoculated leaves. The number of responding whiteflies increased gradually, from 70% after 1 hour to over 90% after 24 hours. Whitefly settling on 4 wpi CYSDV-infected was positively affected by leaf symptoms (yellow discoloration) up until a discoloration of ~70% then the preference is reduced (GAM model, $F = 8.097$; estimated $df = 7.143$; $P < 0.001$; $R\text{-}sq(adj) = 0.763$) (Fig. 2a). A complementary bioassay presenting only volatile cues in the absence of treatment-specific visual or contact cues indicates that whitefly preferences for 4 wpi CYSDV-infected plants are not driven by odors (Student t-test, $t = 0.91$, $P = 0.376$) (Fig. 2b).

CYSDV-infection on 2 wpi melon leaves did not significantly influence apterous and alate aphid settlement preference after 1, 2 and 24 hours (Wilcoxon signed rank tests, $P > 0.05$) (Fig. 3a). Alate aphids exhibited a slight preference for sham-inoculated leaves over 4 wpi CYSDV-infected melon leaves at 1 hour and after 24 hours (Wilcoxon signed rank test, $V = 34.5$, $P = 0.015$ and $V = 21.5$, $P = 0.003$, respectively) while apterous aphids settled evenly on both sham-inoculated and CYSDV-infected leaves (Wilcoxon signed rank tests, $P > 0.05$) (Fig. 3b). The number of responding aphids, either apterous or alatae, increased gradually from 80% after 1 hour to over 90% after 24 hours.

Whitefly and aphid feeding behavior

For whiteflies, the durations of pathway phases and salivation in phloem on melon plants were not affected by CYSDV-infection at both 2 wpi and 4 wpi time points (GLM, “virus”: $P = 0.723$, “week”: $P = 0.052$, interaction “virus:week”: $P = 0.085$ and “virus”: $P = 0.677$, “week”: $P = 0.104$, interaction “virus:week”: $P = 0.793$, for pathway and salivation phases, respectively) (Fig. 4a). However, whiteflies performed longer phloem sap ingestion on CYSDV-infected melon plants regardless of the stage of disease progression (GLM, “virus”: $P = 0.011$, “week”: $P = 0.579$, interaction “virus:week”: $P = 0.537$) (Fig. 4a) (see ESM for detailed Table S2).

For *A. gossypii*, durations of pathway phases melon plants were increased on CYSDV-infected plants in both the 2 wpi and 4 wpi time points (GLM, “virus”: $P = 0.001$, “week”: $P = 0.475$, interaction “virus:week”: $P = 0.263$) (Fig. 4b). Aphids performed longer salivation phases in phloem at 4 wpi time point (GLM, “virus”: $P < 0.001$, “week”: $P = 0.373$, interaction “virus:week”: $P = 0.589$). However, aphids performed shorter phloem sap

ingestions on CYSDV-infected melon plants in both time points (GLM, “virus”: P = 0.002, “week”: P = 0.509, interaction “virus:week”: P = 0.332) (Fig. 4b).

Plant quality assessments

Whitefly fecundity on CYSDV-infected melon plants was reduced by 20-30% after feeding on plants in both the 2 wpi (GLM, χ^2 = 12.075, P < 0.001) (Fig. 5a) and 4 wpi time points (GLM, χ^2 = 4.091, P < 0.043) (Fig. 5b). We observed an effect of the repetition for both 2 weeks post-inoculation (GLM, χ^2 = 29.127, P < 0.001) and 4 weeks post-inoculation fertility experiments (GLM, χ^2 = 7.098, P = 0.008). At 4 weeks post-inoculation, the fertility of whiteflies was higher on the third leaf than the fourth leaf (factor: “clip-cage”) (GLM, χ^2 = 6.125, P = 0.013).

Population growth for *Aphis gossypii* on CYSDV-infected plants was significantly reduced relative to sham-inoculated plants during the transition from 2 wpi to 4 wpi (GLM, χ^2 = 494.7, P < 0.001) (Fig. 6). Significant temporal effects were also detected, with higher aphid fecundity during the second replication of the experiment relative to the first (GLM, χ^2 = 5209.1, P < 0.001). Aphids established on the fourth leaf of 4 wpi CYSDV-infected plants elicited rapid senescence in the leaf tissue; most infected leaves became unsuitable early on in the experiment (6/8), but most sham-inoculated leaves (5/6) continued to support aphids until day 11 post-infestation (data not shown).

Quantification of primary metabolites and volatile emissions

We detected glucose, fructose, and sucrose as well as sixteen proteinogenic amino acids in the analysis of primary metabolites in leaf tissue (Fig. 7a, Table S3 and S4 in ESM). For upper leaves (asymptomatic in both disease progression stages), sucrose concentration was influenced by infection status (GLM, F = 4.49, P = 0.039) and time point (wpi for infected and weeks post sham-inoculation for controls) (GLM, F = 13.50, P = 0.001) but not by their interaction (Fig. 7a). Glucose concentration in upper leaves was influenced by time point (GLM, F = 8.12, P = 0.006), with infection status marginally non-significant (GLM, F = 3.78, P = 0.057) (Fig. 7c). Fructose concentration in upper leaves was influenced by infection status (GLM, F = 6.89, P = 0.011) with a significant interactions of infection status and time point (GLM, F = 5.57, P = 0.022) and a marginally non-significant effect of time point (GLM, F = 3.49, P = 0.067) (Fig. 7e). For lower leaves (symptomatic in 4 wpi and asymptomatic in 2 wpi treatment groups) sucrose concentration was significantly influenced by time point (GLM, F = 10.34, P = 0.002) (Fig. 7b). There was a marginally non-significant trend of time point having an effect on glucose concentration (GLM, F = 3.88, P = 0.054) with a significant interaction between infection status and time point (GLM, F = 4.08, P = 0.048) (Fig. 7d).

Fructose concentration was significantly influenced by time point (GLM, $F = 6.80$, $P = 0.012$) and the interaction of infection status and time point (GLM, $F = 9.02$, $P = 0.004$) (Fig. 7f).

