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La atracción de un insecto no-vector (e.g., mosca blanca, *Trialeurodes vaporariorum*) hacia plantas hospederas infectadas (e.g., chile, *Capsicum annuum*) reduce el 'fitness' del begomovirus, *Pepper golden mosaic virus* (PepGMV)

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M. C. Yesenia Ithaí Ángeles López

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Dr. Martin Heil

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Attracting a non-vector insect (whitefly, *Trialeurodes vaporariorum*) to infected host plants (chilo, *Capsicum annuum*) reduces fitness parameters of the begomovirus, *Pepper golden mosaic virus* (PepGMV)

Thesis presented by

M. Sc. Yesenia Ithaí Ángeles López

In order to obtain the degree of

Doctor in Sciences in the specialty of Plant Biotechnology

Thesis supervisor

Martin Heil, PhD

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Albert Einstein

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<<Somos granos de maíz

De una misma mazorca

Misma es nuestra raíz

Mismo nuestro camino. >>

Otomi fragment poem "HIN GI 'BU: HSE: HU". Thaayrohyadi

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Abbreviations

AA	Amino acids
Asx	Aspartic acid/asparagine
BSTFA	N, O-bis (trimethylsilyl) trifluoroacetamide
CP	*Coat protein* gene
DHJA	Dihydroxy jasmonic acid
dpi	days post inoculation
DW	Dry weight
EDTA	Ethylenediaminetetraacetic acid
FW	Fresh weight
GC-MS	Gas chromatography-mass spectrometry
Glx	Glutamic acid/glutamine
HPLC	High-pressure liquid chromatography
JA	Jasmonic acid
mosmol	milliosmole
MPa	Mega Pascal
PCA	Principal Components Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase chain reaction
PepGMV	*Pepper golden mosaic virus*
PFB-Br	Pentafluorobenzyl bromide
PR	Pathogenesis Related
REn	*Replication enhancer* gene
SA	Salicylic acid
SDS	Sodium dodecyl sulfate

Abstract

Many plant viruses can manipulate their host plants by changing their odor or their quality for herbivores (e.g. changed contents of amino acids (AA), sugars, or defense compounds) and, thereby, attract their insect vectors. Although in some cases the plants emit 'deceptive' volatile signals, in other cases the insect vectors benefit from these changes, using them to evaluate the quality of the host-plant and thereby, improve the survival of their offspring. However, non-vector insects can also benefit of the phenotypic changes of the host plants. It remains an open question whether the attraction of a non-vector can feed back to the manipulating virus. Here, we aimed to evaluate the level of specificity of the attractive effect of virus-infected plants and the effect of the putative attraction of a non-vector on the fitness of the virus. We used the tripartite interaction among the non-vector whitefly (*Trialeurodes vaporariorum*), the begomovirus *Pepper golden mosaic virus* (PepGMV) and chili plants (*Capsicum annuum* L.). We found that the virus-infected plants emitted volatiles that were attractive for the whitefly and that infected plants supported higher reproduction of the whitefly, an effect associated with an almost 30-fold increase in the AA content of the phloem. However, the colonization of the plants by the non-vector caused a very strong (~100-fold) reduction in viral loads in these plants. The whitefly neutralized the changes that were caused by PepGMV in the nutritional quality to attract the vector insect and suppress salicylic (SA) and jasmonic (JA) acids in leaf tissue. These patterns were similar to control plants colonized by whiteflies. These results demonstrate that phenotypic changes in virus-infected plants can be exploited by non- vector insects and that the attraction of such non-vectors can impair the fitness of the virus.

Resumen

Muchos virus de plantas manipulan a sus plantas hospederas cambiando su aroma y calidad nutricional para los herbívoros (e.g., modificando el contenido de aminoácidos (AA), azúcares o compuestos de defensa) y así atraer a sus insectos vectores. Aunque en algunos casos la planta emite señales 'engañosas', en otros casos el insecto vector se beneficia de estos cambios, usándolos para evaluar la calidad de su planta hospedera y así mejorar la supervivencia de su progenie. Sin embargo, insectos no-vectores pueden beneficiarse de los cambios fenotípicos de las plantas hospederas. Hasta ahora, permanece abierta la siguiente pregunta: ¿puede la atracción de un no-vector afectar el "fitness" del virus que manipula la interacción? En este trabajo, evaluamos el nivel de especificidad de la atracción de la planta infectada con el virus y el efecto de la posible atracción de un insecto no-vector en la acumulación del virus. Usamos la interacción tripartita entre una mosca blanca, insecto no-vector (*Trialeurodes vaporariorum*), el begomovirus *Pepper golden mosaic virus* (PepGMV) y plantas de chile (*Capsicum annuum* L.). Encontramos que las plantas infectadas emiten volátiles que fueron atractivos para la mosca blanca y que la planta infectada mantiene una alta reproducción en estas plantas, un efecto asociado con un incremento de casi 30 veces en el contenido de AA en el floema. Sin embargo, la colonización de las plantas por el insecto no-vector causa una severa reducción (~100 veces) en la carga viral en estas plantas. La mosca blanca neutraliza los cambios causados por PepGMV en la calidad nutricional para atraer al vector y suprime la acumulación de los ácidos salicílico (AS) y jasmónico (AJ) en el tejido de las hojas, siendo los patrones similares a las plantas control colonizadas únicamente por la mosca blanca. Éstos resultados demuestran que los cambios fenotípicos en las plantas infectadas con virus pueden ser explotados por insectos no-vectores y que su atracción perjudica el 'fitness' del virus.

1. Introduction

Most pathogens and parasites such as viruses use so-called vectors to gain entry to their hosts. These are organisms that usually do not permit the parasite to conclude its life-cycle but that facilitate the transmission of the parasite from one host to the other (Stout, Thaler, and Thomma 2006; Colvin et al. 2006; Fereres and Moreno 2009). The most important vectors of pathogens and parasites are arthropods and among them, the main dominating group is the insects (Kluth, Kruess, and Tscharntke 2002). Therefore, the study of tripartite pathogen-vector-host interaction is of great interest due to their role in the dispersal of human, animal and plant diseases throughout the world.

Herbivorous insects require host plants as their major food source, either for the adults or the larvae, or both. In species with herbivorous larvae, the adults usually seek the host plant to lay eggs, and as a virus vector, can obtain additional benefits in the 'fitness', such as an increase in the number of eggs laid by the female and/or prolonged lifetime (Kennedy 1951; Castle and Berger 1993; Belliure et al. 2005; Jiu et al. 2007). On the other hand, the virus needs to be transported by the insect and needs the host plant for its replication. As such, it can be considered to be the most dependent partner of the interaction. In fact, the pathogens and parasites transmitted by vectors are seen as manipulators of the interaction because they alter the fitness of the vector and the physiology of the host in order to enhance their transmission rates (Hurd 2003; Moreno-Delafuente et al. 2013). The changes described so far that are induced by the virus in the host plant in order to help it to attract their insect vector, are the emission of volatile organic compounds (VOCs) and modified host quality (e.g., in defense status and/or nutritional status).

Plant VOCs are involved in plant-plant signaling (communication) and in plant defense. However, plant VOCs can also attract herbivore insects (Ryan 2001; Felton and Tumlinson 2008), a feature for which a role of VOCs as attractants of insect vectors in the virus-insect-plant interaction was proposed. This effect has been demonstrated in several pathogen-vector-host interactions, where it was observed that VOCs released by infected hosts were more attractive to vectors than the VOCs of control hosts (Eigenbrode et al. 2002; Jiménez-Martínez et al. 2004; Mauck et al. 2010). However, attracting the insect vector is only the first step that the virus requires to be transmitted. Consecutively, it is necessary that the herbivorous insect is able to overcome the defenses of the hosts.

In pathogen-vector-host interactions, both organisms need to avoid the plant defenses that are triggered by the insect attack and/ or the virus infection to establish the interaction. These defenses are mediated by several hormones: salicylic acid (SA) and jasmonic acid (JA) are of primordial importance, as they regulate defense pathways that are directed against pathogens and herbivores. These hormones interact via a crosstalk that has been intensively investigated (Smith, De Moraes, and Mescher 2009; Pieterse et al. 2009). For pathogen-vector-host interactions, it has been observed that the antagonism between SA and JA facilitates the interaction in some cases, while in others, a lacking antagonism favors the interaction (Abe et al. 2012; Nachappa et al. 2013).

Besides specific defense responses, the nutritional status of the host plant is important for female insects to select a host for oviposition, in order to maximize its own fecundity and the performance of its offspring (Thorsteinson 1960; Awmack and Leather 2002). In most cases, it has been observed that the host quality for the offspring is more important than for the adults themselves, a phenomenon that has been termed the 'mother knows best' principle

(Bernays and Graham 1988; Gripenberg et al. 2010). In order to achieve optimal host selection in relatively short time, insects use visual and olfactory cues to localize and select the best host plant to obtain food and for egg deposition (Thorsteinson 1960; Bruce, Wadhams, and Woodcock 2005; Döring and Chittka 2007). Therefore, the changes in VOC profiles that are caused by previous infection could provide information about the current quality of the host plant for the adult herbivore that engages in a virus-insect-plant interaction. However, it has been observed that non-vector insects also can obtain benefits from feeding on infected hosts and thereby improve their performance, although they do not act as parasite vectors (Belliure et al. 2005; Belliure, Sabelis, and Janssen 2010). Moreover, VOCs in particular represent openly available information and, thus, are particularly prone to be used by non-vector herbivores as well, to assess host plant quality. This situation opens the question: which are the implications it might have for the fitness of the parasite if the parasite-induced changes in the host plant attract the wrong insect?

In the present work, we aimed at investigating the specificity of pathogen-induced changes in the VOC profiles of virus-infected plants by using a non-vector insect and studying its interaction with control vs. infected plants, and how this interaction can affect the virus fitness in the host plant. We used chili plants (*Capsicum annuum*, Solanaceae) infected with the begomovirus *Pepper golden mosaic virus* (PepGMV, *Geminiviridae*) and a non-vector insect: the whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). This species is vector of the *Crinivirus* genus but does not transmit begomovirus (Jones 2003; Navas-Castillo, Fiallo-Olivé, and Sánchez-Campos 2011).

Specifically, we asked: (i) whether *T. vaporariorum* can use visual or olfactory cues to distinguish PepGMV-infected over control host plants; (ii) whether the long-distance

choice predicts the oviposition behavior; (iii) whether the insects show different performances on control vs. infected plants; (iv) whether such differences in performance can be explained by changes in the SA/ JA profile of the infected plants or by changes in their nutritional quality (e.g., the content and composition of amino acids (AA) in the phloem), and finally (v) how does whitefly colonization affect the virus accumulation in the host plant.

2. Theoretical framework

2.1 VOCs in the virus-insect-plant interaction

VOCs have a low molecular weight and are lipophilic; there is also a great chemical diversity of these compounds. The most important groups are those derived from fatty acids such as the green leaf volatiles (GLVs) and jasmonic acid methyl ester (MeJA), the derivatives of the shikimic acid pathway, such as salicylic acid methyl ester (MeSA), and terpenes (Dudareva et al. 2013).

The role of VOCs in the attraction of vectors in the virus-insect-plant interaction has been documented in the last years in several interactions, but so far, three biological systems have been most studied. The first interaction studied was the one between PLRV (*Potato leaf roll virus*, a Polerovirus) - *Solanum tuberosum/ S. sarrochoide* - *Myzus persicae* (Eigenbrode et al. 2002; Srinivasan et al. 2006). The second was between BYDV (*Barley yellow dwarf virus*, a Luteovirus) - *Triticum aestivum* L. - *Rhopalosiphum padi* (Jiménez-Martínez et al. 2004) and the third, between CMV (*Cucumber mosaic virus*, a Bromovirus) - *Cucurbita pepo- Myzus persicae*/ *Aphis gossypii* (Mauck, De Moraes, and Mescher 2010; Mauck, De Moraes, and Mescher 2014).

In all of those interactions, changes in VOCs profiles emitted by infected plants were observed, being the VOCs profiles of infected plants more attractive to the insect vector. Importantly, it was shown that a single volatile was generally not enough to maintain the attraction (Eigenbrode et al. 2002; Jiménez-Martínez et al. 2004; Srinivasan et al. 2006; Ngumbi et al. 2007; Mauck, De Moraes, and Mescher 2010). It was also reported that the age of the plants and leaves had an influence on vector attraction, because the VOCs profiles and

their concentrations changed with respect to these ontogenetic factors (Alvarez et al. 2007). Another important point to remark is that the stage of the plant infection with the virus also changed the VOCs profiles, making them less or more attractive to the insect vector. Most interesting, the underlying mechanisms appear to function in a quantitative manner, since in two studies, a positive correlation between the virus titer in the infected plants and the amount of VOCs emitted by these plants was found (Werner et al. 2009; Medina-Ortega et al. 2009). However, even though the VOCs of infected plants are more attractive to the insect vector (Mauck, De Moraes, and Mescher 2010), in the case of the plants infected with CMV, the insects spend less time on these plants because CMV also changed the plant quality of the host plant, making it less suitable as a host for the insects (Mauck, De Moraes, and Mescher 2014). Although these effects appear to be contradictory, the authors attribute this emission of a "deceptive volatile signal" to the transmission mode of CMV which, in difference to many other viruses, is non-propagative and non-circulative in the vector. Therefore, successful acquisition of the virus by the vector does not require that the insect vector spends a long time feeding on the host plant, like it is the case of other viruses, and transmission is consequently optimized by the attraction of vectors which shortly probe on the plant and then leave it to continue feeding on healthy plants, to which they transmit the virus.

Recently, a work that shows us how the virus changes the VOCs profiles was performed using the *Tomato yellow leaf curl China virus* (TYLCCNV, a *Begomovirus*)-*B. tabaci*-*Nicotiana tabacum* interaction (Luan et al. 2013). In this work, the authors found lower amounts of terpenes such as camphor, α-cedrene and β-cedrene in VOCs profiles of TYLCCNV-infected plants. In addition, the suppression in the expression of genes involved in terpene biosynthesis such as terpene synthases (pinene synthase, *5-epi*-aristolochene and

5-epi-aristolochene 12 synthases) in TYLCCNV-infected plants was observed. When control plants were sprayed with synthetic α-cedrene and β-cedrene, the authors found a lower percentage of whiteflies feeding on these plants and a decrease in the percentage of survivors. These results highlight the role of VOCs, particularly terpenes, in the establishment of the virus-insect-plant interaction.

2.2 The importance of the quality of the host plant for the herbivore insect on virus-insect-plant interactions

2.2.1 The defensive status of the host plant

Plants have different mechanisms to respond to the attack by pathogens and herbivores. Several hormones, including SA and JA, mediate these defense mechanisms. SA is known to regulate the responses against biotrophic bacteria and fungi, viruses and piercing-sucking insects such as aphids and whiteflies. In contrast, JA is associated with the regulation of responses against necrotrophic bacteria and fungi, and chewing insects. Both hormones are involved in complex regulatory networks, being in most cases antagonistic to each other (Thaler et al. 2002; Loake and Grant 2007; Koornneef and Pieterse 2008; Smith, De Moraes, and Mescher 2009; Pieterse et al. 2009). However, little is known about the roles of these hormones in the establishment of the virus-vector/ non vector-plant interaction.

Only a few studies have explored how the defensive status of the host affects the interaction virus-vector/ non vector-plant. One of these studies reported the enhanced survival rates of the offspring of the insect vector *Frankiniella occidentalis* (thrips) on plants infected (*C. annuum*) with *Tomato spotted wilt virus* (TSWV, a *Tospovirus*), independently

of whether or not the insects carried the virus, suggesting that plant pathogens suppress host plant defenses against the vectors (Belliure et al. 2005). In another study, the authors observed that the antagonism between SA and JA favored the establishment of the TSWV–*F. occidentalis*–Arabidopsis interaction (Abe et al. 2012). Concretely, the authors observed that TSWV promoted an accumulation of SA and enhanced the defense responses that depend on this hormone, thereby repressing the defense responses mediated by JA. In consequence, the feeding and performance of *F. occidentalis* on the host plant was facilitated (Abe et al. 2012). Similarly, another study reported the same increase in the accumulation of SA that caused an alteration in the JA levels in CMV-infected plants; also, the hormone ethylene was induced by CMV in those plants (Mauck, De Moraes, and Mescher 2014).

A study that indicated that non-vector insects are capable to take advantage of defense status of virus-vector insect-plant interactions was reported by Belliure and coworkers (Belliure, Sabelis, and Janssen 2010). The authors showed that *T. urticae*, a non-vector insect, enhanced its performance and oviposition rate on TSWV- infected plants and on *F. occidentalis*-damaged plants, attributing these effects to the crosstalk between SA and JA, but also to the nutritional status of the host plant. However, and in contrast to the above mentioned reports, in the interaction between TSWV-*T. urticae*-tomato plants (*S. lycopersicum*), no antagonistic crosstalk between the SA and JA pathways was found in the transcriptome profiles of TSWV-infected plants (Nachappa et al. 2013). In this work, an up-regulation of SA-dependent genes was observed in infected plants without any apparent down-regulation of the JA-dependent genes, whereas an up-regulation of both SA and JA-dependent genes was found in plants that interacted with both TSWV and *T. urticae*. In the case of the *Rice black streak dwarf virus* (RBSDV, a *Fijivirus)-Nilaparvata lugens* (non-

vector plant hopper)-*Oriza sativa* interaction, changes in the expression of genes involved in defense and detoxification of reactive oxygen species (e.g. peroxidase, catalase, glutathione peroxidase, etc.) were observed (Xu et al. 2014).

2.2.2 Nutritional status of the host plant

The nutritional quality of the host plant is crucial for herbivorous insects because it determines their own fitness and the performance of their offspring (Thorsteinson 1960; Awmack and Leather 2002). For females, meeting both requirements frequently represents a dilemma, because the nutritional requirements of the adults and the larvae are not identical. Think, for example, of the diverse family of Lepidoptera: adult butterflies feed on floral nectar whereas larvae feed on leaves, in many cases even leaves of other plant species. In most of cases that have been investigated so far, the requirements of the offspring fitness turned out to be more important for decision-taking by the females than their own fitness, a phenomenon coined as 'mother knows best' principle (Gripenberg et al. 2010; García-Robledo and Horvitz 2012). In general, to select a host plant to oviposit is not an easy task; females need to take into account visual and olfactory cues to localize a possible host and to obtain information about its defensive and nutritional status. Finally, females usually taste the tentative host to make the final choice. In the case of phloem-feeding sucking insects, the nutritional quality of the host plant is particularly relevant to select the best choice because its food source, the phloem content, is poor in AA but contains high amounts of sugars (Dinant et al. 2010).