Redundancy analysis with permutation testing indicates that the main drivers of variation in leaf amino acid composition (consisting of compound identity and quantity) are the time point at which the samples are taken (2 wpi vs. 4 wpi, $F = 9.49$, $P = 0.001$) and the leaf position (upper vs. lower, $F = 5.81$, $P = 0.001$) (Table 3). We also detected a significant interaction between infection status and time point ($F = 3.08$, $P = 0.004$), a significant interaction between infection status and leaf position ($F = 2.43$, $P = 0.017$), and a significant interaction between time point and leaf position ($F = 3.57$, $P = 0.003$) (Table 3). Constrained ordination plots (Fig. 8) illustrate clustering of treatment groups based on significant and marginally non-significant interaction effects.

Volatile collections were only performed for the time point in which we detected significant differences in whitefly preferences among infected and non-infected hosts (4 wpi). Blend compositions (compound identities and quantities) were analyzed using PPLS-DA, which detected significant differences in blends based on the infection status factor (CER = 14.3%, $P = 0.002$). The first two ordination axes explained 78.13% (44.16% and 33.97% respectively) of variation in volatile blends and clearly separated infected from sham-inoculated plants (Fig. 9a). A complementary random forest analysis also clearly separated treatments based on blend features (out-of-bag error rate 28.57%, Fig. 9b) and identified two compounds that were strong predictors of infection status (3-hexen-1-ol and 4-hexen-1-ol, isomers not discernible).

Discussion

Repeated documentation of transmission-conducive phenotypic changes in hosts has led to the hypothesis that plant viruses evolve specific adaptations for “manipulating” host-vector interactions to facilitate their own transmission (Mauck et al. 2012, 2018; Eigenbrode et al. 2017). However, the taxonomic diversity of viruses examined for evidence of manipulative effects remains limited, with many emerging pathogens of concern not yet studied. Additionally, limited evidence suggests that effects of viruses on their hosts and vectors are not static, but change over the course of plant phenology and disease progression (Werner et al. 2009; Rajabaskar et al. 2013; Lu et al. 2016; Shrestha et al. 2019). “Manipulations” can also change how hosts resist abiotic stressors and interact with other, non-vector organisms (Davis et al. 2015; Mauck et al. 2015). Thus, to determine whether putative virus manipulations are biologically meaningful in managed and unmanaged communities, we must begin to consider virus-induced phenotypes in a broader ecological context.

Our results indicate that CYSDV induces changes in *C. melo*, its main agricultural host, that are consistent with host and vector manipulation: CYSDV infection significantly increased whitefly settling and phloem sap uptake. Given that CYSDV is only acquired and inoculated from the phloem, these effects should increase both the number of viruliferous whiteflies on infected hosts and the probability of each whitefly obtaining sufficient virions to subsequently inoculate (Ng and Zhou 2015). Virus-induced phenotypes and their effects on vector behavior were also strongly influenced by the stage of disease progression, with the most pronounced transmission-conducive phenotype evident at 4 wpi (increased attraction and phloem sap uptake) relative to 2 wpi (only increased phloem sap uptake). This finding lends further support to a growing body of evidence that virus effects on host phenotypes and vector behavior are not static (Blua and Perring 1992a , b ; Shrestha et al. 2019), but change dynamically over time, with significant implications for virus evolution and management (Mauck and Chesnais 2020).

Even though whiteflies preferred and fed more easily on infected hosts, whitefly females produced fewer eggs on infected plants in both stages of disease progression during no-choice feeding trials. Although this may appear to be detrimental for the virus, on the contrary, lower host quality may encourage whiteflies to emigrate after feeding for long enough to become viruliferous. This finding highlights the insights we can gain from studying viruses with semi-persistent transmission modes; as a semi-persistent virus, prolonged feeding and settling on infected hosts after virus acquisition is more likely to hinder rather than enhance new CYSDV infections (Ng and Zhou 2015). And mathematical models have shown that the benefits of attracting and retaining vectors depend on there being a mechanism for dispersal through a reversal of the preference for infected hosts (Roosien et al. 2013; Shaw et al. 2017). Although we did not observe defection in the 24-hour time frame of our tests, the fecundity measurements suggest that a slower-acting, inducible antibiosis may encourage later dispersal. An interesting next step in studying the CYSDV-melon pathosystem would be to perform further experiments that quantify post-acquisition effects of CYSDV on vector behavior (Chesnais et al. 2020), as well as effects of vector feeding on the expression of virus-induced phenotypes.

Parallel experiments showed that the same symptoms that induce greater visitation and settling by whiteflies on infected hosts had opposite effects on the behavior of a non-vector competitor (*A. gossypii*), even though both whiteflies and aphids must locate and ingest nutrients from the same host tissue (phloem elements). Regardless of the time point in disease progression, *A. gossypii* was largely indifferent to disease status in free choice tests, with a slight preference for sham-inoculated plants. EPG recordings revealed that this

preference may be linked to greater difficulty in feeding on infected plants during both asymptomatic and symptomatic disease stages. Subsequent aphid performance experiments carried out across the transition from the asymptomatic to symptomatic condition indicate that this difficulty in feeding (antixenosis) may contribute to reductions in fecundity and overall aphid population size on infected relative to non-infected hosts.

Reduced feeding and reproduction by *A. gossypii* is biologically significant because it suggests dual benefits of the CYSDV-induced host phenotype for the virus: attraction and retention of vectors plus repellence and resistance against a damaging non-vector that competes directly with the vector. We previously documented a similar effect of infection by *Cucumber mosaic virus* (CMV) (family *Bromoviridae*, genus *Cucumovirus*) on non-vector herbivores of squash; phenotypic changes that encourage virion acquisition and dispersal by vectors also discourage feeding and oviposition by non-vector herbivores (Mauck et al. 2015). Based on this work, we hypothesized that virus-induced changes that reduce damage from herbivores are conducive to transmission because infected hosts will remain in the landscape for longer periods of time and continue to serve as sources of inoculum (Mauck et al. 2015, 2018). By exploring impacts of CYSDV infection on host interactions with a non-vector, we provide evidence that a virus can induce a phenotype that both facilitates transmission-conducive interactions with vectors and hinders feeding and exploitation by a non-vector.

Our selected plant trait analyses provided insight into the mechanisms underlying CYSDV effects on hosts, vectors, and non-vectors, but do not provide a complete explanation for all observed patterns. CYSDV infection induced changes in both leaf volatiles and leaf appearance (degree of yellowing) at the most attractive time point (4wpi). However, whiteflies exhibited no preference for 4wpi infected hosts based on odor cues alone, while the number of whiteflies selecting 4wpi infected hosts when color cues were accessible was more than twice the number choosing sham inoculated hosts of the same age, or asymptomatic 2wpi infected hosts. When we analyzed the relationship between the degree of symptom severity (yellowing) and whitefly preference (percentage selecting that leaf) using a subset of the data that included only 4wpi infected hosts, we detected a tight relationship between the percentage of yellowing and whitefly settling. Although we did not focus on 2wpi hosts for volatile analysis, it should be noted that there was also a slight preference for leaves of 4wpi sham-inoculated plants over leaves of 2wpi sham-inoculated plants in preference tests. We suspect this preference is also driven by slight color differences between the older leaves of 4wpi sham plants, which we observed to be a lighter green color relative to darker green leaves in the same vine position on 2wpi sham plants. In

587 future experiments, it would be interesting to use plant age and infection status as a basis for
588 further dissecting the relative importance of different types of cues used by whiteflies under
589 varying conditions. Overall, whitefly preferences in our experiments are consistent with
590 prior studies documenting strong whitefly attraction to the color yellow (Coombe 1981;
591 Stukenberg and Poehling 2019) with yellow or yellow-green traps being a primary means of
592 whitefly monitoring in agricultural settings (Berlinger and Others 1980; Gillespie and Quiring
593 1987).

594 Our results are also congruent with those of another study documenting effects of
595 a related crinivirus, *Tomato chlorosis virus* (ToCV) (family *Closteroviridae*, genus *Crinivirus*)
596 on vision-based preferences and odor-based preferences of *B. tabaci* (Fereres et al.
597 2016) . This study reported attraction of non-viruliferous whiteflies to ToCV-infected
598 tomato plants based on visual cues presented in the absence of contact or odor cues
599 (Fereres et al. 2016). When only odor cues were permitted, non-viruliferous whiteflies were
600 instead slightly repelled by odors of ToCV-infected plants. Like CYSDV, ToCV induces
601 yellowing of host foliage when infecting highly susceptible crops but does not cause rugosity
602 (wrinkling/puckering) leaf rolling, or other size reductions (Wintermantel and Wisler 2006).
603 The study by Fereres et al. (2016) suggests that ToCV-infected tomato plants exhibit
604 symptoms that are visually attractive and do not suffer decreased apparency
605 due to severe reductions in size or leaf area. However, a follow-up study using near-identical
606 plant ages and culture conditions (Maluta et al. 2017) found that non-viruliferous whitefly
607 preferences for ToCV-infected tomatoes were reversed when access to all cues (visual,
608 odor, and contact) was permitted. Additionally, both studies found that whitefly preferences
609 often depend on viruliferous status, even when the virus being acquired (ToCV) does not
610 enter and circulate in insect hemolymph. Thus, the relative importance of different cues may
611 vary across situations, vector conditions, and bioassay designs. This will be important to
612 consider in future efforts to extrapolate results for ToCV or CYSDV to whole plants in field
613 settings.

614 Although it is difficult to clarify the relative importance of different cues in the
615 laboratory, the benefits, for the virus, of manipulating leaf appearance are readily
616 apparent when you consider that whiteflies are minute and poor flyers. In a field
617 environment, v olatile blends are less likely to be constant across the space between a
618 vector and an infected plant (Byrne et al. 1988; Byrne 1999; Aartsma et al. 2017). Virus-
619 induced changes in volatiles are also more subject to perturbations due to feeding by other
620 organisms or co-occurring pathogens (Salvaudon et al. 2013) as well as abiotic conditions
621 (Blanc and Michalakis 2016). In contrast, a visual source remains fixed in space and, to

some degree, more constant over time. This is the case for CYSDV infection in melons; yellowing becomes apparent 21-28 days after successful inoculation and this phenotype (represented by our 4 wpi time point) persists for weeks (Wintermantel et al. 2017). Based on the present results, we hypothesize that changes in visual cues are an essential component of virus manipulations that enhance whitefly attraction to infected hosts. Disrupting these changes may be a viable route for reducing virus spread in agricultural settings (Kenney et al. 2020).

Our study also quantified changes in primary metabolites associated with infection status, disease progression, and leaf age within disease and time point categories. Surprisingly, these analyses did not reveal any strong connections among drivers of variation in leaf tissue metabolites, vector and non-vector behavioral preferences, and stylet activities inside plant tissues. Amino acid composition and quantities varied primarily based on time point (2wpi vs. 4wpi), with little separation based on infection status. Leaf sugar concentrations also varied based on time point: for both upper and lower leaves, glucose, fructose, and sucrose were higher in leaves of 4wpi vs. 2wpi sham-inoculated plants. The most significant change due to CYSDV infection was increased variation in sugar quantities and nullification of differences between the 2wpi and 4wpi time points; glucose, fructose and sucrose quantities in 2wpi infected plants do not differ from those in 4wpi infected plants, but all three compounds are significantly different by time point for sham-inoculated plants. While this is interesting, there is no clear connection to the outcomes of behavior experiments. For example, in choice tests, whiteflies exhibited only a slight preference for 4wpi sham-inoculated plants over 2wpi sham-inoculated plants. This outcome could be partially driven by the higher quantities of sugars in leaf tissues, or a combination of differences in sugar quantities and amino acid composition. But differences in stylet activities consistent with metabolites being involved in this preference were not evident in whitefly EPG experiments. And aphid stylet activities were similarly unaffected by the time point, with CYSDV infection status being the only significant term in the model. Collectively, these results show that the two h emipterans studied here are not strongly responsive to the range of variation in melon leaf tissue primary metabolites we observed.