Using artificial diets, (Thompson 2006) observed that different concentrations of AAs and sugars affect the survival and oviposition of *Bemisia tabaci* and aphids. Similarly,

several studies showed an improvement in fitness of insect vectors when feeding on infected plants (Maris et al. 2004; Belliure et al. 2005; Hodge and Powell 2008; Xu et al. 2014) and few reported the opposite effect (Donaldson and Gratton 2007). However, a direct link to phloem quality was not made in these studies

Nevertheless, the first evidence that viruses can alter the nutritional status of the host plants in favor to an insect vector was reported by Blua and coworkers (Blua, Perring, and Madore 1994). In this work, sugars and AAs levels were quantified in the phloem of control and *Zucchini yellow mosaic virus* (ZYMV, a *Potyvirus*)-infected cucumber (*Cucurbita pepo* L.) plants at different days of the infection (2-9 and 13-37 days). The authors found significant differences in some individual AA between control and infected plants, but in general, the total AAs content was not different. In contrast, total protein and sugars levels were lower in the phloem of infected plants. Additionally, the longevity and fecundity of the vector *A. gossypii* was enhanced in infected plants. By contrast, a recent study showed that virus infection not always improves the quality of the host plant for the vector: free AAs levels and the AA: sugars ratio in the phloem of CMV-infected cucumber plants were reduced as compared to controls, resulting in the phloem being a poor food for the vector, *M. persicae* (Mauck, De Moraes, and Mescher 2014).

As mentioned before, the non-vector insects can take advantage of these changes in the nutritional status of the host plant. For example, the total free AA levels in leaf tissue were increased by TSWV infection in the TSWV-*T. urticae*-tomato interaction and resulted in benefits for the non-vector insect (Nachappa et al. 2013), and similar observations were reported in RBSDV-infected plants, showing also an increase in sugar levels in the leaf tissue (Xu et al. 2014).

2.3 PepGMV- non vector whitefly- chili plant interaction

2.3.1 The Begomovirus *PepGMV*

Begomoviruses belong to the family *Geminiviridae*. As general characteristics these viruses have a genome of single-stranded DNA which, depending on the family, is distributed over one or two components with a size between 2.6-2.8 Kb. Its physical form is two fused incomplete icosahedra. In particular, the *Begomoviruses* have one or two components and correspondingly are called monopartite or bipartite viruses; additionally, the latter can count with a satellite (a mini component) (Fauquet and Stanley 2003; Yadava, Suyal, and Mukherjee 2010). As opposed to others virus families that have multiple insect vectors, *Begomoviruses* are exclusively transmitted by the cryptic 'specie complex' *B. tabaci*, covering more than 28 morphologically indistinguishable species (Brown, Frohlich, and Rosell 1995; De Barro et al. 2011; De Barro 2012). Until now, *B. tabaci* is reported as the main vector (Markham et al. 1994; Brown 2000; Jones 2003; Seal, Jeger, and Van den Bosch 2006; Navas-Castillo, Fiallo-Olivé, and Sánchez-Campos 2011).

In Mexico, begomoviruses have been reported to infect diverse chili species. Two major viruses are the *Pepper golden mosaic virus* (PepGMV) and *Pepper huasteco yellow vein virus* (PHYVV); both are bipartite (contain components A and B) and can be transmitted by *B. tabaci* at the same time (Méndez-Lozano et al. 2003; Carrillo-Tripp, Lozoya-Gloria, and Rivera-Bustamante 2007; Medina-Ramos et al. 2008). PepGMV was reported for the first time in Mexico in the year 2000 and was found in infected chili plants, *Capsicum* spp. (Morales and Jones 2004). The symptoms of PepGMV infection are the presence of a yellow mosaics along the leaf and a leaf deformation known as curly leaf (Carrillo-Tripp, Lozoya-

Gloria, and Rivera-Bustamante 2007). Although several aspects are known of PepGMV infection on chili plants, the interaction with the vector or with non-vector insects has been poorly explored.

2.3.2 The whitefly, *Trialeurodes vaporariorum*

Whiteflies are sucking-piercing insects that usually use large number of species plants (around 900 species) from diverse families, as hosts. The two main species of whiteflies that cause important losses in agricultural crops in field and greenhouse are the cryptic 'species complexes' *B. tabaci* and *T. vaporariorum*. Both whiteflies share a large number of host plants, including chili (Inbar and Gerling 2008). The whitefly *T. vaporariorum* is a vector of viruses of the *Criniviruses* genera (*Closteroviridae* family), but it does not vector *Begomoviruses* (Jones 2003; Navas-Castillo, Fiallo-Olivé, and Sánchez-Campos 2011). The center of origin of this whitefly is in the Americas, Central or South America. The life cycle comprises six stages of development: egg, four nymph instars, and the adult insect. Depending of its host plant and the environmental conditions, the development of the whitefly can be complete between 19-55 days at 18-33 °C (Byrne and Bellows 1991). *T. vaporariorum* coexists with *B. tabaci* in the field and have been found to share the same host plant in the field (Arnó, Albajes, and Gabarra 2006; G.-F. Zhang and Wan 2012). Therefore, the exploration of the behavior of *T. vaporariorum* towards begomovirus-infected plants can provide information about the specificity of the changes in the host plants that are caused by the virus. Does PepGMV specifically manipulate its host to attract its vector *B. tabaci*, or can the changes in virus-infected plants also be exploited by a non-vector such as *T. vaporariorum*?

2.3.3 Chili, a host plant of *PepGMV*

The chili is a crop of economic importance in Mexico because the fruit has a major role in the gastronomic culture of the country. Several species of *Capsicum* spp. are susceptible to the infection of begomoviruses such as PepGMV and PHYVV. In particular, the cultivar *C. annuum* cv. Sonora Anaheim has been studied during the infection with PepGMV and PHYVV (Carrillo-Tripp, Lozoya-Gloria, and Rivera-Bustamante 2007). An important characteristic to remark is the loss of symptoms in the younger leaves of chili plants several days after the infection with PepGMV, this "symptom remission" is unusual in virus-infected plants (Carrillo-Tripp, Lozoya-Gloria, and Rivera-Bustamante 2007).

3. Objectives

3.1 General objective

The general objective of this work was to evaluate the effect of PepGMV infection of chili host plants on their nutritional quality and the emitted volatile blends as well as the significance of these changes for a non-vector insect, *Trialeurodes vaporariorum*, and the impact that *T. vaporariorum* attraction has on the titer of the virus in the host plant.

3.2 Particular objectives

I. Confirm that *T. vaporariorum* is not a vector of PepGMV.

II. Evaluate the *T. vaporariorum* preference towards control and PepGMV-infected plants at different days post inoculation, in cage and olfactometer, and choose a time post inoculation for subsequent trials.

III. Evaluate the *T. vaporariorum* preference towards control and PepGMV-infected plants to oviposite and determine the VOC profiles of these plants.

IV. Quantify SA, JA, total and free AA in leaf tissue, and free AA in phloem, in individual leaves of control and PepGMV-infected plants at 20 dpi.

V. Quantify SA and JA in leaf tissue and free AA in phloem in individual leaves of control and PepGMV-infected plants in response to whitefly colonization.

VI. Correlate the AA concentration in phloem with the predicted composition of AA of PepGMV and PR proteins identified as differentially expressed in the transcriptome of chili plants.

VII. Quantify PepGMV in symptomatic leaf tissue of PepGMV-infected plants in response to whitefly colonization.

4. Materials and Methods

4.1 Plants and Insects

Chili seeds of *Capsicum annuum* L. var. Sonora Anaheim (Seminis) were planted in pots of 350 cm^3 with sterile soil mixture (3 parts Sunshine Mix 3™ [SunGro Horticulture, Bellevue, WA], 1 part loam, 2 parts mulch, 1 part vermiculite [SunGro Horticulture] and 1 part perlite [Termolita S.A., Nuevo León, México]). The plants grew under greenhouse conditions under a natural photoperiod, were watered every two days and fertilized weekly.

The whitefly *Trialeurodes vaporariorum* was donated by Dr. Guadalupe Peña Chora (Universidad Autónoma del Estado de Morelos) and maintained on tomato plants (*Solanum licopersicum*, cv. Río Fuego [Cal-Oro, Vegetable Seeds, United Genetics, Inc., Gilroy, USA]) in a growth room (3.2 m [width] × 4.2 m [length] × 3 m [height]), under controlled conditions of light intensity (≈300 $\mu mol^{-1}m^2\ s^{-1}$) and photoperiod (16 h light/ 8h dark, at 28 °C).

4.2 Virus inoculation

The two plasmids that contain the genome (components A and B) of the begomovirus PepGMV (Tamaulipas isolate, *Geminiviridae*) were inoculated into young chili plants having four extended leaves using a low-pressure biobalistic device as reported (Garzón-Tiznado et al. 1993; Torres-Pacheco et al. 1996; Carrillo-Tripp, Lozoya-Gloria, and Rivera-Bustamante 2007). Control plants were mock-inoculated with the carrier mix with no PepGMV plasmids.

4.3 Transmission assays

Six infected plants (20 days post inoculation) were introduced in a cage with 100 whitefly adults. After five days, the whiteflies were removed and reintroduced in a cage that contained six control plants. After five days, the insects were removed and subsequently stored at -70°C, and the plants were harvested 20 days after to check for the appearance of symptoms. The presence of the virus in plant and insect tissues was determined via the amplification of the *REn* (Replication Enhancer) gene by PCR, using the primers PepGMVRen5' (5'-GCCTGATGCACAGTGATGCTCTC-3') and PepGMVRen3' (5'-GTGGAGTATAACGTCATTGATG-3'), yielding a PCR product of expected 408 bp (Carrillo-Tripp, Lozoya-Gloria, and Rivera-Bustamante 2007). We used the following program in the thermocycler (Bio-Rad): 3 min at 95°C; 30 s at 95°C, 30 s at 55°C, 40 s at 72°C (30 cycles); and the final extension at 7 min at 72°C. The product of the amplification was run in agarose gels 1% (previously dyed with ethidium bromide) in an electrophoresis chamber at 90 mV.

4.4 DNA extraction of plants and insects

The insects and the leaves of the infected and control plants were shock-frozen in liquid nitrogen and ground. The Power Soil ™ DNA Isolation kit (MoBio, Carlsbad, CA, USA) was used for the DNA extraction from insects. Groups of 30 whiteflies were introduced in the mixture tube and extracted following the protocol as suggested by the manufacturer.

For the DNA extraction from leaves, 100 mg of the ground tissue was placed in an Eppendorf tube and 600 µL of Dellaporta buffer (Tris-HCl 7.4 mM, EDTA 50 mM, NaCl 0.5

M, SDS 1%, β-mercaptoethanol 2 mM) was added. The samples were incubated at 65°C for 10 min. After the incubation, 600 µL of potassium acetate 3M (Sigma) were added and incubated on ice for 10 min. The samples were centrifuged at 14,000 rpm for 10 min. The supernatant was separated and 600 µL of isopropanol (Keral) were added, and the samples were incubated at -20 °C for 10 min. Subsequently, the samples were centrifuged at 14,000 for 10 min. The supernatant was discarded and the excess isopropanol was evaporated at room temperature. The pellet was washed twice with 1 mL of ethanol 70% and dried at room temperature. Finally, the pellet was re-suspended on 20 µL of MilliQ sterile water. The DNA was quantified in a nanodrop equipment and used for the amplification of the *REn* gene as mentioned above or for coat protein (*CP*) gene by qPCR, as mentioned below.

4.5 Cage and olfactometer assays

For the cage assay, one infected and one control plant were put inside the cage, locating the plants at the extremes of the cage extremes, and a glass jar was placed in the center that contained the whiteflies. We used 100-300 whiteflies for each days post inoculation (dpi) tested. We counted the number of whiteflies present on the leaves six h later. These assays were performed in similar conditions as those under which the colony was maintained, in a growth room as described before.

For the olfactometer assays we used a "Y" tube (20 cm long , and a 120 mm diameter) and we performed the assays in a dark room with a temperature of 28°C, using as light source a 40-watt bulb (see Figure S1 for more details). Infected or control plants were placed in each branch of the "Y" tube at different dpi. A waiting period of 25 min was defined as the limit

for the insect to make a choice. A total of 100-300 whiteflies were tested. After every 50 whiteflies the position of the plants at the ends of the olfactometer was changed to avoid experimental bias.

4.6 Volatile collection and analysis

Volatiles were collected using solid-phase-micro-extraction (SPME) fibers (2 cm, Carboxen/ Polydimethylsiloxane/ Carboxen; Supelco) from infected and control plants at 20 dpi. To collect volatiles from the headspace, the plants were covered with PET bags (Toppits) and the fibers were exposed to the headspace during 18 h. Subsequently, the samples were run in a GC-MS (7890A GC System, 5975C EI/CI MSD; Agilent Technologies) equipped with a HP-5 column (30 m length, 0.25 mm diameter, 0.25 µm film; Agilent Technologies), using the following temperature program: 60°C as initial temperature then, an increase of 5°C/ min to 80°C; then, 80°C for 1 min; then, increase 8°C/ min to 210°C, and hold for 5 min. To identify the VOCs, the NIST mass spectral library and pure standards of some volatiles were used. Results are reported as mean percentages of the total peak area obtained by the normalization of peak area.

4.7 Extraction and analysis of total and free amino acids (AA) from phloem and leaf tissues

Phloem fluid was collected as described in a previous study (Deeken et al. 2008). In short, the petioles of the leaves were put in tubes with 1.5 mL of an EDTA/ sorbitol sterile solution (270 mosmol: Sorbitol [Sigma], 5 mM EDTA, pH 7.5, [Sigma]) to which protease

inhibitor (one tablet per 50 mL of the solution; Roche) had been added. The tubes were put in a box with a CO_2 saturated atmosphere under light for two h, and the exudates were then immediately frozen in liquid nitrogen and stored at -70°C until further use.

For the quantification of free AA in phloem, 300 µL of the phloem exudate were mixed with 500 µL of acetonitrile (HPLC degree; Baker, México) for 12 h at 4°C, following a published method (Bidlingmeyer, Cohen, and Tarvin 1984). For the quantification of free AA in leaf tissue, 100 mg of frozen leaf tissue from individual leaves were used and mixed with 500 µL of acetonitrile following the same method mentioned for the exudates. For total AA quantification in leaf tissue, a previous step was made using 100 µL of HCl 8 N to hydrolyze the proteins and peptides of 20 mg of lyophilized leaf tissue and following the same method mentioned above. Two microliters of each sample were injected in an HPLC (Agilent Technologies 1200 Series) equipment with a Pico-Tag column (3.9 x 150 mm [C18], Particle 3 µm; Waters) and detected at 254 nm wavelength. The AA standards (Sigma-Aldrich) for identification and quantification were run in the same conditions. The standard aspartic acid/ asparagine (Asx) was used for the quantification of Asp and Asn, similarly for the standard glutamic acid/ glutamine (Glx) that was used for the quantification of Glu and Gln.

4.8 Extraction and quantification of SA and JA

4.8.1 SA quantification

The extraction of SA was performed according to previous studies with some modifications (Malamy, Hennig, and Klessig 1992; Meuwly and Métraux 1993), using the

five youngest leaves of control and infected plants. For extraction, 250 mg of ground tissue was mixed with 750 µL of methanol 90% and 250 ng/mL of ortho-anisic acid (internal standard), and incubated at 4°C all night. After incubation, the samples were centrifuged at 13,000 rpm for 15 min, the supernatant was recovered and stored, and the pellet was re-suspended on 750 µL of 100% methanol (Sigma) and centrifuged again as mentioned before. Both supernatants were combined and dried in a *Concentrator plus* (Eppendorf). The pellet was re-suspended with 500 µL of TCA 5% and centrifuged at 6,000 rpm for 10 min. The resulting supernatant was mixed with two volumes of ethyl acetate-hexane (1: 1 v/ v) and incubated at room temperature for 10 min. The organic phase was recovered and dried with gaseous nitrogen. The resulting pellet was mixed with 20 µL of pyridine and 80 µL of BSTFA (Sigma-Aldrich) and incubated at 80°C for 1h for the derivatization to proceed (in a volume of 100 µL) and one microliter was injected in the GC-MS. The samples were analyzed with a GC-MS (Agilent Technologies) equipment with the column DB-1MS (60 m length, 0.25 mm diameter, 0.25 µm film; Agilent Technologies), using the following oven program: 150°C for 3 min; then, 4°C/ min to 260°C; then hold at 260°C for 25 min. For quantification, a standard curve of pure SA (Baker) was run and peak areas were evaluated with reference to the internal standard.

4.8.2 JA quantification.

For the extraction of JA, a previously reported protocol was followed, with some modifications (Mueller and Brodschelmt 1994) using the five youngest leaves of control and infected plants. For extraction, 250 mg of fresh ground tissue were mixed with 500 µL of ethyl acetate and 100 mg^{-1} mL of dihydroxy jasmonic acid (DHJA), and incubated overnight. The samples were centrifuged at 13,000 rpm at 4°C for 15 min, and the supernatant was

recovered and stored. The pellet was mixed with 500 µL of ethyl acetate and centrifuged again. The supernatant was recovered, combined with the first, and dried with gaseous nitrogen. The resulting pellet was mixed with 100 µL de N'N' disopropylethylamine, 100 µL of chloroform, and 10 µL of PFB-Br. The samples were incubated at 60°C for 30 min, the solvent was evaporated with gaseous nitrogen and the pellet was re-suspended with 100 µL of methanol (HPLC degree, Sigma-Aldrich). This mix was placed in GC-vials for the GC-MS analysis. The samples were run in a GC-MS (Agilent Technologies) with the column DB-1MS and the following oven program: 150°C for 3 min; then, 4°C/ min to 280°C; then hold at 280°C for 25 min. For the quantification, a standard curve using pure JA (Sigma) was performed normalized to the internal standard.

4.9 Oviposition assays

For the oviposition assay, six infected plants (20 dpi) and six control plants were introduced in a cage with 100 whitefly adults. After 5 days, the whiteflies were removed and after a 15 day-incubation period, the nymphs (in second/ third development stages) present on the leaves of infected plants and control plants were counted. These experiments were conducted in ambient conditions, as mentioned above.

4.10 Effect of the whitefly colonization in host plant quality

Groups of six control plants or six PepGMV-infected plants (T0 = 20 dpi) were placed in cages to which groups of 500 adult whiteflies were introduced. The whiteflies were allowed to oviposit for seven days, time after which they were removed (T2 = 27 dpi). After

removal of the whiteflies, a waiting time of 5 days was allowed for the appearance of first nymphal stage (T2 = 32 dpi). Then, samples of leaf tissue and phloem were collected at T0, at the egg stage (T1) and at T2. Hormones (SA and JA) were quantified in leaf tissue and free AA were quantified in the phloem for each individual leaf of control and infected plants, with leaves number 1 (Leaf 1) and 8 (Leaf 8) representing the youngest and oldest leaves, respectively. These assays were performed under greenhouse conditions. Additionally, the quantification of free and total AA in leaf tissue was performed only for control and infected plants, at T0.

4.11 PepGMV quantification by qPCR

For this purpose we used a pool of the four symptomatic youngest leaves collected from the above-mentioned experiment. The relative level of PepGMV DNA was evaluated by qPCR according to a previous study (Carrillo-Tripp, Lozoya-Gloria, and Rivera-Bustamante 2007). The samples were analyzed in a StepOne (Applied Technologies) equipment using the reactive Platinum SYBR Green qPCR SuperMix-UDG with ROX (Invitrogen Life Technologies) for the DNA quantification. The primers used for the *CP* gene were PepGMVCPq5' (5'-CCCATCGTGTAGGCAAGCGTTTCTG-3') and PepGMVCP3' (5'-CATGACGCTGTTGGTGTGGTTCTTG-3'). A 104 bp amplicon was the expected PCR product. The primers used for the *Elongation factor 1α* (*EF-1α*) housekeeping gene were EF-1α forward (5'-TCCAGTGTTCTGTGACATCCCGCCTAG-3') and EF-1α reverse (5'-CTCCATTTCGTCCATTCCTTCACCTGTG -3').

4.12 Heat map construction

The data used for the heat map were obtained from the whitefly colonization assay (T0 = 20 dpi; T1 = 27 dpi, with/ without eggs; T2 = 32 dpi, with/ without nymphs) from control and PepGMV-infected plants (see Tables S9a/ b-S13a/ b for more details). In the heat map, the quantitative data of the concentration of AA in phloem were transformed to relative units, which were visualized using a code of colors (dark blue to yellow), as indicated in Figures 5.10 A and B. The latter represent the changes in AA contents between the different treatments and in leaves of control and infected plants. Also, the fold changes of these AA were calculated using the original dataset. As 'controls' plants not colonized by the whiteflies at T0, T1 and T3 were used (as are represented in Figures 5.10 A and B). These fold changes are represented in logarithmic scales in the color bar (dark blue to red) where the number -4 in the color bar represents -40 fold-change with respect to the control, and the 4 represents 40 fold-change with respect to the control (Figure 5.10 C). Additionally, two vertical bars were inserted, one with six blocks with a different color that represent the treatments, and the second bar, which represents the individual leaves (eight), represented in a scale of gray. In the latter, the black represents the youngest leaf (Leaf 1), and the light gray represents the oldest leaf (Leaf 8). This pattern was repeated by each color in the first bar. The horizontal bar represents the classification of the AA, in which unessential AA are represented in gray, semi-essential AA in purple and essential AA in aquamarine. The heat map was constructed using the R program (R Core Team 2014) via the command 'heatmap3' from the package 'heatmap3' (Zhao et al. 2014).

4.13 Statistical analysis

The data obtained from the cage and olfactometer assays in were analyzed using a binomial test. For the oviposition assays, an ANOVA test followed by a posthoc Tukey HDS was used. The same test was used to determine the significance of SA, JA and AA data at T0, T1 and T2. A t-student test was used for the analysis of AA concentrations in the phloem and leaf tissues, in which control plants vs. infected plants, colonized-control plants vs. non colonized-control plants and colonized-infected plants vs. non colonized-infected plants were compared. The same test was used for the SA, JA and VOCs analysis in which control plants vs. infected plants were compared. PCA analysis was performed for VOCs profiles of infected and control plants and for the AAs content of six PepGMV proteins, three PR proteins (PR1a, PR1b and PR5) and the observed concentrations of AA in the phloem (three replicates) of infected plants at 20 dpi. A principal coordinate analysis (PCoA) analysis was performed using all the data on AA content in the phloem of control and infected plants at T0, T1 and T2. Here, the data were transformed into a logarithmic scale to calculate 'Euclidean' distances. The 'pcoa' command was activated to perform the PCoA analysis in the R program (Figure 5.11). All the statistical analysis, with the exception of the PCoA, were performed using SPSS 13.0 (SPSS Inc., Chicago, USA) using 95% (alpha = 0.05) as the confidence level. The complete data can be found in various Tables included in 'Annexes 1', in which the p-values of the tests are included.

5. Results

5.1 The whitefly T. vaporariorum is not a vector of PepGMV

To corroborate that *T. vaporariorum* is not a vector of PepGMV, transmission assays were performed and the genes *REn* and *Trap* were amplified by PCR from plants and insects (Figure 5.1). The genes *REn* and *Trap* were amplified from material of experimentally infected plants, but not from whiteflies that were in contact with these plants (Figure 5.1). Additionally, we could not amplify the genes in plants that had contact with whiteflies that had fed previously on infected plants (Figure 5.1). This result shows that *T. vaporariorum* does not transmit PepGMV and, thus, is most probably a non-vector insect.

5.2 T. vaporariorum is attracted by VOC emitted by infected plants

Choice assays were performed using plants at different days after the inoculation with PepGMV or with the mock solution (control plants) to see if the non-vector whitefly is attracted by the VOCs emitted from infected plants (Figure 5.2). In the cage, where the whiteflies can perceive visual and olfactory stimuli and even taste the plants, we observed a preference for infected plants at 4, 12, 20 and 22 dpi, but not at 18 dpi (Figure 5.2 A). Regarding the olfactometer, where the whiteflies can only use olfactory stimuli, a preference for infected plants was also observed at all times examined (Figure 5.2 B). These results show that infected plants can be attractive for a non-vector insect and that VOCs are the main contributing factor for the attraction of *T. vaporariorum*.

Figure 5.1. Expression of PepGMV *REn* and *TRap* genes in chili plants and in the whitefly *T. vaporariorum*. The expression of *REn* and *Trap* was analyzed using PCR in the tissue of insects exposed to infected plants, in virus-infected plants and in tissue of control plants before and after contact with the whiteflies that had been exposed to infected plants. The respective positive controls included the component A for both genes.

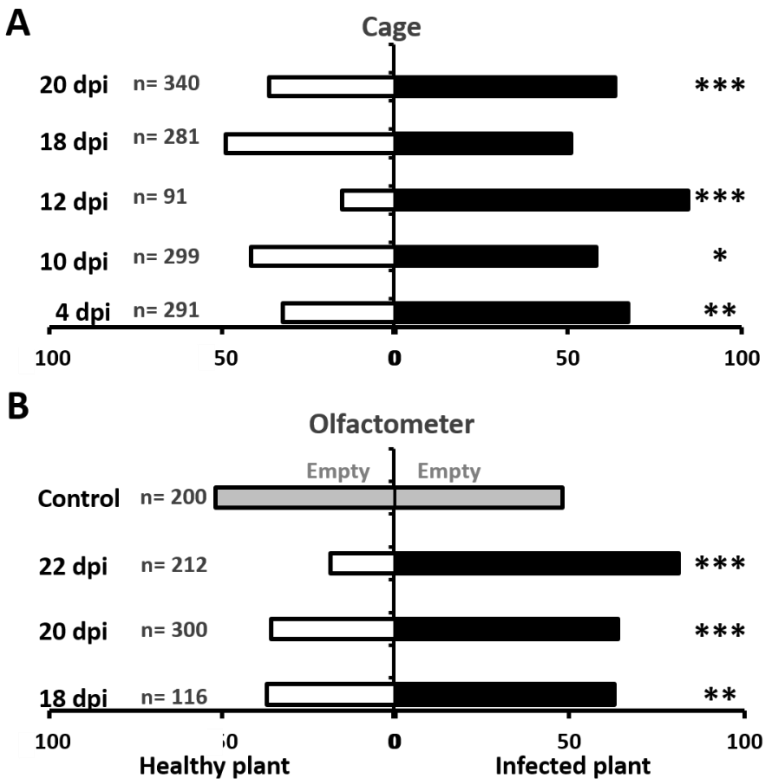

Figure 5.2 Cage and olfactometer behavioral assays with the non-vector *T. vaporariorum* at different days post inoculation (dpi) with PepGMV. A, choice assays in cage; **B**, choice assays in olfactometer. The whiteflies were introduced in the cage or olfactometer, as required, with control and infected plants sampled at different dpi. Binomial tests ($p<0.05$ *, $p<0.01$ ***, $p<0.001$ ***, n= 100-350) is specified in the figure for each time point.

5.3 VOCs profiles of control and infected plants at 20 dpi are different

To observe which VOCs characterized the odor of infected plants that were attractive to *T. vaporariorum*, these compounds were collected by SPME from blends of control and infected plants at 20 dpi. The 26 VOCs found in these profiles are reported in Table 5.1. Most of the compounds were tentatively identified as terpenes. However, five volatiles could not be identified using the NIST library (Table 5.1). Five of the compounds (α-terpineol, 2 (H) Naphthalenone, 3, 5, 6, 7, 8, 8a-hexahydro-4, 8a-ditmethyl-6-(1-methylethenyl), dendrasaline, 8-cedren-13-ol, and unknown 4) were found only in the profiles of infected plants, while six compounds (9 dimethyl-7-oxabicyclo [4.2.1] nona-2,4-dien-8-one, 2-ethenyl-bicyclo[3.1.1]hex-2-ene, ocimene, β-*cis*-terpineol, β-guaiene, and unknown 5) were exclusively detected in the profiles of control plants. Interestingly, most of the volatiles that showed a high percentage in the VOCs profiles of control plants were found at low percentages in the profiles of infected plants (see the p-values in Table 5.1), with the exception of D-limonene, which was found at a high percentage in infected plant-profiles.

In order to compare the complete profiles of control and infected plants, a PCA was performed using the 26 volatiles mentioned in Table 5.1. The profiles of the infected plants clustered in a more compact group than those of control plants (Figure 5.3). All the differences observed in the VOCs profiles of infected plants confirm that PepGMV modifies the odor of its chili host plant.

Table 5. 1 Volatile organic compounds emitted from chili pepper controls and PepGMV-infected plants (20 dpi) collected by SPME and analyzed by GC-MS.

RT (min)	Compound	Control plant Area* (%)	Infected plant Area* (%)	*P*-value
4.00	9 dimethyl-7-Oxabicyclo[4.2.1] nona-2,4-dien-8-one	n.d.[2]	24.8 ± 4.21	**0.000[1]**
4.55	2-Ethenyl-bicyclo[3.1.1]hex-2-ene	n.d.	1.76 ± 0.72	**0.001**
4.78	Ocimene	n.d.	2.57 ± 2.41	**0.047**
6.65	β-*cis*-Terpineol	n.d.	1.25 ± 1.00	**0.027**
6.99	D-Limonene	15.5 ± 8.02	35.4 ± 8.35	**0.002**
7.52	*cis*-p-Mentha-2,8-dien-1-ol	5.21 ± 8.71	6.39 ± 2.44	0.778
8.33	7-Dimethyl-Bicyclo[3.1.1]hep-3-ene-spiro-2,4'-(1',3'-dioxane),7	4.25 ± 2.89	7.37 ± 1.50	0.065
8.55	Undecane	2.12 ± 1.55	3.75 ± 2.98	0.279
10.04	Limonen-6-ol-pivalate	5.46 ± 2.01	5.42 ± 2.76	0.976
10.55	α-Terpineol	4.30 ± 1.02	n.d.	**0.000**
10.56	5-Carenol	2.26 ± 1.02	0.80 ± 0.66	**0.024**
12.90	Bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene	2.07 ± 0.90	0.27 ± 0.28	**0.005**
14.31	β-elemene	8.15 ± 1.80	2.28 ± 2.14	**0.000**
14.61	D-Longifolene	3.18 ± 1.55	1.54 ± 0.18	0.061
14.83	Unknown 1	2.51 ± 0.98	0.50 ± 0.36	**0.004**
15.54	Unknown 2	7.48 ± 3.69	0.49 ± 0.26	**0.008**
15.70	2(H)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-ditmethyl-6-(1-methylethenyl)	2.97 ± 1.00	n.d.	**0.001**
15.73	β-Guaiene	n.d.	0.43 ± 0.35	**0.029**
16.04	α-Selinene	5.15 ± 1.23	0.91 ± 0.94	**0.000**
16.40	Globulol	2.04 ± 0.90	0.15 ± 0.12	**0.005**
16.46	1,4-Methanoazulen-3-ol,decahydro-1,5,5,8a-tetramethyl-,[18(1a.3b.3ab.4a.8a)]-	3.34 ± 1.51	0.14 ± 0.12	**0.004**
17.17	Dendrasaline	1.85 ± 0.62	n.d.	**0.001**
17.47	8-Cedren-13-ol	5.91 ± 2.98	n.d.	**0.006**
18.55	Unknown 3	7.51 ± 4.72	0.19 ± 0.14	**0.017**
18.64	Unknown 4	3.92 ± 2.36	n.d.	**0.013**
19.17	Unknown 5	n.d.	0.47 ± 0.60	0.114

[1]Each value represents the mean ± SD, of n = 6 independent samples. The *p*-values with statistical significance (t-student test) are in bold.
[2]n.d=not detected.
*Area under the curve

5.4 Infected plants are preferred for oviposition by T. vaporariorum female adults

To determine if *T. vaporariorum* has a preference for infected plants, oviposition assays were performed using infected and control plants at 20 dpi (Figure 5.4 A). A higher number of nymphs was found on leaves of infected plants compared to leaves of control plants (Figure 5.4 B). More interestingly, a larger proportion of nymphs was observed on leaves that showed severe symptoms, whereas much lower numbers were observed on leaves of symptomless infected plants and on the leaves of the control plants (Figure 5.4 A and B). These observations show that infected plants are a better host for oviposition by *T. vaporariorum* than control plants and provide a better support for nymph development.

5.5 PepGMV changes the quality of the host plant

5.5.1 SA and JA contents in leaves of control and infected plants at 20 dpi

SA and JA were quantified to characterize the defense status of control and infected plants at 20 dpi, the initial time for the oviposition experiments. The results obtained show that the accumulation of SA was higher in the second youngest leaf of the infected plants as compared to the leaf in the same position of control plants (Figure 5.5 A). In the other leaves, no significant differences in the levels of SA could be detected (Figure 5.5 A). In contrast, the accumulation of JA was not affected by the infection, as no significant difference in the JA content of leaves of infected and control plants was observed (Figure 5.5 B). These observations suggest that the defenses dependent of SA and JA, at least at 20 dpi, are not playing an important defensive role during this stage of the infection by PepGMV.

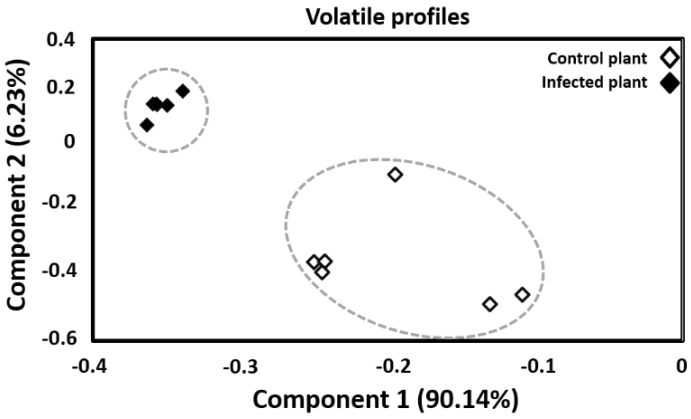

Figure 5.3. PCA analysis of VOCs profiles of chili pepper control and PepGMV-infected plants at 20 dpi. The PCA was performed with the volatiles emitted by control and infected plants collected with SPME fibers and analyzed by GC-MS. Black diamonds, infected plants; open diamonds, control plants. Components 1 and 2 explain 96.37% of the variance.

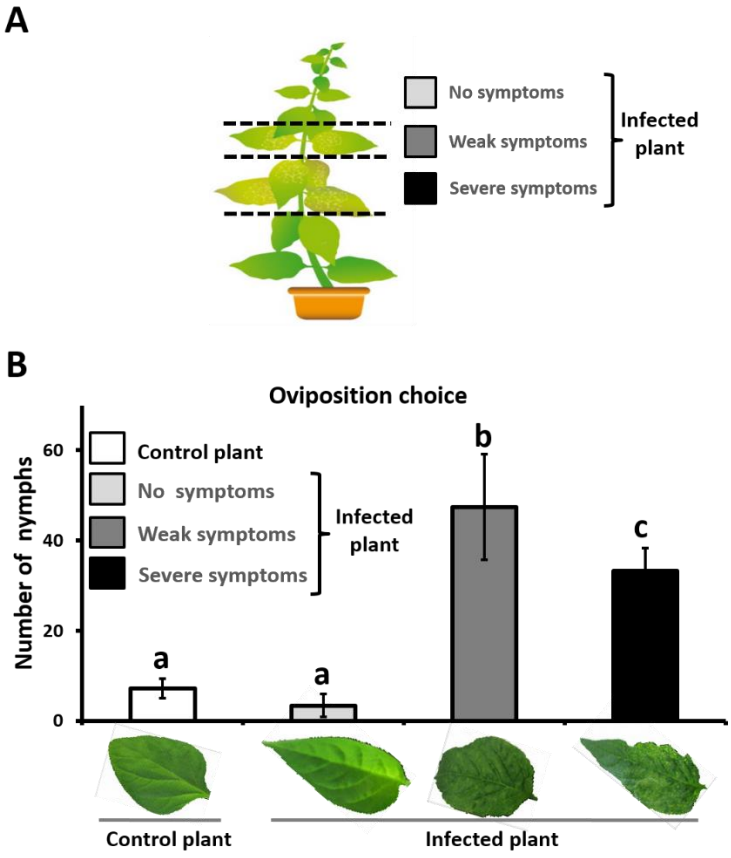

Figure 5.4. Oviposition assays with *T. vaporariorum*. A, illustration of the symptomatic leaves position in the PepGMV-infected plants. B. Oviposition preference of whitefly adult females. Open bar, control plants; light gray bar, symptomless leaves in infected plants; gray bar, leaves with weak symptoms in infected plants; black bar, leaves with severe symptoms in infected plants. Bars represent mean ± SD (n = 5). Different letters over the bars represent statistically significant differences between treatments (ANOVA test, Tukey HSD post-hoc, $p < 0.001$).