Based on this, we hypothesize that primary metabolic pathways in leaves are not targets for manipulation by CYSDV and that the phenotypes observed manifest via mechanisms not explored in our study. We observed most post-contact behavioral effects (e.g., EPG) over short time frames (a few hours), suggesting that the phenotype underlying these effects may involve changes initiated by infection prior to vectors contacting infected hosts rather than a slow activation of defenses over time following vector feeding. Effects of

this sort could be mediated by constitutively produced compounds not measured in this study and by changes in plant architecture. There is some evidence for the latter mechanism from prior work on CYSDV pathology. In *C. melo*, CYSDV virions are present in phloem sieve elements, as well as phloem parenchyma, bundle sheath, and companion cells. Within these tissues, infection can induce vesicles, cell wall overgrowths, lipid bodies, plasmalemma deposits and cytopathological effects on organelles, particularly chloroplasts and mitochondria (Medina et al. 2003). Thus, CYSDV and other criniviruses possess adaptations for inducing drastic changes in the architecture of cells that form the interface between the site of nutrient acquisition for whiteflies and aphids (sieve elements) and the tissue that must be bypassed to reach this site (mesophyll). The importance of focusing on these mechanisms in future work was directly revealed by our comparative approach exploring behavior of two hemipterans in the context of metabolomics.

Overall, our study makes several important contributions to our understanding of the ecology of plant virus manipulation of host phenotypes and vector behavior in monoculture crops. We found that CYSDV infection discourages colonization by a non-vector competitor while inducing a suite of changes that encourage virion acquisition from infected hosts by the vector, with the most effective manipulation occurring at the latter stage of disease progression due to the appearance of a visually attractive phenotype. This same phenotype is characteristic of infections in the field (Wintermantel et al. 2017) and can be disrupted by manipulating host resistance and tolerance to infection with commercially available plant defense priming agents (Kenney et al. 2020). Thus, our study has the potential to directly inform management options that target a putative virus manipulation of vector behavior. It also provides new insight into the hierarchies of cues used by different phloem-feeding Hemipterans and the ways that virus infection alters vector-competitor interactions. Importantly, this knowledge, and its potential for real-world applications, would not have been discovered if we focused solely on behavioral responses of only vectors at a single time point in disease progression.

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Figure captions

Figure 1. Whitefly behavioral responses to contact, volatile and visual cues of sham-inoculated (i.e., non-infected) and CYSDV-infected melon plants after 1h, 2h and 24h. Thirty

whiteflies were allowed to settle on melon leaves of two non-infected and two infected plants either two- or four- weeks post-inoculation. Twenty-four replicates were performed (N=24). Letters indicate significant differences associated with Friedman tests followed by pairwise comparisons using Wilcoxon signed rank tests.

Figure 2. Effect of 4 wpi CYSDV-infected melon leaves symptoms (yellow discoloration) on whitefly settlement preferences (data from tests in Fig. 1) (a) and response of whiteflies to volatile cues from 4 wpi plants in contact and visual-cue free choice tests (N=16) (b).

Figure 3. Aphid behavioral responses to contact, volatile and visual cues of sham-inoculated (i.e., non-infected) and CYSDV-infected melon plants after 1h, 2h and 24h. Twenty aphids were allowed to choose between a leaf from each of one non-infected and one infected plant either (a) two weeks post-inoculation or (b) four weeks post-inoculation. Between twenty and twenty-two replicates were performed for each modality. Asterisks indicate significant differences (** $P < 0.01$, NS: not significant) as determined using Wilcoxon tests.

Figure 4. Durations of pathway phases, phloem salivation phase, and phloem sap ingestion phase of (a) *Bemisia tabac*i and (b) *Aphis gossypii* on CYSDV-infected or sham-inoculated melon plants after two- or four-weeks post-inoculation (wpi) (N=20-24).

Figure 5. Effect of CYSDV-infection after (a) two weeks post-inoculation (wpi) or (b) four weeks post-inoculation (wpi) on whitefly fecundity. Data shown are the means +/- standard errors of the means of data from 22 to 32 repetitions. Asterisks indicate significant differences between CYSDV-infected plants and sham-inoculated plants (EMMeans pairwise comparisons, * $P < 0.05$, ** $P < 0.01$).

Figure 6. Effect of CYSDV infection on *Aphis gossypii* population size. Aphids were allowed to reproduce on plants between 18 dpi and 29 dpi (transition from pre-symptomatic 2 wpi to symptomatic 4 wpi period). Data shown are mean +/- standard errors for two temporally separated repetitions of the experiment (batch 1 and batch 2), each with 6-8 replicate plants in each treatment. Letters indicate significant differences between CYSDV-infected plants and Sham-inoculated plants (EMMeans pairwise comparisons, $P < 0.05$).

Figure 7. Quantifications of sucrose, glucose, and fructose in leaf tissue samples taken from upper leaves (asymptomatic across time points) (a, b and c) and the lower leaves (same as those used in all bioassays for each disease progression time point) (d, e, f). Data displayed as means +/- standard errors with 8 replicate plants in each treatment x disease progression x leaf position combination. Analyses on upper and lower leaves were performed separately, with post-hoc Tukey tests when significant main effects were detected. Letters within each

graph indicate significant differences at $P < 0.05$.

Figure 8. Constrained PCA score plots of multivariate analyses (RDA) for amino acids only, illustrating interactions of infection status with time point (a), infection status with leaf position (b) and leaf position with time point (c). CY and SH designate CYSDV-infected and sham-inoculated, respectively, in both plots. In graphs (a) and (b) these treatments also maintain the green (SH) and yellow (CY) color codes used throughout the other figures. Graph (c) pools data across the SH and CY treatments. In this graph U (in red) and L (in blue) refer to upper and lower leaf samples and 2wk and 4wk refer to stages of disease progression (2 wpi and 4wpi).

Figure 9. Volatile blend analyses illustrating effects of CYSDV infection (4 wpi) on blend composition. Plot (a) is a score plot from a multivariate analysis (PPLS-DA) with infection status as the factor (analysis details in ESM). Plot (b) shows sample clustering for the random forest analysis (decision-tree based method, analysis details in ESM). Means +/- SE for individual volatile components of each blend are included in Table S5 in ESM).

948 **Table 1:** GC-MS operating parameters and non-volatile metabolite quantification

GC-MS Parameter	Details
Sample volume	1µL
Inlet temperature; mode	230°C; splitless mode
Carrier gas; inlet flow rate	Helium (99.9999% UHP200); 1ml/min constant
Split flow rate; splitless time	25mL/min; 0.8min
Purge flow rate; septum purge	5mL/min; constant
Gas saver	Enabled at 25mL/min, initiated at 2 min
Column	Thermo Scientific TG-5MS (0.25 mm i.d. × 28.33 m, 0.25 µm film thickness)
Temperature program	70 °C for 5 min, followed by a 5 °C/min ramp to 325 °C, and a hold at this temperature for 1 min (total time 57 min)
Transfer line/MS source temps	250 °C/230 °C
MS mode	Single quadrupole, electron ionization, general acquisition (scan) mode starting at 5.95 minutes
Mass range for scanning	50-600
Dwell time	0.2s
Quality control for identifications and major ion selection	Commercial standards for each metabolite
Quantification	Individual channels for each compound were extracted from Total Ion Chromatogram (TIC) by specifying the mass range for the major ion detected in each standard
Standardization	Individual metabolite amount (µg/g tissue) = Total peak areas (counts*min) of each compound / peak area of internal standard (ribitol) * 12 (12 µg ribitol spiked in each sample) / tissue weight (g)

949

950 **Table 2:** GC-MS operating parameters and volatile metabolite quantification

GC-MS Parameter	Details
Sample volume	1µL
Inlet temperature; mode	280°C; splitless mode
Carrier gas; inlet flow rate	Helium (99.9999% UHP200); 3ml/min constant
Split flow rate; splitless time	24mL/min; 0.8min
Purge flow rate; septum purge; vacuum compensation	5mL/min; constant; constant
Gas saver	Enabled at 25mL/min, initiated at 2 min
Column	Thermo Scientific TG-5MS (0.25 mm i.d. × 28.33 m, 0.25 µm film thickness)
Temperature program	40°C for 1 min, ramp to 100°C at a rate of 4°C/min, ramp to 280°C at a rate of 8°C/min, hold at 280°C for 1 min
Transfer line/MS source temps	280°C/250°C
MS mode	Single quadrupole, electron ionization, general acquisition (scan) mode starting at 2.95 minutes
Mass range for scanning	50-600
Dwell time	0.2s
Identifications	NIST 2014 library and commercial standards for each metabolite if available
Quantification	Peak areas in resulting chromatograms were integrated to calculate area using Chromeleon software
Standardization	Individual metabolite amount (ng/g tissue) = Total peak areas (counts*min) of each compound / peak area of internal standard (nonyl acetate) * 600 (600ng ribitol spiked in each sample) / tissue weight (g)

951

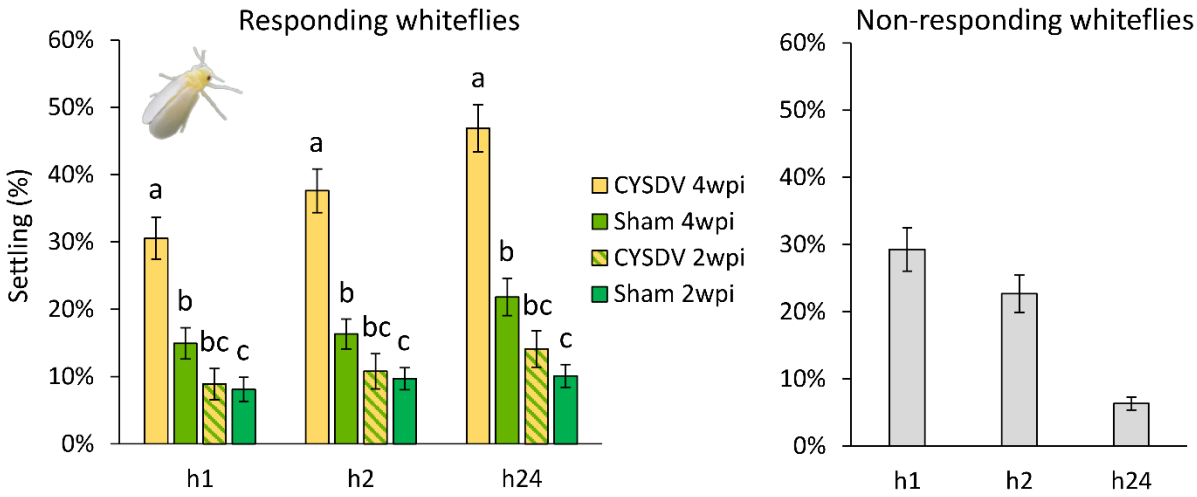
952

953 **Table 3:** Permutation F-tests of the factors included in Redundancy Analysis (RDA) (999
954 permutations) to identify main drivers of variation in leaf metabolite composition (compound
955 identity and quantity).

	F	*P*
Infection status	0.464	0.906
Time point	9.491	**0.001 *** **
Leaf position	5.810	**0.001 *** **
Infection x Time	3.084	**0.004 ** **
Infection x Leaf	2.431	**0.017 ***
Time x Leaf	3.572	**0.003 ** **
Infection x Time x Leaf	1.117	0.294

956 Significant P-values are indicated in bold (* P < 0.05; ** P < 0.01; *** P < 0.001). Pairwise
957 comparisons are available in the ESM (Table S1).