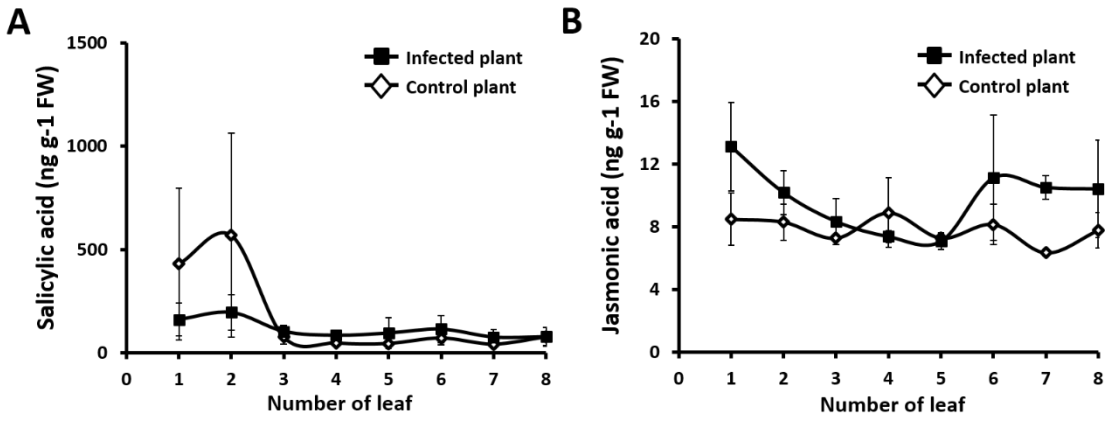

Figure 5.5. SA and JA contents in chili pepper control and PepGMV-infected plants at 20 dpi. A, SA concentration. B, JA concentration. The leaf number progresses from 1, which was the youngest leaf (leaf 1), to the oldest leaf (leaf 8). Black squares, infected plants; open diamonds, control plants. Each value represents mean ± SD (n = 3, pool of 6 plants).

5.5.2 Amino acid content in leaves of control and infected plants at 20 dpi

To characterize the nutritional status of control and infected plants, the free AAs in the phloem were quantified for each individual leaf and also the total and free AAs of leaf tissues. The total concentration of free AAs in the phloem of infected plants showed a significant increase (ca. 3-fold) as compared with control plants. The highest concentration of free AAs was found in the three younger leaves of infected plants, where both the youngest (number 1) and the third youngest leaves (number 3) showed a ca. 3-fold increase. Interestingly, the AA content in the phloem of the second youngest leaf was more than 4-fold higher than its counterpart in control plants (Figure 5.6 A and B). Most of the free AAs showed a significant increase in the phloem of infected plants. This increment included the semi-essential AA cysteine (with an increase of ca. 4-fold) and most of the essential AAs, such as methionine, lysine, threonine, isoleucine, and phenylalanine (with an increase of more that 2-fold, more that 3-fold, ca. 4-fold, more that 30-fold, ca. 4-fold, and more that 5 fold, respectively). Additionally, non-essential AAs such as proline, alanine, tyrosine, serine,

glicyne and leucine, also increased considerably in the phloem of infected plants (more that 7-fold, more that 8-fold, more that 30-fold, more that 30-fold, ca. 3-fold, and 3-fold respectively) (Table 5.2). Asx (ca. 2-fold) and Glx (ca. 2-fold) showed considerable but not significant increases, and the semi-essential AA histidine and the essential AA, valine, increased only slightly, ca. 0.5-fold each (See Tables S1a, S1b for more details).

In contrast, no significant differences were found between the total concentration of free AAs in the leaf tissue of control and infected plants, neither for entire plants nor when individual leaves were evaluated (Figure 5.6 C, D, E and F; Tables S2a, S2b, S3a and S3b). A high (ca. 4-fold) significant increase in AAs was only detected in the second youngest leaf of infected plants that was significant (Figure 5.6 F). From these results, it may be concluded that the infection of PepGMV modified the transport of AAs through the phloem and, thus, enriched the food source for *T. vaporariorum* with essential and semi-essential AAs, at least in the phloem of the younger and most strongly infected leaves.

5.6 Whitefly infestation modifies the quality of the host plant independently of PepGMV infection

5.6.1. SA and JA contents

In order to evaluate the effect of *T. vaporariorum* infestation on the defense status of the plants, SA and JA were quantified in the five youngest leaves at T0, T1 and T2 (see Tables S4-S7 for more details). No differences in SA content between control and infected plants were found at T0 (Figure 5.7 A). In contrast, the levels of SA at T1 were lower than T0 in control and infected plants, the difference being more pronounced in whitefly-infested

plants at T1. SA levels were observed to increase again, particularly in the infected plant, however, whitefly infestation also decreased SA levels in T2 (Figure 5.7 A). No relevant changes in JA in control and infested plants content were detected at T0 and T2, even when nymphs of *T. vaporariorum* were already present in the latter. However, JA levels significantly increased by ca. 0.7-fold at T1 (Figure 5.7 B).

In general, the three younger leaves of control and infected plants showed high amounts of SA at T0 with respect to the other leaves. In T1 and T2, the levels of SA increased as the result of virus infection. However, when eggs and nymphs were present in the leaves of control and infected plants, a dramatically decreased SA levels were observed (Figure 5.8 A). In contrast, the viral infection decreased JA levels in the three younger leaves, similarly to what was observed at T1, whereas the level of JA were similar in the leaves of control and infected plants at T2. In like manner to SA, the presence of eggs and nymphs in the leaves, also led to a drastic decrease of JA the levels (Figure 5.8 B). With these results, it is valid to propose that PepGMV infection affects the accumulation of SA and JA hormones at the level of individual leaves, although whitefly infestation has the strongest impact on the accumulation of both hormones, independently of the virus infection (See Tables S4-S5).

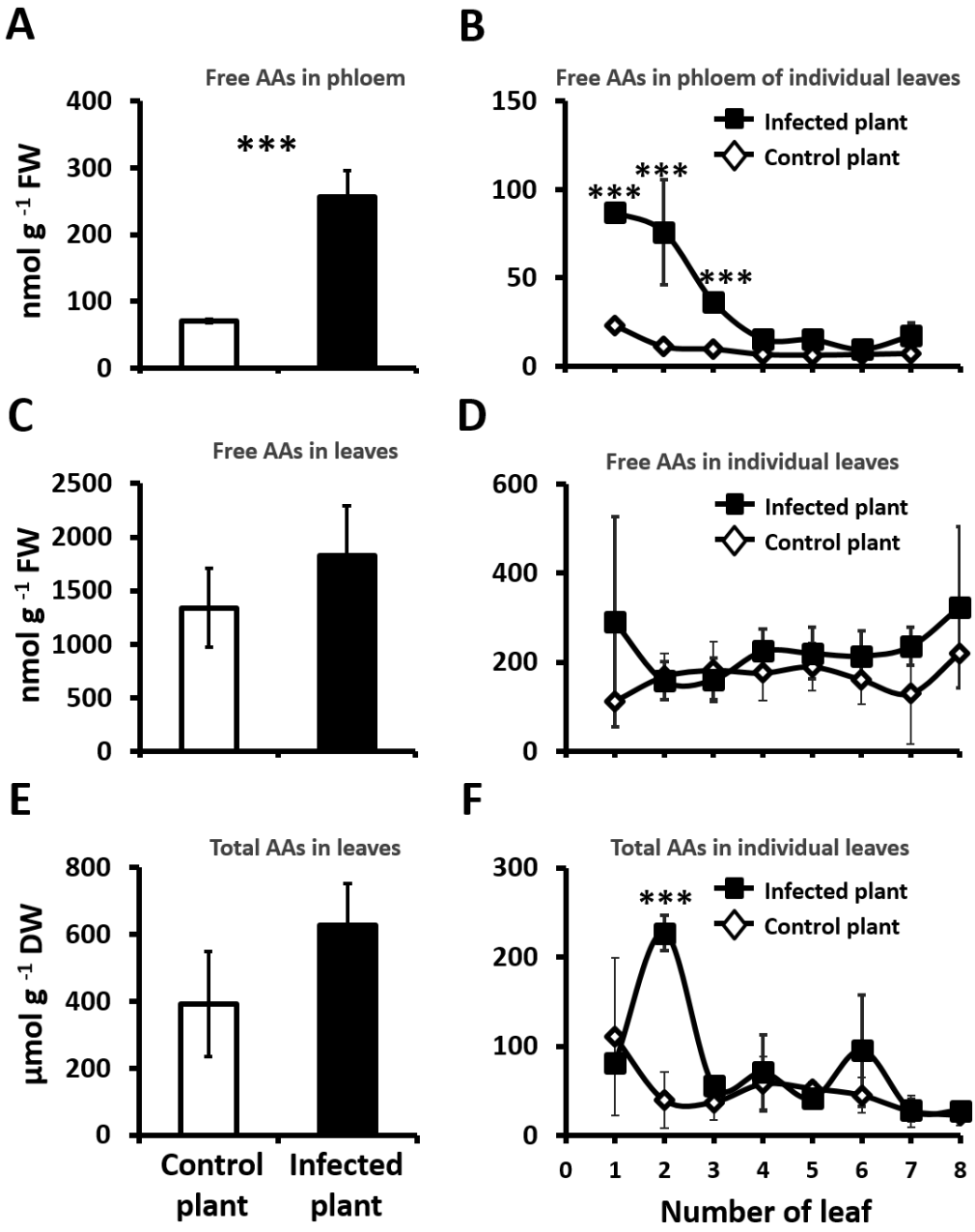

Figure 5.6. Free amino acids (AAs) content in the phloem of control and PepGMV-infected plants at 20 dpi. A, Free AAs in phloem; C, Free AAs in leaf tissue; E, Total AAs in leaf tissue. Open bars, control plants; black bars, infected plants. B, Free AAs in phloem of individual leaves; D, Free AAs in leaf tissue of individual leaves; F, Total AAs in leaf tissues of individual leaves. Open diamonds, control plants; black squares, infected plants. Number 1 represents the youngest leaf, whereas number 8 is the oldest leaf. Each value represents the mean ± SD (n = 3, pool of 6 plants). Significance was determined by t-student tests at $p < 0.05$ *, $p < 0.01$ ***, and $p < 0.001$ ***.

Table 5. 2 Free amino acids (AAs) in the phloem of chili pepper control and PepGMV-infected plants (20 dpi).

AA	Healthy plant (nmol g^{-1} FW)	Infected plant (nmol g^{-1} FW)	P-value
Met*	102.53 ± 4.0	293.49 ± 67.65	**0.038**
Lys*	74.64 ± 5.03	240.64 ± 7.09	**<0.001**
Ala	2.30 ± 0.18	40.24 ± 23.00	**0.046**
Cys†	8.20 ± 0.79	37.80 ± 10.71	**0.040**
Tyr	0.0001 ± 0	37.41 ± 10.55	**0.025**
Thr*	0.12 ± 0.05	33.02 ± 9.69	**0.027**
Pro	0.03 ± 0.03	7.27 ± 2.27	0.414
[1]Asx	3.02 ± 0.10	6.65 ± 2.11	0.097
[1]Glx	1.75 ± 0.05	3.93 ± 1.54	0.071
Arg	0.16 ± 0.23	3.63 ± 1.96	**0.038**
Ser	0.0001 ± 0	2.84 ± 0.83	**0.027**
His†	1.09 ± 0.29	1.81 ± 0.28	**0.036**
Ile*	0.32 ± 0.01	1.58 ± 0.26	**0.013**
Val*	0.99 ± 0.31	1.49 ± 0.17	0.070
Gly	0.53 ± 0.08	1.43 ± 0.47	**0.032**
Leu	0.12 ± 0.01	0.68 ± 0.15	**0.022**
Phe*	0.06 ± 0.02	0.35 ± 0.06	**0.001**
Total	**195.5 ± 6.61**	**714.2 ± 109.76**	**0.014**

[1]Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants). The p-values with statistical significance are in bold (t-student test).
* Essential amino acid
† Semi-essential amino acid

Table 5. 3 Free amino acids (AAs) in the leaf tissue of chili pepper control and PepGMV-infected plants (20 dpi).

AA	Healthy plant (nmol g^{-1} FW)	Infected plant (nmol g^{-1} FW)	P-value
Met*	173.0 ± 26.8	165.5 ± 25.5	0.743
Lys*	8.8 ± 0.6	7.7 ± 0.8	0.141
Ala	42.7 ± 9.4	52.6 ± 9.2	0.262
Cys†	183.0 ± 22.7	201.0 ± 42.7	0.565
Tyr	67.8 ± 77.8	30.7 ± 5.8	0.496
Thr*	53.1 ± 3.5	82.9 ± 10.4	**0.028**
Pro	318.9 ± 127.3	437.5 ± 199.5	0.442
[1]Asx	60.8 ± 4.0	68.1 ± 17.5	0.550
[1]Glx	177.7 ± 13.9	223.0 ± 77.2	0.416
Arg	7.6 ± 1.6	17.1 ± 4.2	**0.045**
Ser	217.9 ± 83.4	377.0 ± 164.6	0.223
His†	n. d.	n. d.	-
Ile*	39.4 ± 7.8	34.6 ± 3.7	0.407
Val*	23.5 ± 2.6	53.1 ± 3.3	**<0.001**
Gly	19.2 ± 6.4	32.0 ± 9.7	0.138
Leu	2.8 ± 0.4	3.8 ± 0.7	0.116
Phe*	8.7 ± 0.4	10.3 ± 0.4	**0.008**
Total	**1404.7 ± 279.7**	**1796.9 ± 432.5**	0.268

[1]Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The statistically significant p-values are in bold (t-student test).

* Essential amino acid

† Semi-essential amino acid

n. d. - not detected

Table 5. 4 Total amino acids (AAs) in the leaf tissue of chili pepper control and PepGMV-infected plants (20 dpi).

AA	Healthy plant (µmol g^{-1} DW)	Infected plant (µmol g^{-1} DW)	P-value
Met*	5.1 ± 1.5	11.2 ± 2.1	**0.023**
Lys*	7.6 ± 3.4	13.0 ± 3.0	0.234
Ala	42.4 ± 16.1	59.4 ± 11.2	0.288
Cys†	34.2 ± 11.4	60.9 ± 9.8	0.064
Tyr	9.0 ± 2.6	16.0 ± 3.1	0.058
Thr*	13.4 ± 6.2	23.8 ± 4.8	0.105
Pro	63.2 ± 32.7	93.5 ± 24.5	0.301
[1]Asx	21.6 ± 8.2	26.7 ± 6.9	0.723
[1]Glx	34.0 ± 13.6	49.7 ± 13.2	0.268
Arg	10.3 ± 4.3	17.5 ± 3.5	0.113
Ser	20.2 ± 7.0	33.3 ± 6.5	0.106
His†	6.7 ± 2.2	12.8 ± 2.5	**0.039**
Ile*	15.1 ± 5.6	28.1 ± 4.4	**0.046**
Val*	21.4 ± 8.6	37.3 ± 6.4	0.080
Gly	43.6 ± 17.4	65.3 ± 14.5	0.201
Leu	28.7 ± 11.1	51.2 ± 8.3	0.068
Phe*	14.8 ± 5.5	27.4 ± 2.8	0.054
Total	**391.3 ± 157.2**	**627.0 ± 124.5**	0.140

[1]Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants). The statistically significant t p-values are in bold (t-student test).
* Essential amino acid
† Semi-essential amino acid

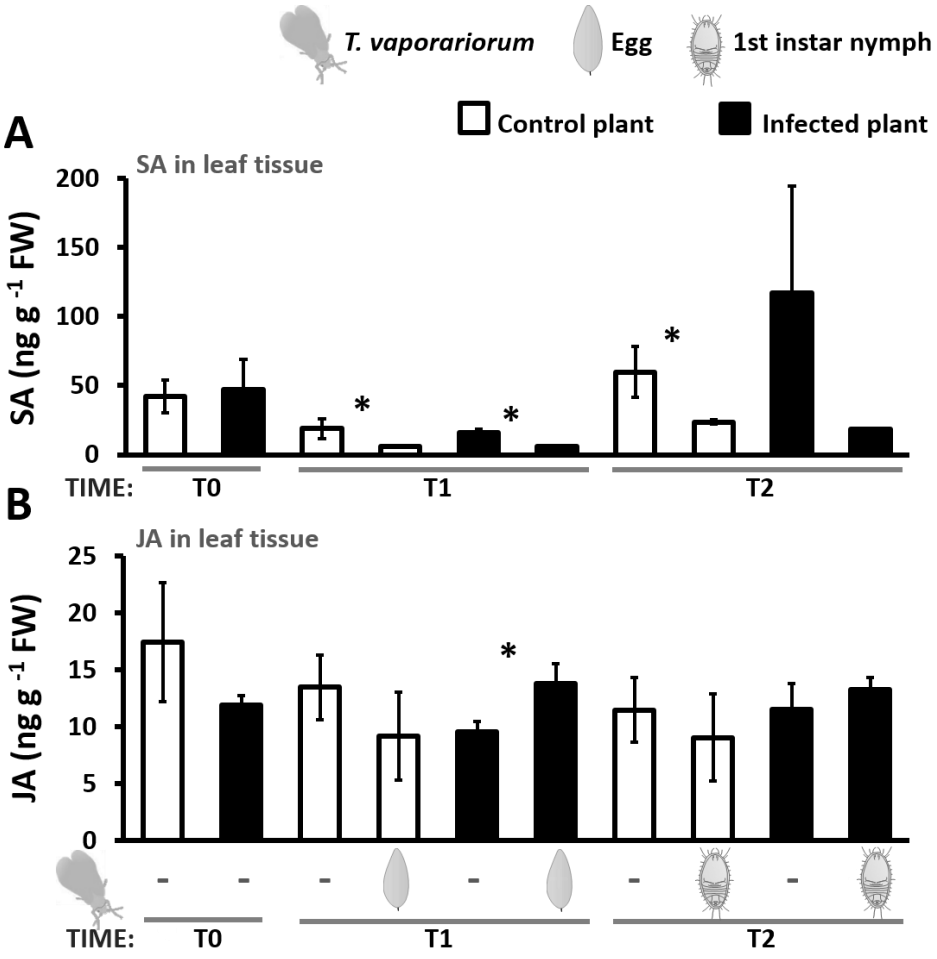

Figure 5.7. SA and JA contents in leaves of chili pepper control and PepGMV-infected plants after *T. vaporariorum* colonization. A, SA concentration in individual leaves; B, JA concentration in individual leaves. Open bars, control plants; black bars, infected plants. T0, plants at 20 dpi, colonized or not colonized with whiteflies; T1, plants at 27 dpi, with or without eggs; T1, plants at 32 dpi, with or without nymphs. Each bar represents the mean ± SD, n = 3 (pool of 6 plants) independent samples. Statistical significance was determined by means of t-student tests at $p < 0.05$ *, $p < 0.01$ ***, $p < 0.001$ ***.