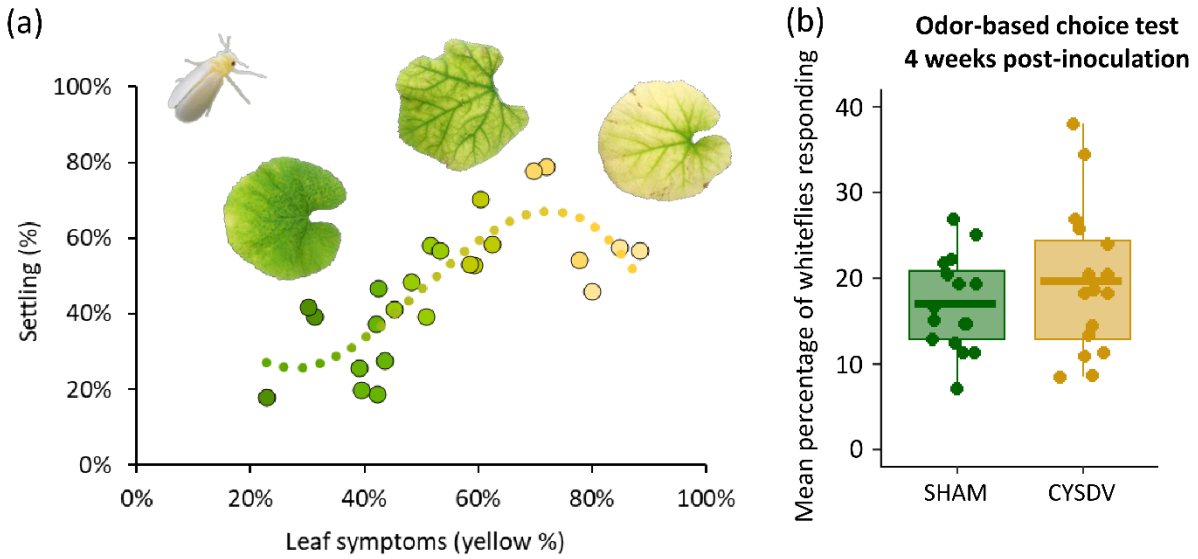
958



959

960 **Figure 1.**

961



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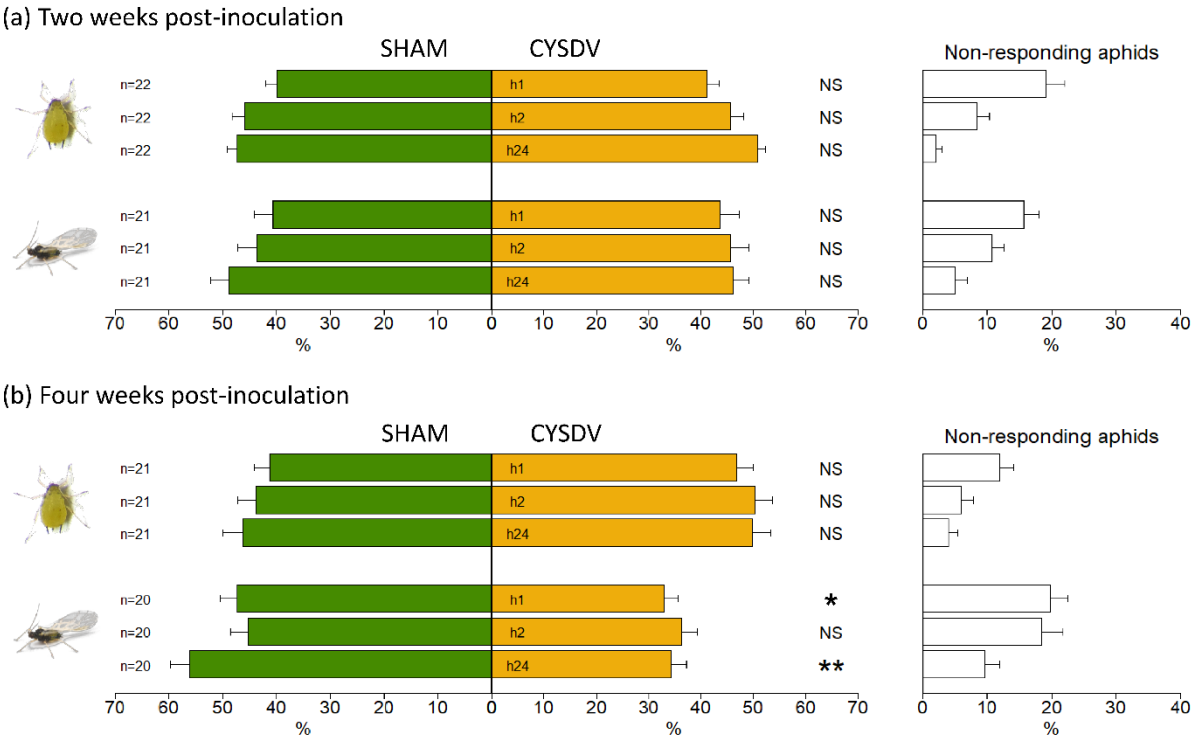

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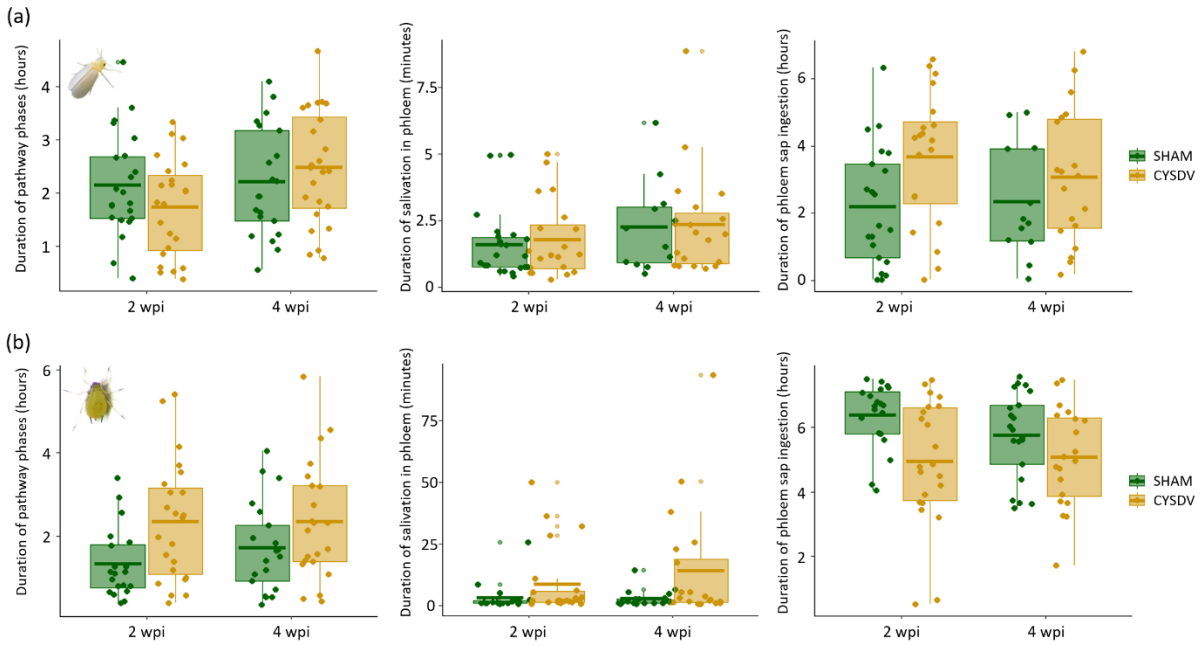

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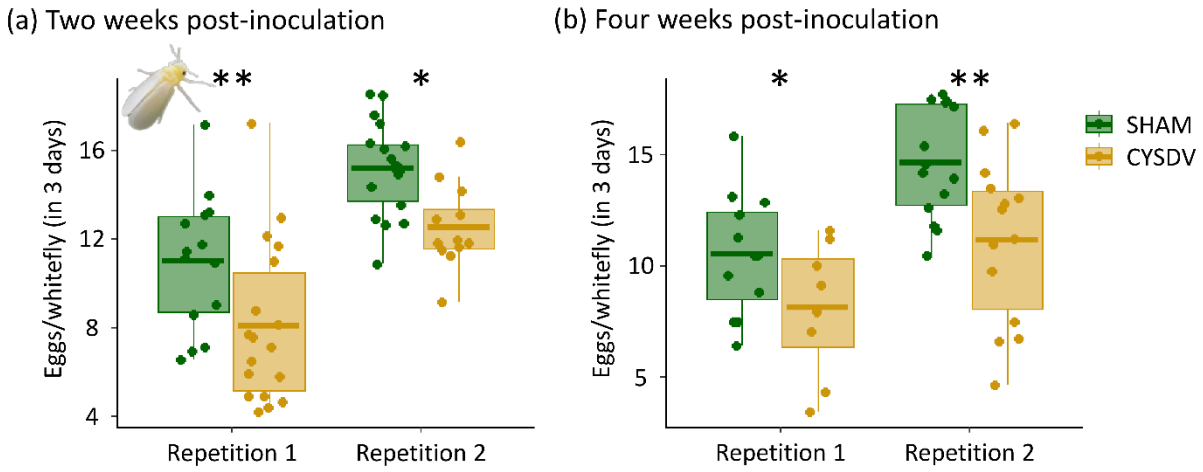

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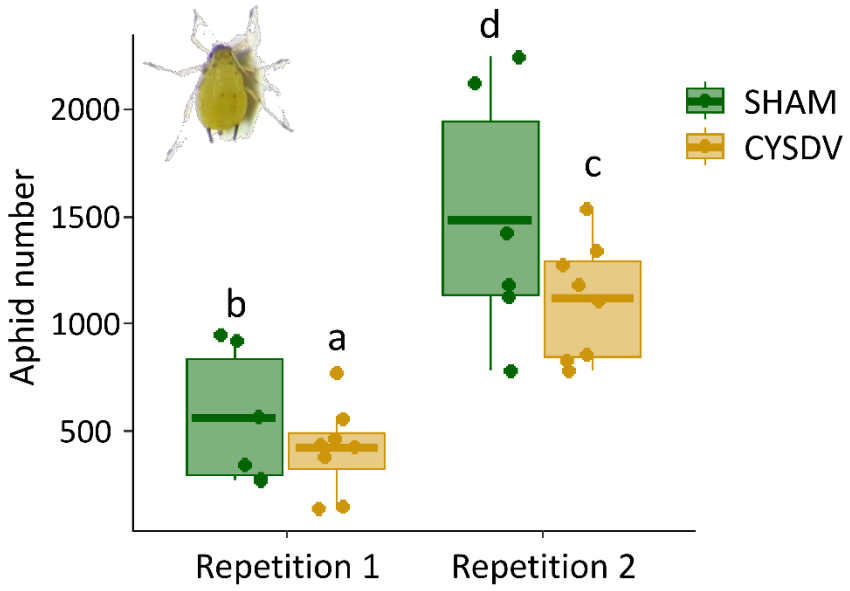
969 **Figure 5.**