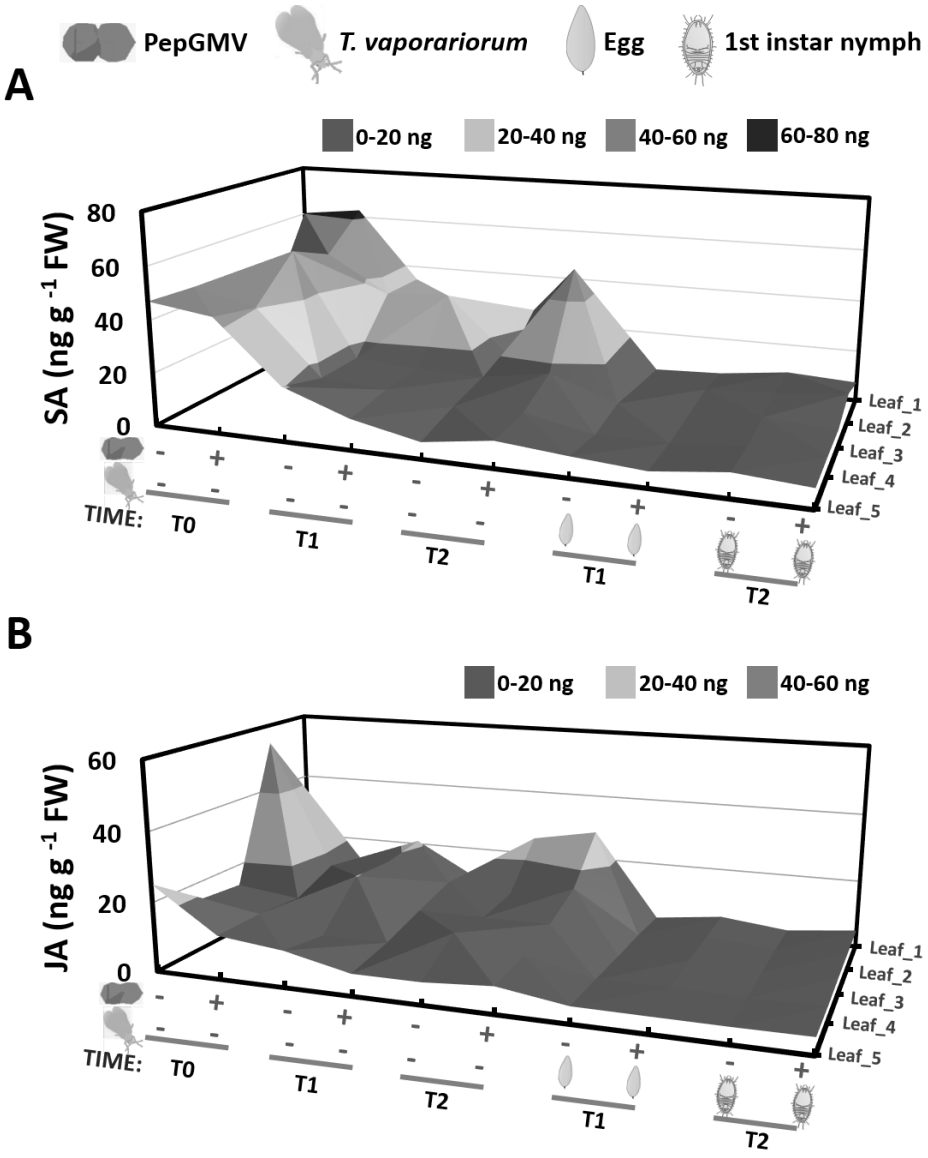

Figure 5.8. SA and JA contents on each leaf of chili pepper control and PepGMV-infected plants after *T. vaporariorum* colonization. A, SA concentration in individual leaves; B, JA concentration in individual leaves. T0, plants at 20 dpi, colonized or not colonized by the whitefly; T1, plants at 27 dpi, with or without eggs; T1, plants at 32 dpi, with or without nymphs. Leaf_1 represents the youngest leaf, whereas Leaf_8, represents the oldest leaf. Each value represented in the graph is the mean of n = 3 (pool of 6 plants) independent samples.

5.6.2. Free AAs in phloem of chili pepper control and infected plants after whitefly colonization

Free AAs were analyzed in the phloem of all eight leaves of control and PepGMV-infected plants. The analysis was performed in bulk (Figure 5.9) or individually, per leaf (Figures 10 and 11) at T0, T1, and T2 (see Tables S9a/ b-S13a/ b for more details).

The heat maps show that virus-infection caused an almost 30-fold increase in the overall AA content in the phloem of chili plants, but this pattern was completely neutralized upon arrival of *T. vaporariorum* (Figure 5.10 A, B). AA such as arginine, cysteine, proline, serine, tyrosine and valine exhibited up to 40 times higher contents in the phloem of PepGMV-infected as compared to control plants at 20 dpi, whereas PepGMV-infected plants contained less alanine, Asx, Glx and histidine. By contrast, all the last mentioned AAs (alanine, Asx, Glx, glycine and histidine) increased their content in PepGMV-infected plants upon colonization by *T. vaporariorum* (Figure 5.10 C). This effect was seen already at 27 dpi and maintained stable until 32 dpi. By contrast, the contents of isoleucine, leucine, phenylalanine, proline, tyrosine and valine dropped up to 30 times below control levels when plants had been colonized by whiteflies (Figure 5.10 C). This latter effect was relieved after removal of the adults, since plants carrying only 1st instar nymphs (32 dpi) did not exhibit any strong reduction in the contents of isoleucine, leucine, phenylalanine or valine. Nevertheless, the presence of nymphs had particularly strong effects on methionine, proline and tryosine: three amino acids that exhibited enhanced contents in response to PepGMV alone, but strongly (10- 40-fold) decreased contents at 32 dpi, that is, when the PepGMV-infected plant carried 1st instar nymphs (Figure 5.10 C).

The overall patterns in phloem AA contents were very similar in control plants that were colonized by *T. vaporariorum* (Figure 5.10 A and B). For example, plants at 27 dpi (carrying eggs, adults removed) exhibited reduced contents of isoleucine, leucine, phenylalanine, proline, tyrosine and valine as compared to controls, whereas contents of alanine, Asx, Glx , glycine and histidine were enhanced as compared to control and no colonized plants. Even more strikingly, the contents of valine (lower than controls during the egg stage) increased over control levels in plants carrying 1st instar nymphs, and methionine, proline and tryosine again exhibited strong decreases when plants shifted from carrying eggs to carrying 1st instar nymphs (Figure 5.10 C).

5.7 Amino acid changes in phloem are not explained by the amino acid composition of the proteins of the PepGMV or of defense proteins of the host plant

In order to find a possible explanation for the increased of free AAs in the phloem of infected plants at 20 dpi, we compared the relative contribution of each amino acid to the sequence of the proteins that were identified as PR proteins (PR1a, PR1b and PR5) in a transcriptome of PepGMV-infected plants. Comparisons with the sequence of six PepGMV proteins (Rep, REn, TRap, CP, MV and NSP) were also performed. A PCA showed that the AA contents of viral and PR proteins of infected plans were different from the AAs that increased in the phloem of infected plants (Figure 5.12). In addition, no correlation was found between the AAs composition of viral or PR proteins and the free AAs content detected in the phloem (Figure 5.13).

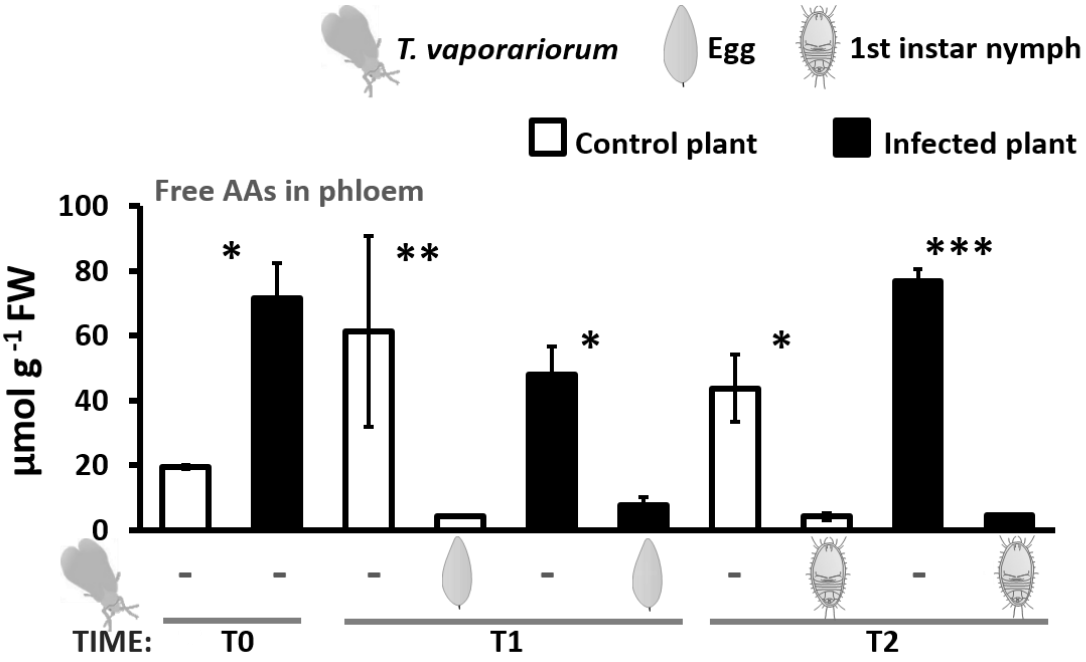

Figure 5.9. Free amino acids (AAs) content in phloem of leaves of control and PepGMV-infected plants after *T. vaporariorum* colonization. Open bars, control plants; black bars, infected plants. T0, plants at 20 dpi, colonized or not colonized with the whitefly; T1, plants at 27 dpi, with or without eggs; T1, plants at 32 dpi, with or without nymphs. Each bar represents the mean ± SD, n=3 (pool of 6 plants) independent samples. Asterisks indicate significant differences at $p < 0.05$ *, $p < 0.01$ ***, $p < 0.001$ *** (t-student test).

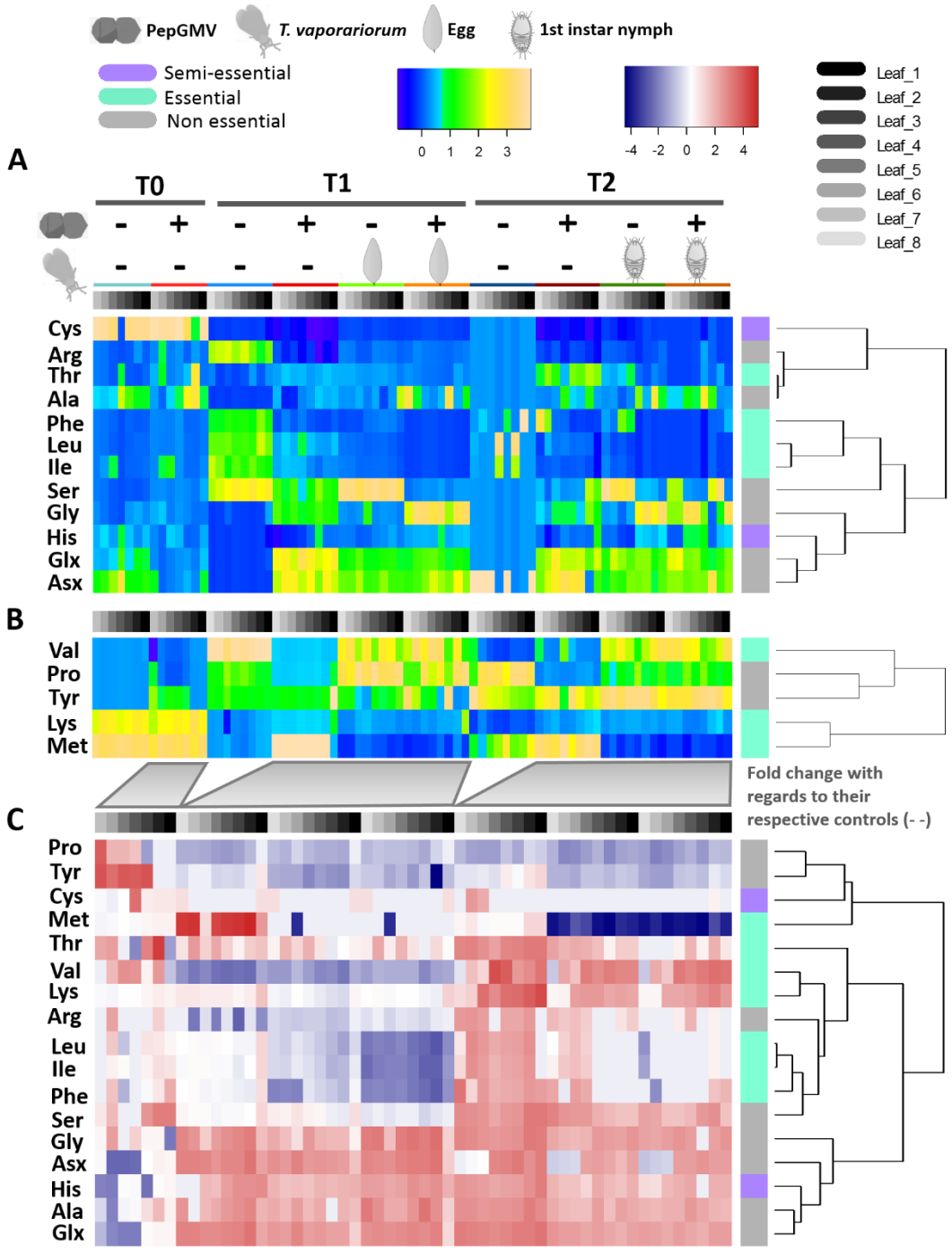

Figure 5.10. Heat map of free amino acid (AAs) content in phloem and fold changes in chili pepper control and PepGMV-infected plants after *T. vaporariorum* colonization. A, amino acids in lower concentrations; B, amino acids in higher concentrations; C, fold changes. T0, plants at 20 dpi, colonized or not colonized with the whitefly; T1, plants at 27 dpi, with or without eggs; T1, plants at 32 dpi, with or without nymphs. Leaf_1 represents the youngest leaf; Leaf_8, represents the oldest leaf. Each relative value represented in the heat map is based in the mean of the replicates; n = 3 (pool of 6 plants) independent samples.

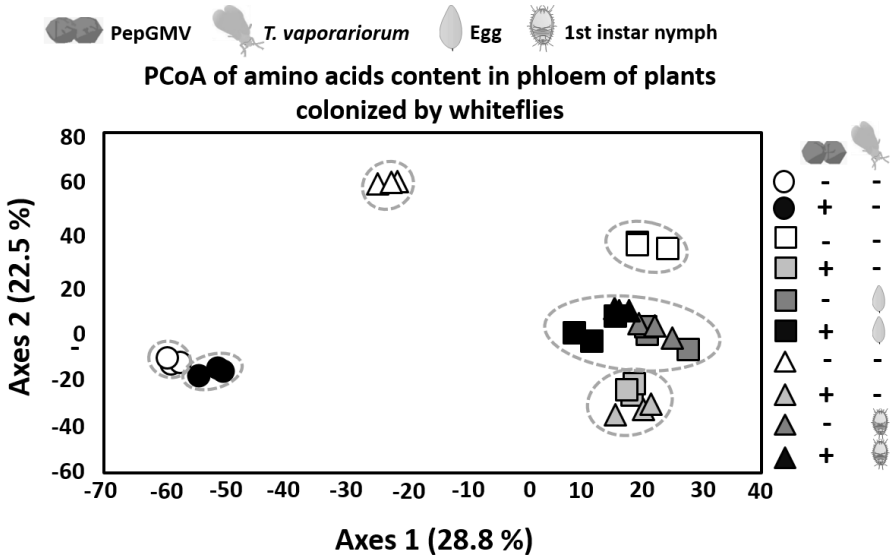

Figure 5.11. PCoA of free amino acids in phloem of chili pepper control and PepGMV-infected plants after *T. vaporariorum* colonization. T0, plants at 20 dpi, colonized or not colonized with the whitefly; T1, plants at 27 dpi, with or without eggs; T1, plants at 32 dpi, with or without nymphs. Each sample represented in the PCoA is based in the concentrations of the eight leaves of each pool of plants; n = 3 (pool of 6 plants) independent samples.

5.8 Viral load is reduced in plants colonized by T. vaporariorum

To evaluate the effect of whitefly colonization on the PepGMV load in the leaf tissue of infected plants, the PepGMV *CP* gene expression levels were quantified by qPCR in symptomatic leaf tissue of infected plants colonized by the non-vector insect, *T. vaporariorum*. The results obtained indicated that viral load was drastically reduced at T2 in plants that carried 1st instar nymphs as compared to plants at same virus inoculation time but without the presence of the nymphs (Figure 5.14). At times T0 (initial time of the colonization) and T1 (eggs presence on infested plants), no significant differences were found between the viral load in infected and colonized as compared to infected but whitefly-free plants (Figure 5.14). These observations indicate that the infestation with *T. vaporariorum* had a negative effect on the proliferation of PepGMV (Table S14).