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971 **Figure 6.**

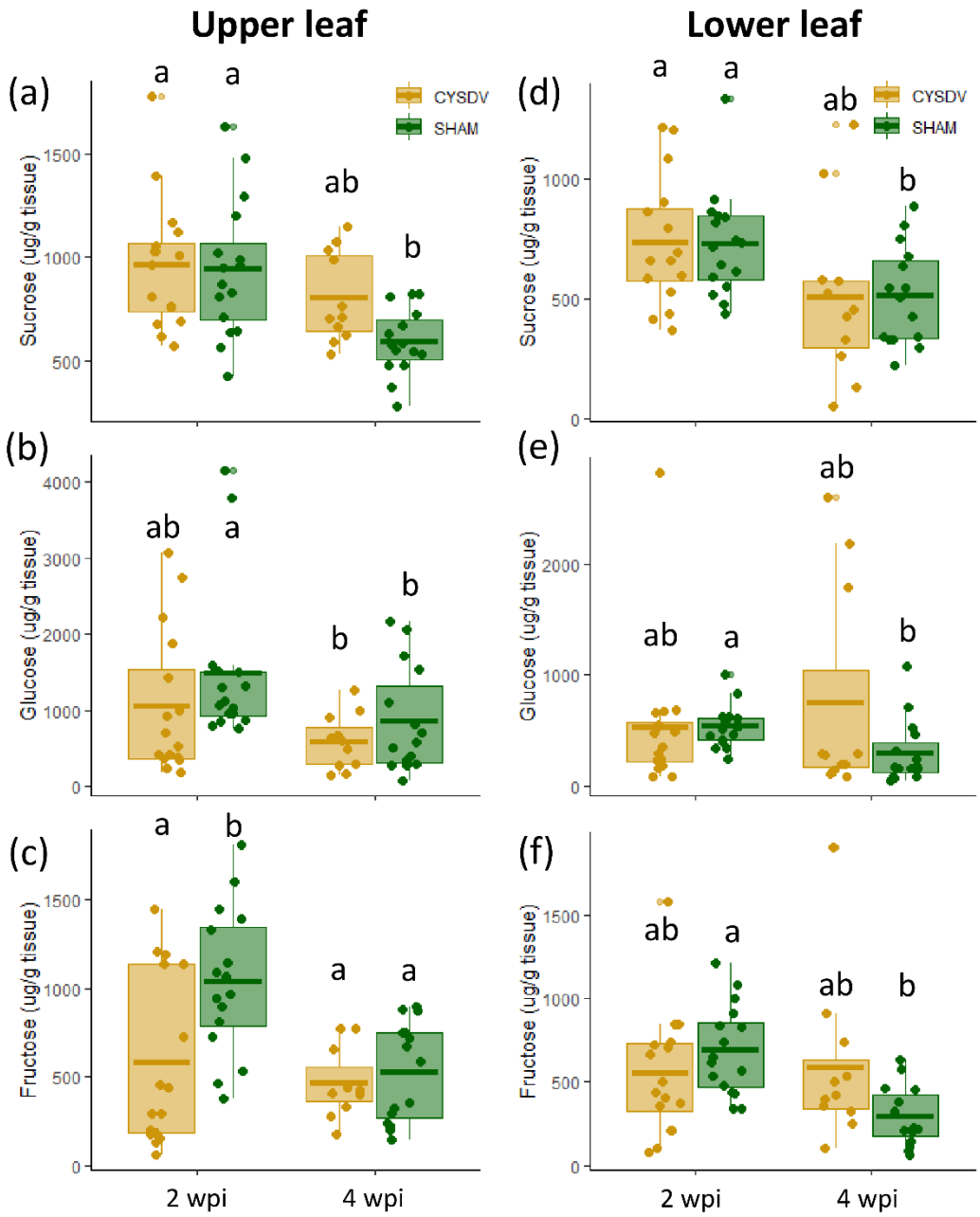
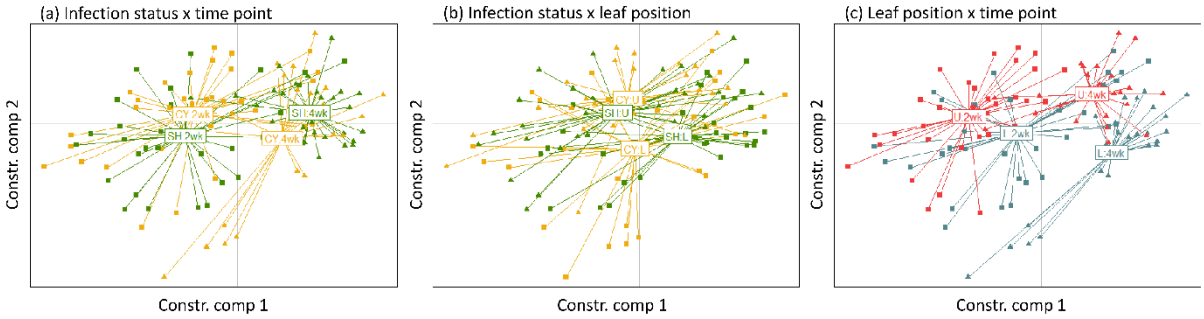

Upper leaf
Lower leaf
(a)
(b)
(c)
(d)
(e)
(f)
CYSDV
SHAM
Sucrose (ug/g tissue)
Glucose (ug/g tissue)
Fructose (ug/g tissue)
2 wpi
4 wpi

Figure 7.



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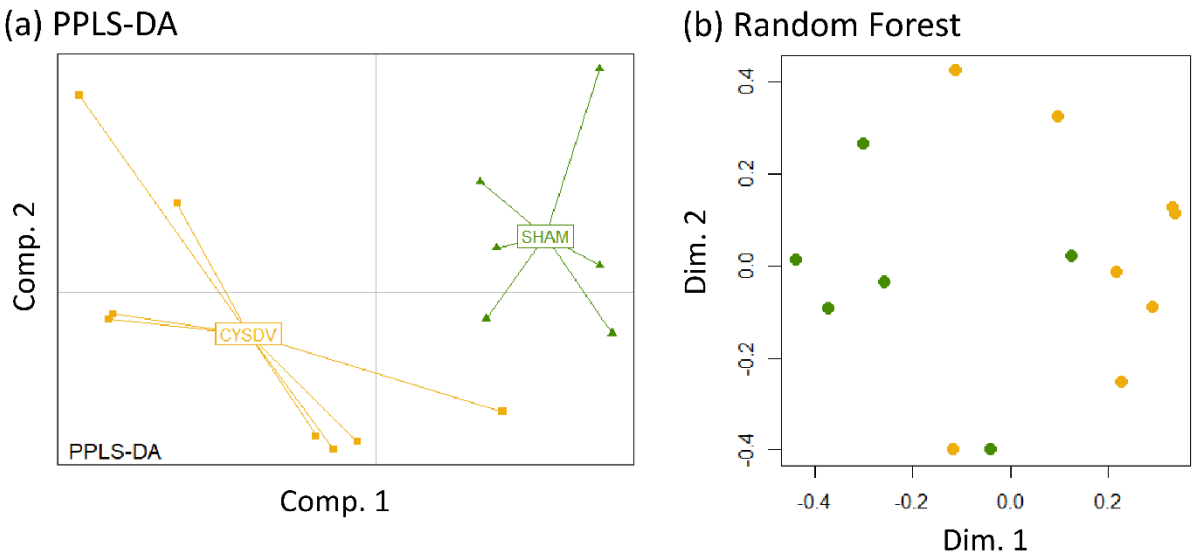
975 **Figure 8.**



976

977 **Figure 9.**