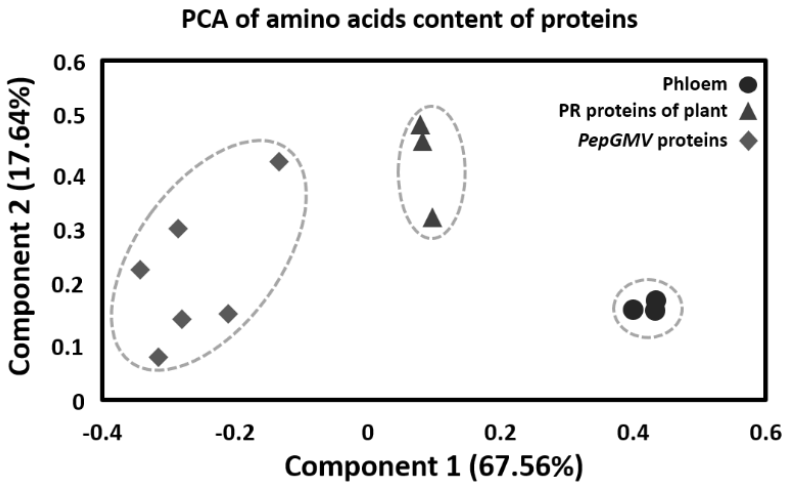

Figure 5.12. PCA of the amino acid composition of the viral PepGMV proteins, chili plant PR proteins and in phloem of infected plants (at 20 dpi). The PCA was performed with the amino acids that compose the primary sequence of the above proteins. Black circles, the three repetitions of phloem; gray triangles, PR1a, PR1b and PR5 proteins induced on infected plants; gray diamonds, the six PepGMV proteins (*Rep*, *REn*, *TRap*, *CP*, *MV* and *NSP)*. The components 1 and 2 explains the 96.37% of the variance.

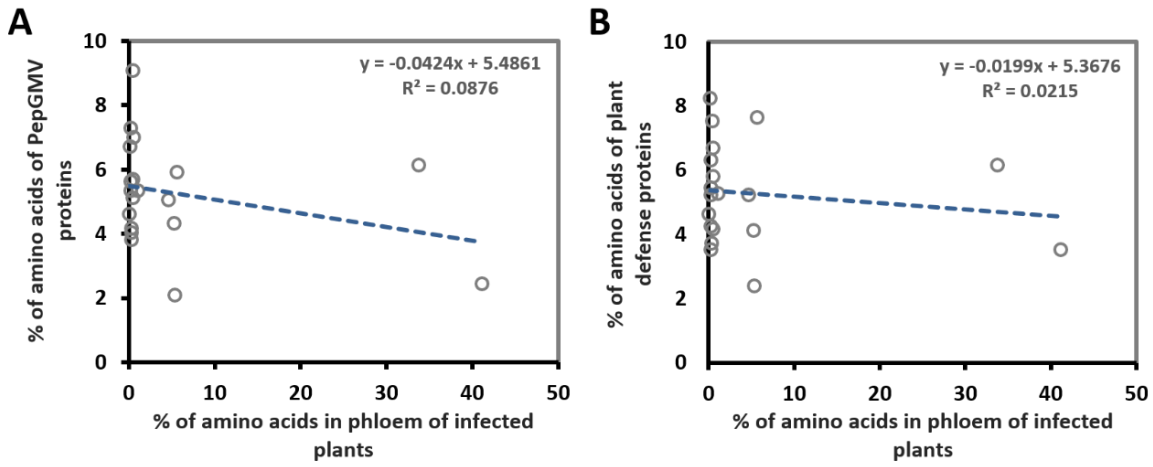

Figure 5.13. Correlation of the percentage of amino acids (AAs) in phloem of PepGMV-infected chili pepper plants with the percentage of amino acids in the sequence of PepGMV or chili pepper PR proteins. A. Correlation between AAs of phloem and AAs in the six proteins of PepGMV. B, Correlation between AAs of phloem and AAs in the chili pepper PR proteins. Peason's index, the equations describe the blue lines, the R2 represents the adjustment of the values, $p > 0.05$.

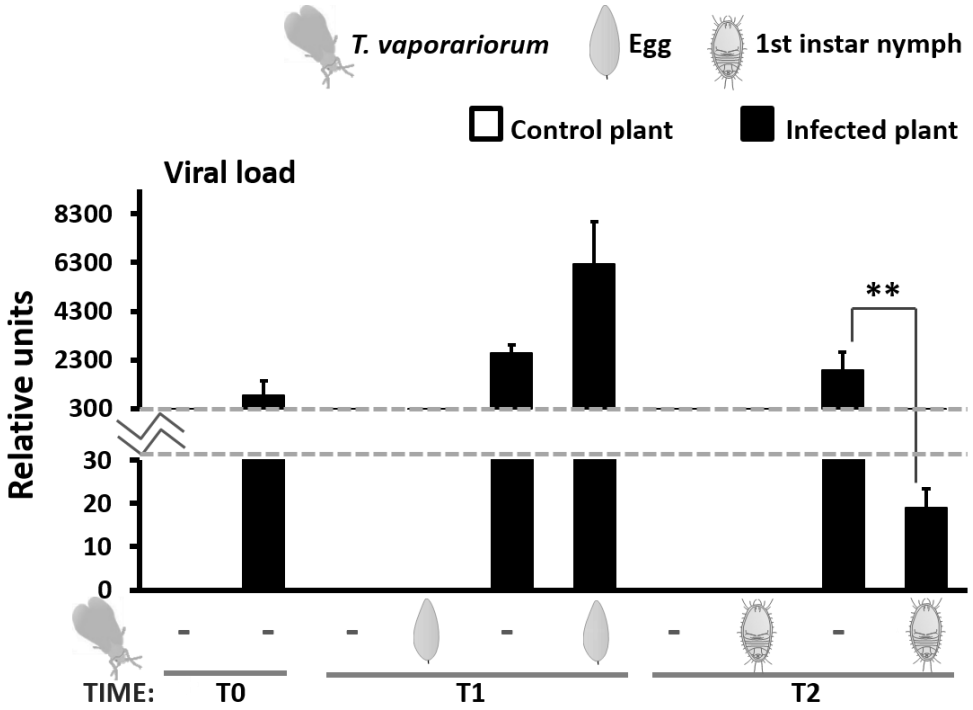

Figure 5.14. PepGMV titer in infected plants after *T. vaporariorum* colonization. The estimation of virus titers was performed by relative qPCR quantification of the viral CP gene, using as references the chili pepper *EF-1α* gene. Open squares, control plants; Black squares, infected plants. T0, plants at 20 dpi; T1, plants at 27 dpi, with or without eggs; T1, plants at 32 dpi, with or without nymphs. Each value represents the mean ± SD; n=3 (pool of 6 plants) independent samples. Asterisks indicate statistically significant differences at $p < 0.05$ *, $p < 0.01$ ***, $p < 0.001$ *** (t-student test).

6. Discussion

In this study, the VOC-mediated behavioral response of the whitefly *T. vaporariorum* to PepGMV-infected host plants was investigated, together with an exploration of how the colonization of the plants by *T. vaporariorum* affects the 'fitness' of PepGMV. Changes in VOCs emissions and in host quality that result from PepGMV infection, which were used by whiteflies to choose these plants as hosts. Moreover, virus-infected plants were identified to be better suited for the development of the nymphs. However, and most importantly, whitefly colonization had a negative effect on the accumulation of PepGMV (Figure 5.4).

We confirmed, using PCR to amplify the viral *REn* and *Trap* genes in plants and animals that were used in transmission assays, that the *T. vaporariorum* whitefly is not a vector of PepGMV (Figure 5.1). Nevertheless, the changes in the VOC profiles of virus-infected plants had the same attractive effect on *T. vaporariorum*, a non-vector insect, as it has been described in multiple other studies for vector insects (Eigenbrode et al. 2002; Jiménez-Martínez et al. 2004; Srinivasan et al. 2006; Alvarez et al. 2007; Medina-Ortega et al. 2009; Mauck, De Moraes, and Mescher 2010). This outcome indicated that these changes do not necessarily represent a specific signal for the attraction of vectors.

Cage and olfactometer behavioral assays demonstrated that the attraction of the non-vector insect *T. vaporariorum* to PepGMV-infected plants changed over time (Figure 5.2).The same phenomenon has been described for vector insects. Such temporal changes in the odor profile of infected plants are consistent with the idea of a parasite that manipulates its host and vector to enhance its transmission rates by allowing the viruliferous insect to migrate to uninfected plants. However, this behavior was derived from a non-vector insect, suggesting that non-vectors are also capable of interpreting the visual, olfactory and

chemotactic cues that in principle were believed to be exclusively activated to attract vectors. We observed that in the cage, in general, the percentage of attraction of the whitefly was higher than in olfactometer, which means that the insects can also make use of non-olfactory cues to obtain information on the quality of their possible host plant and make a choice. However, the olfactory cues still provided enough information to the insects to evaluate the host plant quality and to make a consistent choice.

Which olfactory cues were perceived by the whiteflies in the olfactometer assays? To answer this question, VOCs were collected from PepGMV-infected plants and control plants at 20 dpi, time when the whitefly showed the same behavior in both cage and olfactometer assays. The VOCs profiles emitted by infected plants at 20 dpi were clearly different from the profiles of control plants, as revealed by the PCA analysis (Figure 5.3). The group of volatiles that showed the most important changes in the blend of infected plants were the terpenes, as it has been described for other virus-plant interactions (Table 5.1) (Eigenbrode et al. 2002; Jiménez-Martínez et al. 2004; Ngumbi et al. 2007; Luan et al. 2013). These results were in accordance with reports showing that terpenes such as the sesquiterpenes, zingiberene and curcumene, and the monoterpenes, *p*-cymene, α-terpinene, and α-phellandrene, act as semiochemicals that mediate, for example, the interaction among tomato plants and *B. tabaci*, the vector of PepGMV, being most of them repellents to the whitefly (Bleeker et al. 2009). According to the results herewith described, most of the terpenes decreased their presence in the blends emitted by infected plants, suggesting that PepGMV infection reduced the emission of potential repellents for *B. tabaci*. This, it is valid to conclude that the reduced emission of these compounds was beneficial to *T. vaporariorum*. In line with this interpretation, Luan and coworkers reported the suppression of terpene

synthases and the subsequent decrease of the emission of some terpenes from infected tobacco plants (e.g., camphor, α-cedrene and β-cedrene) and found that these changes enhanced the performance of *B. tabaci* and permitted a more efficient infestation of these plants (Luan et al. 2013). However, in order to confirm that the observations derived from the present study were caused by the same mechanisms, future behavioral assays using individual volatiles will be required.

Nevertheless, these olfactory cues helped the non-vector whitefly to find the best host plants to lay eggs onto, and these were the infected plants, as was observed in the oviposition assays (Figure 5.4). Interestingly, symptomatic leaves of infected plants were preferred by the whiteflies for oviposition, suggesting that PepGMV was capable of changing the quality of the host plant at the level of individual leaves. This interpretation was confirmed with the analysis of SA, JA in leaf tissue and free AAs in the phloem of control and infected plants at 20 dpi (Figures 5.5 and 5-6). As described for other pathogen-vector/ non-vector-host interactions, these changes in the nutritional composition of the phloem and the defensive state of the leaves benefited the whitefly (Abe et al. 2012; Nachappa et al. 2013; Wang et al. 2014; Mauck, De Moraes, and Mescher 2014). Interestingly, only small changes in the levels of defense hormones, SA and JA, were detected after infection with PepGMV, at 20 dpi, in the leaves (Figure 5.5). This absence of strong differences in the SA and JA concentrations indicated that the mechanism that underlies the attraction of the whitefly is not linked to the trade-off between the major defense pathways that are dependent on these hormones. Nevertheless, a role of these hormones cannot be ruled out completely, for example during the early stages of the infection which were not considered in this work. However, these observations were in accordance with other studies, were the JA did not change considerably

in response to the virus infection, as well as, the expression of JA-dependent genes (Nachappa et al. 2013; Mauck, De Moraes, and Mescher 2014). Despite this pattern, other workers observed an antagonistic effect with SA in other interactions, which was evidenced as an up-regulation of JA-dependent genes (Abe et al. 2012). On the other hand, an interesting study of the interaction between the begomovirus TYLCCNV, plus a β-satellite (an accessory DNA 'plasmid' that codifies for one protein), with tobacco plants and the vector whitefly *B. tabaci*, showed that the virus infection, by itself, did not strongly modify SA and JA contents (similarly to the results obtained in the present study). However, the co-infection with the satellite could suppress JA accumulation as well as the expression of JA-dependent genes. This effect contributed to enhance the performance of *B. tabaci* on those plants, suggesting a mutualism among begomovirus and its vector (T. Zhang et al. 2012).

Nevertheless, the presence of the insect vector, or in our case a non-vector insect, could change the pattern in the accumulation of both hormones in the leaves of infected plants (Abe et al. 2012; Nachappa et al. 2013).

More important than effect on the concentration of defense hormones was the influence of PepGMV on host nutritive quality, since infection with the virus significantly and strongly (ca. 3-fold) increased the concentration of free AAs in the phloem of PepGMV-infected plants at 20 dpi (Figure 5. 6 A and B). Individual AAs, such as cysteine, histidine, methionine, lysine, threonine, isoleucine, phenylalanine, alanine, tyrosine, serine, glicyne and leucine, increased their concentration from 2-fold to almost 30-fold in the phloem of the leaves of infected plants (Table 5.2). Phloem represents the only food source used by *T. vaporariorum* and AAs in the phloem represent, in general, the most limiting factor in the diet of phloem-feeding insects (Thorsteinson 1960; Awmack and Leather 2002). Moreover,

some of the individual AAs that exhibited an increased content in the phloem of the infected plants (i.e., methionine, lysine, threonine, isoleucine and phenylalanine) are considered essential for animals, whereas other AAs are considered as semi-essential (i.e., cysteine and histidine). Thus, the results obtained indicate that the nutritional status of the infected plants as a food source for the non-vector whitefly *T. vaporariorum* was much higher than the nutritional status of the control plants (Table 5.2; Figure 5.10). Similar virus-induced changes in the composition of AAs in the phloem of virus-infected host plants have been reported by others (Blua, Perring, and Madore 1994; Nachappa et al. 2013; Mauck, De Moraes, and Mescher 2014). The above information supports the idea that viruses, or parasites in general, can manipulate the tripartite interaction parasite/ pathogen-vector-host to enhance their transmission rates, attracting the vector via VOCs and rewarding it via a modified host quality, and that all these changes can also be exploited by other species for their own benefit.

A surprising observation was the capacity of the non-vector insect to override the changes in phloem AA composition that had been caused by PepGMV infection. In fact, the content of free AA levels in the phloem of control and infected plants was very similar when the infected plants were colonized by *T. vaporariorum* (see the heatmaps and the PCoA analysis, Figures 5.10 and 5.11; consult Annexes 1 for more details).

An interesting fact to be considered is that all the changes in the AA levels in the host plant caused by the parasite appeared to be restricted to specific tissues or organs of the host, which correspond with the feeding mode of the insect vectors (Figure 5.6a). For example, the food source of mites is the cell content of the leaf, while for aphids and whiteflies, it is the phloem content of the leaves. Several studies found the main changes in the levels of AAs and/ or sugars exactly in these tissues, and in the present work, the leaf tissue did not show

the same changes in the free AA content as it occurred in phloem (Figure 5.6, Table 5.3, 5.4) (Nachappa et al. 2013; Mauck, De Moraes, and Mescher 2014). These observations suggest that parasites such as viruses, share an overall strategy to attract the vector and that the specific way to achieve their goal can differ, depending on the family of viruses, the feeding mode of the vectors and of other characteristics that remain to be identified.

An open question remains: Why does virus infection enhance the free AAs in the phloem? Possible explanations include that the virus manipulated the transport of AAs required for its own replication in the younger tissues; that the plant directed the AAs transport to the infected tissues to mount a resistance response, or that the plant mobilizes the AAs in the infected leaves to allocate them towards other tissues. Nevertheless, no reciprocity was observed when the AA content in the phloem of infected plants (20 dpi) was compared to the AA composition of viral PepGMV and plant PR proteins (e.g., PR1a, PR1b and PR5) which were found to be up-regulated in an accompanying transcriptomic analysis (results not shown), (Figure 5.12). Likewise, no correspondence was found between the percentages of individual AAs in phloem of infected plants with those recorded in viral or plant PR proteins (Figure 5.13). Thus, the ultimate mechanism that explains the increased AA content in the phloem of PepGMV-infected chili plants remains to be identified.

7. Conclusions

- The whitefly *Trialeurodes vaporariorum* cannot transmit PepGMV and, thereby, is not a vector for this begomovirus.
- The changes in the VOCs profile of infected plants are not specifically attractive insect vectors and can be used as cues for host choice by a non-vector insects, such as *T. vaporariorum*.
- Infected plants are better host plants for *T. vaporariorum* feeding and oviposition than control plants.
- The changes in the quality of infected plants were predominantly found in the younger leaves.
- Both, PepGMV infection and the colonization by the whitefly had a strong impact on the quality of the host plant, in particular on the accumulation of certain free AAs in the phloem of infected plants, whereas the levels of SA and JA in the leaf tissue hardly differed between control and infected plants.
- The fitness of the virus PepGMV was negatively affected by the infestation of the plant with the non-vector whitefly, *T. vaporariorum*. Thus, attracting the wrong insect via olfactory cues can causes a higher cost of manipulation for the virus.

8. Perspectives

i. Quantify defense-related secondary metabolites in the phloem of control and infected plants.

ii. Determine the expression of defense genes of control and infected plants at the level of individual leaves at different day post inoculation with PepGMV.

iii. Compare the feeding behavior of the insect vector (*B. tabaci*) of PepGMV and the non-vector insect (*T. vaporariorum*) at different days post inoculation with the virus.

iv. Perform co-infestation assays with both whitefly species in control and infected plants to determine if they compete for the same host plant.

9. References

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Annexes 1

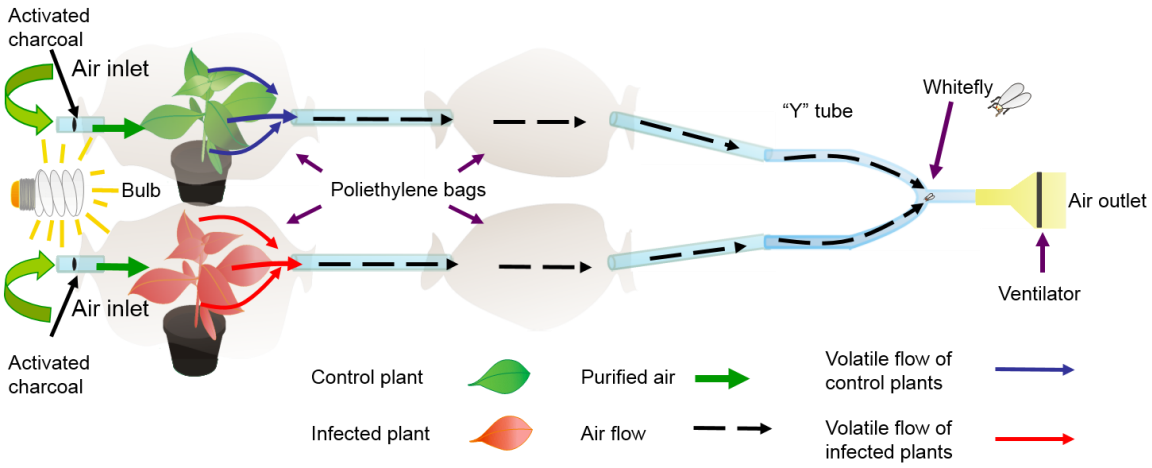

Figure S1. Olfactometer design diagram used in the choice assays with the whitefly *T. vaporariorum*. The "Y" tube is glass.

Table S1a Free amino acids in the tissue of the leaves of control and PepGMV-infected plants of chili at 20 dpi.

	Leaf 1			Leaf 2			Leaf 3			Leaf 4		
AA	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	11.967 ± 1.018	44.625 ± 3.492	**0.002**	5.290 ± 1.252	21.906 ± 18.228	0.255	5.171 ± 0.923	15.928 ± 6.807	0.109	3.910 ± 0.047	6.418 ± 2.290	*0.061*
Lys*	8.775 ± 0.498	33.232 ± 0.589	**0.000**	4.386 ± 0.099	23.761 ± 3.911	**0.013**	3.469 ± 0.569	11.768 ± 0.254	**0.000**	2.488 ± 0.929	5.410 ± 0.318	**0.022**
Ala	0.237 ± 0.112	0.901 ± 0.634	0.208	0.174 ± 0.158	11.627 ± 9.655	0.176	0.192 ± 0.195	1.755 ± 2.578	0.404	0.070 ± 0.012	0.114 ± 0.059	0.328
Cys†	0.896 ± 0.623	5.454 ± 2.631	0.088	0.594 ± 0.211	3.449 ± 1.778	0.107	0.437 0.181	2.181 ± 0.472	**0.014**	n. d.	0.871 ± 0.390	*0.061*
Tyr	n. d.	n. d.	---	n. d.	n. d.	----	n. d.	3.493 ± 0.288	**0.002**	n. d.	2.521 ± 0.557	**0.016**
Thr*	0.012 ± 0.022	n. d.	0.423	0.000	11.387 ± 3.361	**0.028**	n. d.	0.357 ± 0.215	0.102	0.030 ± 0.007	n. d.	**0.019**
Pro	n. d.	n. d.	---	n. d.	n. d.	---	0.012 ± 0.011	n. d.	0.184	n. d.	0.014 ± 0.024	0.423
Asx	0.319 ± 0.014	1.112 ± 0.284	**0.040**	0.187 ± 0.099	0.791 ± 0.651	0.248	0.145 ± 0.097	0.309 ± 0.183	0.262	0.091 ± 0.015	n. d.	**0.009**
Glx	0.203 ± 0.050	0.644 ± 0.124	**0.015**	0.176 ± 0.087	0.554 ± 0.0340	0.188	0.089 ± 0.099	0.210 ± 0.134	0.280	0.047 ± 0.017	n. d.	**0.037**
Arg	n. d.	n. d.	---	0.058 ± 0.082	1.237 ± 0.708	0.100	n. d.	n. d.	---	n. d.	n. d.	---
Ser	n. d.	0.448 ± 0-086	**0.012**	n. d.	0.408 ± 0.200	0.072	n. d.	0.142 ± 0.080	0.091	n. d.	n. d.	---
His†	0.128 ± 0.033	0.432 ± 0.068	**0.007**	0.051 ± 0.002	0.119 ± 0.065	0.215	0.073 ± 0.032	n. d.	*0.057*	0.031 ± 0.010	0.049 ± 0.002	0.073
Ile*	0.022 ± 0.006	0.168 ± 0.174	0.281	0.017 ± 0.007	0.155 ± 0.106	0.152	0.009 ± 0.003	0.038 ± 0.024	0.167	n. d.	n. d.	---
Val*	0.350 ± 0.106	0.062 ± 0.028	**0.035**	n. d.	0.107 ± 0.022	**0.014**	0.008 ± 0.004	0.034 ± 0.009	**0.019**	n. d.	0.132 ± 0.030	**0.016**
Gly	0.057 ± 0.017	n. d.	**0.028**	0.077 ± 0.013	0.265 ± 0.110	0.096	0.043 ± 0.029	0.142 ± 0.056	0.071	n. d.	0.008 ± 0.007	0.193
Leu	0.014 ± 0.005	0.048 ± 0.038	0.262	0.020 ± 0.005	0.134 ± 0.051	*0.060*	0.006 ± 0.007	0.048 ± 0.014	**0.020**	n. d.	n. d.	---
Phe*	n. d.	0.047 ± 0.018	**0.044**	0.008 ± 0.005	0.050 ± 0.008	**0.003**	0.008 ± 0.009	0.013 ± 0.012	0.652	n. d.	n. d.	---
Total	22.980 ± 1.153	87.174 ± 1.958	**0.000**	11.037 ± 1.440	75.950 ± 29.671	*0.063*	9.663 ± 2.063	36.420 ± 4.673	**0.004**	6.667 ± 0.855	15.537 ± 3.047	**0.030**

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the younger and Leaf 8, de oldest leaf.

* Essential amino acid

† Semi-essential amino acid

n. d. – not detected

Table S1b Free amino acids in the tissue of the leaves of control and PepGMV-infected plants of chili at 20 dpi.

	Leaf 5			Leaf 6			Leaf 7			Leaf 8		
AA	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	3.291 ± 1.375	6.682 ± 0.987	**0.030**	3.316 ± 1.296	4.793 ± 0.521	0.177	3.950 ± 0.344	5.304 ± 0.684	*0.057*	3.573 ± 0.310	4.711 ± 0.607	*0.064*
Lys*	2.529 ± 0.489	4.962 ± 0.874	**0.022**	2.797 ± 0.629	3.279 ± 0.709	0.429	2.426 ± 1.186	4.216 ± 0.104	0.104	2.187 ± 1.068	3.745 ± 0.471	0.112
Ala	0.043 ± 0.019	0.058 ± 0.052	0.672	0.04 ± 0.017	n. d.	*0.054*	0.073 ± 0.030	0.029 ± 0.050	0.276	0.066 ± 0.027	0.026 ± 0.045	0.267
Cys†	0.202 ± 0.096	0.438 ± 0.139	0.096	0.313 ± 0.025	0.348 ± 0.042	0.293	0.509 ± 0.274	0.866 ± 0.139	0.139	0.459 ± 0.247	0.769 ± 0.123	0.148
Tyr	n. d.	2.608 ± 1.227	0.067	n. d.	0.881 ± 0.085	**0.003**	n. d.	3.965 ± 0.106	0.106	n. d.	3.521 ± 2.162	0.106
Thr*	n. d.	n. d.	---	n. d.	0.089 ± 0.071	0.162	n. d.	0.053 ± 0.019	**0.041**	n. d.	0.047 ± 0.017	**0.041**
Pro	n. d.	0.029 ± 0.050	0.423	n. d.	n. d.	0.423	n. d.	2.548 ± 4.364	0.418	n. d.	2.263 ± 3.876	0.418
Asx	0.113 ± 0.015	n. d.	**0.006**	0.106 ± 0.024	n. d.	**0.017**	0.128 ± 0.036	0.182 ± 0.012	**0.036**	0.115 ± 0.021	0.162 ± 0.011	**0.041**
Glx	0.041 ± 0.014	n. d.	**0.037**	0.027 ± 0.005	n. d.	**0.013**	0.046 ± 0.018	0.005 ± 0.008	**0.039**	0.042 ± 0.016	0.004 ± 0.007	**0.039**
Arg	n. d.	n. d.	---	n. d.	0.068 ± 0.004	**0.001**	n. d.	n. d.	---	n. d.	n. d.	---
Ser	n. d.	n. d.	---	n. d.	0.021 ± 0.037	0.423	n. d.	0.002 ± 0.004	0.423	n. d.	0.002 ± 0.004	0.423
His†	0.023 ± 0.006	0.053 ± 0.010	**0.019**	0.049 ± 0.039	n. d.	0.163	0.039 ± 0.016	n. d.	*0.051*	0.035 ± 0.014	n. d.	*0.051*
Ile*	0.048 ± 0.037	0.135 ± 0.017	**0.038**	0.019 ± 0.033	0.072 ± 0.034	0.126	n. d.	n. d.	---	n. d.	n. d.	---
Val*	n. d.	0.179 ± 0.046	**0.022**	n. d.	0.021 ± 0.015	0.130	n. d.	n. d.	---	n. d.	n. d.	---
Gly	n. d.	n. d.	---	n. d.	0.050 ± 0.008	**0.008**	0.015 ± 0.014	0.049 ± 0.003	**0.045**	0.013 ± 0.013	0.044 ± 0.003	**0.047**
Leu	0.002 ± 0.002	n. d.	0.184	n. d.	0.013 ± 0.011	0.194	n. d.	n. d.	---	n. d.	n. d.	---
Phe*	0.004 ± 0.004	n. d.	0.196	n. d.	0.015 ± 0.015	0.235	n. d.	n. d.	---	n. d.	n. d.	---
Total	6.295 ± 1.925	15.144 ± 2.789	**0.014**	6.666 ± 0.097	9.678 ± 0.962	0.097	7.201 ± 1.749	17.219 ± 7.114	0.128	6.490 ± 1.585	15.293 ± 76.318	0.130

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the younger and Leaf 8, de oldest leaf.

* Essential amino acid

† Semi-essential amino acid

n. d. – not detected

Table S2a Free amino acids in the tissue of the leaves of control and PepGMV-infected plants of chili at 20 dpi.

	Leaf 1			Leaf 2			Leaf 3			Leaf 4		
AA	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	13.0±7.6	25.0±16.7	0.346	23.5±7.6	18.6±1.3	0.280	24.0±3.5	20.0±8.4	0.508	22.2±7.2	27.5±4.1	0.348
Lys*	0.8±0.2	0.9±0.1	0.845	1.2±0.2	0.7±0.1	0.396	1.1±0.2	0.9±0.3	0.322	1.5±0.4	1.3±0.4	0.609
Ala	3.0±0.7	7.2±5.5	0.314	4.4±0.7	4.7±1.4	0.804	4.6±1.3	4.8±1.6	0.864	4.6±2.2	7.6±1.9	0.145
Cys†	21.2±5.9	40.6±25.7	0.320	25.1±5.9	25.3±3.4	0.913	22.7±4.5	21.4±5.6	0.764	21.7±2.0	28.0±2.9	**0.040**
Tyr	2.1±1.5	6.0±6.0	0.381	3.4±1.5	3.4±0.4	0.991	3.9±0.5	2.7±1.5	0.318	1.1±0.7	4.3±0.6	**0.003**
Thr*	4.1±1.1	9.3±6.7	0.306	6.5±1.1	5.9±0.6	0.592	6.2±1.8	8.0±2.7	0.389	6.2±2.0	9.6±1.8	0.096
Pro	19.0±11.9	54.7±47.6	0.322	37.9±11.9	32.4±20.3	0.820	50.4±34.4	37.7±19.5	0.615	40.7±31.1	45.6±27.9	0.847
Asx	6.9±2.1	13.9±11.5	0.403	5.6±2.1	10.5±4.6	0.202	6.4±2.2	7.1±4.1	0.811	9.7±5.1	9.6±2.5	0.987
Glx	16.6±3.7	62.5±70.3	0.376	21.4±3.7	19.9±5.5	0.707	21.5±5.1	18.9±4.8	0.551	25.0±8.6	29.5±8.1	0.541
Arg	0.6±0.2	1.8±1.9	0.390	0.9±0.2	0.8±0.1	0.922	0.8±0.2	0.9±0.3	0.706	0.9±0.3	1.7±0.3	**0.024**
Ser	17.8±9.3	53.4±33.1	0.197	26.8±9.3	26.7±10.3	0.994	29.0±12.1	28.3±12.5	0.948	31.0±22.9	35.1±12.0	0.802
His†	n. d.	n. d.	---	n. d.	n. d.	---	n. d.	n. d.	---	n. d.	n. d.	---
Ile*	2.2±0.8	4.0±2.2	0.307	4.7±0.8	2.9±0.3	0.250	4.5±1.0	3.8±1.4	0.571	5.2±0.7	5.5±0.6	0.622
Val*	1.8±0.3	3.2±1.6	0.276	2.2±0.3	2.3±0.7	0.901	2.5±0.8	2.2±0.7	0.682	2.4±0.8	13.9±3.8	**0.030**
Gly	2.3±0.8	6.7±±7.4	0.413	3.0±0.8	3.1±1.2	0.983	3.3±1.8	2.3±1.2	0.499	2.5±0.8	3.2±0.6	0.274
Leu	0.2±0.1	0.3±0.1	0.177	0.2±0.1	0.2±0.1	0.577	0.3±0.1	0.3±0.1	0.898	0.3±0.03	0.4±0.2	0.426
Phe*	0.7±0.3	1.3±0.4	0.135	1.1±0.3	1.1±0.2	0.801	1.0±0.1	1.2±0.3	0.369	1.1±0.4	1.7±0.2	0.111
Total	112.4±16.6	290.7±236.5	0.321	167.9±51.6	158.3±43.3	0.817	182.0±63.4	160.4±48.7	0.666	176.1±62.0	224.7±50.3	0.353

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the younger and Leaf 8, de oldest leaf.

* Essential amino acid

† Semi-essential amino acid

n. d. – not detected

Table S2b Free amino acids in the tissue of the leaves of control and PepGMV-infected plants of chili at 20 dpi.

	Leaf 5			Leaf 6			Leaf 7			Leaf 8		
AA	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Healthy plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	25.2±4.3	24.6±8.4	0.931	24.8±7.7	18.8±4.6	0.326	22.2±2.7	16.9±5.8	0.241	18.0±4.5	11.8±1.7	0.127
Lys*	1.1±0.4	1.2±0.7	0.776	0.6±0.03	0.9±0.1	0.008	2.0±0.1	1.0±0.1	**<0.000**	0.6±0.1	1.1±0.7	0.278
Ala	6.4±0.8	7.5±1.9	0.434	5.2±0.9	6.8±0.7	0.071	6.8±2.5	7.6±1.7	0.633	7.6±3.7	7.3±3.6	0.925
Cys†	25.0±7.5	23.8±3.4	0.827	21.9±6.1	20.0±4.1	0.671	23.5±4.9	21.4±6.9	0.674	22.0±2.8	18.8±3.7	0.302
Tyr	3.8±2.4	4.0±1.7	0.081	2.6±1.2	2.4±2.0	0.867	2.2±0.1	4.8±2.1	0.164	48.6±78.6	3.4±2.2	0.424
Thr*	7.3±1.0	10.4±1.7	0.069	7.8±2.0	12.3±4.1	0.192	7.5±2.9	14.0±1.8	**0.014**	7.5±5.1	14.2±3.2	0.139
Pro	49.6±24.1	54.8±29.6	0.825	47.7±40.0	57.8±33.6	0.755	31.7±16.6	57.6±22.1	0.170	41.8±38.2	112.9±75.8	0.244
Asx	8.7±4.0	8.8±5.7	0.985	4.3±0.3	7.3±0.4	**<0.000**	14.3±6.7	6.3±1.5	**0.048**	4.9±2.6	6.2±5.4	0.744
Glx	23.7±8.2	25.7±9.3	0.789	14.7±2.5	22.1±1.1	**0.023**	35.5±1.6	23.5±3.3	**0.015**	19.2±5.9	25.8±14.0	0.514
Arg	1.4±0.1	1.4±0.2	0.852	1.1±0.2	2.2±1.3	0.278	1.2±0.5	3.2±0.7	**0.018**	0.8±1.1	5.2±3.2	0.131
Ser	25.2±7.7	41.9±20.3	0.290	20.5±10.2	47.0±29.1	0.250	31.0±4.0	54.8±22.1	0.201	36.6±18.3	97.2±81.7	0.326
His†	n. d.	n. d.	---	n. d.	n. d.	---	n. d.	n. d.	---	n. d.	n. d.	---
Ile*	6.1±1.6	4.9±0.5	0.346	5.0±0.9	4.3±0.1	0.308	6.1±2.0	4.9±1.4	0.354	5.6±1.6	4.4±0.4	0.327
Val*	2.9±0.7	6.2±5.6	0.407	2.2±0.4	6.8±6.2	0.331	6.6±5.2	13.3±8.5	0.307	3.0±0.3	7.0±3.6	0.195
Gly	2.0±0.6	2.9±0.7	0.183	1.6±0.7	3.7±0.7	**0.017**	2.2±0.8	4.0±0.3	**0.015**	2.3±1.2	6.4±3.3	0.158
Leu	0.3±0.1	0.5±0.1	0.138	0.2±0.01	0.5±0.2	0.046	0.6±0.02	0.7±0.02	**0.007**	0.6±0.2	1.1±0.5	**0.034**
Phe*	1.3±0.2	1.4±0.1	0.384	1.0±0.1	1.3±0.3	0.193	1.1±0.1	1.2±0.2	0.469	1.3±0.6	1.2±0.3	0.829
Total	189.9±53.3	220.1±58.4	0.544	161.3±56.5	214.2±56.4	0.314	194.7±113.0	235.2±42.3	0.235	220.4±79.1	323.9±181.1	0.437

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the younger and Leaf 8, de oldest leaf.

* Essential amino acid

† Semi-essential amino acid

n. d. – not detected

Table S3a Total amino acids in the tissue of the leaves of control and PepGMV-infected plants of chili at 20 dpi.

	Leaf 1			Leaf 2			Leaf 3			Leaf 4		
AA	**Healthy plant** (µmol g^{-1} DW)	**Infected plant** (µmol g^{-1} DW)	**P-value**	**Healthy plant** (µmol g^{-1} DW)	**Infected plant** (µmol g^{-1} DW)	**P-value**	**Healthy plant** (µmol g^{-1} DW)	**Infected plant** (µmol g^{-1} DW)	**P-value**	**Healthy plant** (µmol g^{-1} DW)	**Infected plant** (µmol g^{-1} DW)	**P-value**
Met*	0.1±0.1	0.4±0.1	**0.050**	0.4±0.4	0.4±0.0	0.812	0.7±0.4	2.0±1.4	0.232	0.8±0.1	0.6±0.1	0.128
Lys*	0.1±0.1	0.4±0.3	0.296	0.4±0.3	0.3±0.0	0.535	1.0±0.6	2.4±1.4	0.445	1.0±0.1	0.6±0.1	**0.004**
Ala	2.4±1.1	2.6±1.5	0.861	3.3±2.1	2.4±0.5	0.534	5.7±3.2	10.5±8.4	0.432	5.7±0.7	3.9±0.5	**0.023**
Cys†	1.8±0.9	3.3±0.5	0.096	2.4±1.4	3.6±0.8	0.256	3.5±0.6	7.8±2.6	0.096	5.0±0.7	5.3±0.9	0.663
Tyr	0.5±0.2	0.8±0.4	0.272	0.8±0.5	0.8±0.2	0.889	1.2±0.6	2.4±1.3	0.245	1.4±0.1	1.1±0.1	**0.027**
Thr*	0.3±0.2	1.0±0.5	0.158	0.8±0.6	1.0±0.3	0.741	1.3±0.6	3.5±2.2	0.218	1.6±0.1	1.4±0.2	0.163
Pro	7.0±5.4	5.2±1.2	0.638	4.1±2.4	5.7±2.0	0.430	6.7±1.4	12.8±5.5	0.190	8.6±3.5	7.6±2.2	0.692
Asx	1.6±0.4	1.2±0.1	0.210	0.8±0.4	1.3±0.3	0.109	1.5±0.5	3.2±3.2	0.451	2.0±0.3	1.3±0.3	**0.061**
Glx	1.8±0.8	2.0±1.2	0.884	2.0±1.6	1.6±0.4	0.659	3.7±2.6	7.8±4.9	0.494	4.3±0.6	3.0±0.4	**0.035**
Arg	0.5±0.3	0.8±0.5	0.512	0.8±0.5	0.7±0.3	0.892	1.2±0.7	2.8±2.2	0.350	1.5±0.2	1.0±0.1	0.077
Ser	1.4±0.6	1.5±0.8	0.828	1.5±0.9	1.4±0.3	0.828	2.6±1.3	5.5±4.2	0.353	2.8±0.2	2.1±0.2	0.015
His†	0.4±0.1	0.6±0.4	0.508	0.6±0.4	0.5±0.1	0.844	0.9±0.4	1.8±1.1	0.254	1.0±0.1	0.8±0.1	0.082
Ile*	0.3±0.1	1.1±0.6	0.145	1.2±1.0	1.0±0.3	0.808	1.9±1.0	4.7±2.9	0.232	2.1±0.1	1.6±0.2	**0.016**
Val*	0.4±0.2	1.5±0.8	0.136	1.5±1.4	1.4±0.4	0.862	2.5±1.3	6.2±4.0	0.247	2.9±0.3	2.2±0.3	**0.035**
Gly	2.2±0.9	3.0±1.8	0.545	3.1±1.9	2.7±0.6	0.186	5.3±2.7	10.5±7.8	0.375	5.6±0.5	4.0±0.5	**0.015**
Leu	0.8±0.4	2.0±1.0	0.175	2.2±1.7	1.9±0.5	0.804	3.5±1.8	8.7±5.9	0.260	3.9±0.4	2.9±0.3	**0.028**
Phe*	0.5±0.2	1.2±0.5	0.126	1.1±0.9	1.2±0.3	0.889	1.6±0.7	2.8±0.7	0.114	2.1±0.2	1.7±0.2	0.107
Total	22.3±11.1	28.5±6.9	0.553	27.0±17.9	27.7±12.2	0.952	44.8±19.9	95.4±62.5	0.293	52.3±7.5	41.2±5.6	0.115

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the younger and Leaf 8, de oldest leaf.

* Essential amino acid

† Semi-essential amino acid

n. d. – not detected

Table S3b Total amino acids in the tissue of the leaves of control and PepGMV-infected plants of chili at 20 dpi.

	Leaf 5			Leaf 6			Leaf 7			Leaf 8		
AA	**Healthy plant** (µmol g^{-1} DW)	**Infected plant** (µmol g^{-1} DW)	**P-value**	**Healthy plant** (µmol g^{-1} DW)	**Infected plant** (µmol g^{-1} DW)	**P-value**	**Healthy plant** (µmol g^{-1} DW)	**Infected plant** (µmol g^{-1} DW)	**P-value**	**Healthy plant** (µmol g^{-1} DW)	**Infected plant** (µmol g^{-1} DW)	**P-value**
Met*	0.9±0.5	1.2±0.7	0.534	0.5±0.3	0.9±0.1	0.118	0.6±0.5	4.7±0.2	**0.001**	1.1±0.9	1.0±0.2	0.903
Lys*	0.8±0.6	1.1±1.1	0.792	0.4±0.3	0.5±0.3	0.595	0.5±0.4	6.7±3.3	0.079	3.4±3.0	1.0±0.6	0.301
Ala	5.5±3.5	7.0±5.4	0.712	3.0±1.8	4.6±0.7	0.268	3.5±2.9	22.3±4.9	**0.008**	13.4±10.9	6.2±2.9	0.374
Cys†	6.1±2.0	7.6±2.3	0.444	4.9±1.6	8.2±2.1	0.102	5.2±4.1	13.3±0.5	0.075	5.2±4.4	11.7±2.9	0.108
Tyr	1.5±0.9	1.9±0.9	0.625	0.8±0.4	1.6±0.1	0.090	1.0±0.8	5.5±0.4	**0.005**	1.7±1.3	1.9±0.1	0.745
Thr*	1.8±1.0	2.4±1.3	0.566	1.1±0.6	1.9±0.2	0.118	1.3±1.0	10.0±0.7	**0.0004**	5.2±4.4	2.7±0.2	0.437
Pro	11.0±3.5	10.6±4.3	0.918	9.0±5.2	10.7±3.5	0.676	7.8±6.9	25.3±7.6	**0.041**	9.0±8.6	15.6±4.1	0.323
Asx	2.5±1.9	2.9±2.7	0.839	1.9±0.3	1.7±0.2	0.381	1.8±1.4	9.3±3.6	0.052	9.5±5.7	5.7±1.8	0.371
Glx	4.6±3.2	5.7±5.0	0.761	2.6±1.5	3.6±0.4	0.374	2.9±2.3	18.8±4.4	**0.011**	12.1±8.9	7.4±2.7	0.462
Arg	1.4±0.9	2.0±1.5	0.608	0.8±0.5	1.3±0.0	0.192	0.9±0.7	6.9±1.2	**0.003**	3.2±2.8	2.0±0.9	0.533
Ser	2.9±1.7	3.5±2.2	0.759	1.7±0.9	2.5±0.2	0.266	1.7±1.4	13.4±2.6	**0.006**	5.5±4.3	3.4±0.8	0.481
His†	1.1±0.6	1.3±0.8	0.714	0.6±0.3	1.0±0.04	0.180	0.6±0.5	5.7±0.1	**0.002**	1.6±1.3	1.2±0.1	0.624
Ile*	2.2±1.3	3.0±1.8	0.560	1.2±0.7	2.3±0.1	0.101	1.5±1.2	11.7±0.7	**0.0007**	4.7±3.8	2.7±0.7	0.450
Val*	3.0±1.8	4.2±2.6	0.545	1.7±0.9	3.1±0.1	0.111	2.0±1.6	15.0±0.7	**0.001**	7.3±6.0	3.6±1.0	0.394
Gly	6.1±3.9	7.4±4.9	0.731	3.4±2.1	5.2±0.3	0.272	3.7±3.0	25.6±2.3	**0.0007**	14.2±11.3	7.1±1.7	0.389
Leu	4.1±2.4	5.6±3.6	0.572	2.2±1.2	4.1±0.3	0.115	2.7±2.2	21.1±2.1	**0.0004**	9.4±7.6	4.8±1.5	0.408
Phe*	2.3±1.1	3.3±1.7	0.449	1.4±0.7	2.6±0.1	0.093	1.7±1.3	11.8±1.0	**0.0006**	4.1±	2.9±0.2	0.593
Total	57.7±30.8	70.7±42.3	0.691	37.1±19.5	55.6±5.0	0.237	39.5±31.6	227.0±19.9	0.002	110.6±88.3	81.0±8.9	0.620

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the younger and Leaf 8, de oldest leaf.

* Essential amino acid

† Semi-essential amino acid

n. d. – not detected

Table S4. ANOVA of SA in leaf tissue of control and infected plants colonized and not colonized by *T. vaporariorum*

	Df	Sum Sq	Mean Sq	F-value	P-value
Virus	1	910	910	1.152	0.29913
Time	1	11094	11094	14.037	**0.00176**
Whitefly	1	9369	9369	11.855	**0.00334**
Virus:Time	1	1154	1154	1.46	0.24453
Virus:Whitefly	1	1347	1347	1.704	0.21019
Time:Whitefly	1	4726	4726	5.98	**0.02641**
Virus:Time:Whitefly	1	1588	1588	2.009	0.17557
Residuals	16	12646	790		

The p-values with statistical significance are in bold

Table S5. ANOVA of JA in leaf tissue of control and infected plants colonized and not colonized by *T. vaporariorum*

	Df	Sum Sq	Mean Sq	F value	P value
Virus	1	9.4	9.37	1.356	0.300
Time	1	0.2	0.2	0.029	0.900
Whitefly	1	0.3	0.28	0.041	0.800
Virus:Time	1	4.68	4.68	0.677	0.4226
Virus:Whitefly	1	60.8	60.8	8.797	**0.0091**
Time:Whitefly	1	0.2	0.2	0.029	0.8665
Virus:Time:Whitefly	1	7.5	7.48	1.083	0.3136
Residuals	16	110.6	6.91		

The p-values with statistical significance are in bold

Table S6 Salicylic acid in the tissue of the leaves of control and PepGMV-infected plants of chili at 20 dpi.

	T0 (20 dpi)			T1 (27 dpi)						T2 (32 dpi)					
	Before infestation			Without eggs			With eggs			Without nymphs			With nymphs		
Leaf	**Healthy plant** (ng g^{-1} FW)	**Infected plant** (ng g^{-1} FW)	**P-value**	**Healthy plant** (ng g^{-1} FW)	**Infected plant** (ng g^{-1} FW)	**P-value**	**Healthy plant** (ng g^{-1} FW)	**Infected plant** (ng g^{-1} FW)	**P-value**	**Healthy plant** (ng g^{-1} FW)	**Infected plant** (ng g^{-1} FW)	**P-value**	**Healthy plant** (ng g^{-1} FW)	**Infected plant** (ng g^{-1} FW)	**P-value**
1	60.2±50.7	63.0±30.9	0.888	34.4±15.4	15.5±0.6	0.341	4.6±0.2	5.6±1.9	0.439	25.3±8.3	30.8±8.4	0.233	8.7±0.6	7.3±1.0	0.189
2	28.4±8.4	49.3±33.6	0.801	34.6±18.9	36.0±16.5	0.825	6.1±2.1	7.6±2.2	0.843	12.8±4.5	50.8±56.4	0.797	9.0±2.6	11.5±5.3	0.629
3	38.1±20.3	56.2±44.7	0.570	15.2±1.2	13.5±4.3	0.574	7.9±3.8	6.3±2.7	0.580	12.8±4.6	17.1±5.5	0.364	8.6±1.1	6.9±0.9	0.102
4	46.1±23.0	40.3±29.8	0.394	15.3±11.6	13.5±4.1	0.927	5.4±0.7	5.5±0.4	0.444	10.1±1.9	10.5±2.3	0.363	7.8±1.5	7.8±0.9	0.520
5	47.2±28.6	43.7±28.5	0.942	20.6±12.6	11.6±1.6	0.165	7.7±2.1	5.9±1.1	0.460	6.3±2.0	10.0±3.9	0.466	9.0±1.6	7.2±0.2	0.125
Total	220.0±56.0	252.4±131.2	0.722	120.1±36.9	90.0±16.2	0.293	31.8±3.5	30.8±3.4	0.749	67.2±14.2	119.1±68.6	0.319	43.1±3.6	40.2±3.1	0.342

Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold.
Leaf 1 is the younger and Leaf 5, de oldest leaf.

Table S7 Jasmonic acid in the tissue of the leaves of control and PepGMV-infected plants of chili at 20 dpi.

	T0 (20 dpi)			T1 (27 dpi)						T2 (32 dpi)					
	Before infestation			Without eggs			With eggs			Without nymphs			With nymphs		
Leaf	**Healthy plant** (ng g^{-1} FW)	**Infected plant** (ng g^{-1} FW)	**P-value**	**Healthy plant** (ng g^{-1} FW)	**Infected plant** (ng g^{-1} FW)	**P-value**	**Healthy plant** (ng g^{-1} FW)	**Infected plant** (ng g^{-1} FW)	**P-value**	**Healthy plant** (ng g^{-1} FW)	**Infected plant** (ng g^{-1} FW)	**P-value**	**Healthy plant** (ng g^{-1} FW)	**Infected plant** (ng g^{-1} FW)	**P-value**
1	18.4±17.5	12.3±5.7	0.434	20.9±5.1	10.4±0.2	0.067	2.8±1.5	5.1±1.1	0.828	25.0±13.5	28.5±10.2	0.392	2.9±1.5	4.9±1.2	0.884
2	54.4±25.1	16.2±2.1	0.610	19.3±8.4	14.4±4.1	0.904	3.7±1.3	5.1±0.4	0.468	12.5±5.5	16.1±9.4	0.065	3.7±1.3	4.9±0.2	0.678
3	13.7±5.3	11.4±4.2	0.584	20.1±12.7	11.5±1.9	0.357	4.0±0.4	5.2±0.7	0.078	11.3±3.5	11.4±3.8	0.991	4.1±0.6	4.9±0.6	0.202
4	14.8±5.7	12.6±4.3	0.118	8.1±1.2	8.3±0.6	0.433	4.5±0.7	4.8±0.3	0.215	13.2±2.1	8.8±0.2	0.600	4.3±0.5	4.6±0.2	0.216
5	25.3±22.8	12.6±1.8	0.616	10.5±2.3	6.3±1.3	0.069	4.8±0.4	4.7±0.9	0.114	6.3±1.4	7.7±1.9	0.742	4.6±0.4	4.7±0.6	0.147
Total	126.6±46.8	65.1±3.5	0.149	79.0±17.5	50.8±45.0	0.098	19.8±3.5	24.9±1.7	0.106	68.3±23.1	72.5±16.7	0.816	19.5±3.5	23.9±0.7	0.155

Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold.
Leaf 1 is the younger and Leaf 5, de oldest leaf.

Table S8. ANOVA of free amino acids in phloem of control and infected plants colonized and not colonized by *T. vaporariorum*

	Df	Sum Sq	Mean Sq	F-value	P-value
Virus	1	2.06E+10	2.06E+10	1.541	0.2324
Time	1	2.41E+09	2.41E+09	0.18	0.6772
Whitefly	1	1.64E+12	1.64E+12	122.321	**6.65E-09**
Virus:Time	1	6.99E+10	6.99E+10	5.217	**0.0364**
Virus:Whitefly	1	9.15E+09	9.15E+09	0.683	0.4206
Time:Whitefly	1	7.89E+09	7.89E+09	0.589	0.4539
Virus:Time:Whitefly	1	9.31E+10	9.31E+10	6.949	**0.018**
Residuals	16	2.14E+11	1.34E+10		

The *p*-values with statistical significance are in bold.

Table S9a Free amino acids in the phloem of the leaves of control and PepGMV-infected plants of chili before colonization by *T. vaporariorum* at 20 dpi.

	Leaf 1			Leaf 2			Leaf 3			Leaf 4		
AA	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	33.242 ± 2.828	123.958 ± 9.700	**0.002**	14.694 ± 3.478	60.851 ± 50.632	0.255	14.364 ± 2.563	44.244 ± 18.907	0.109	10.861 ± 0.114	17.829 ± 6.362	0.198
Pro	n. d.	n. d.	-	n. d.	n. d.	-	0.034 ± 0.029	n. d.	0.184	n. d.	0.039 ± 0.068	0.423
Tyr	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	9.704 ± 0.800	**0.002**	n. d.	7.002 ± 1.547	**0.016**
Cys†	2.489 ± 1.732	15.149 ± 7.307	0.088	1.650 ± 0.587	9.581 ± 4.938	0.107	1.215 ± 0.502	6.057 ± 1.312	**0.014**	n. d.	2.420 ± 1.083	*0.061*
Thr*	0.035 ± 0.060	n. d.	0.423	n. d.	31.631 ± 9.336	**0.028**	n. d.	0.992 ± 0.597	0.102	0.084 ± 0.020	n. d.	**0.019**
Leu	0.038 ± 0.014	0.133 ± 0.107	0.262	0.055 ± 0.014	0.373 ± 0.143	*0.060*	0.017 ± 0.020	0.134 ± 0.039	**0.020**	n. d.	n. d.	-
Ile*	0.061 ± 0.017	0.467 ± 0.483	0.281	0.047 ± 0.020	0.430 ± 0.295	0.152	0.025 ± 0.008	0.105 ± 0.066	0.167	n. d.	n. d.	-
Phe*	n. d.	0.130 ± 0.049	**0.044**	0.021 ± 0.013	0.140 ± 0.023	**0.003**	0.024 ± 0.025	0.035 ± 0.032	0.652	n. d.	n. d.	-
Arg	n. d.	n. d.	-	0.161 ± 0.227	3.436 ± 1.968	0.100	n. d.	n. d.	-	n. d.	n. d.	-
Ser	n. d.	1.246 ± 0.238	**0.012**	n. d.	1.132 ± 0.557	0.072	n. d.	0.396 ± 0.222	0.091	n. d.	n. d.	-
Val*	0.971 ± 0.294	0.171 ± 0.078	**0.035**	n. d.	0.296 ± 0.061	**0.014**	0.023 ± 0.012	0.097 ± 0.024	**0.019**	n. d.	0.367 ± 0.083	**0.016**
Lys*	24.375 ± 1.384	92.311 ± 1.636	**<0.000**	12.183 ± 0.274	66.002 ± 10.863	**0.013**	9.637 ± 1.580	32.689 ± 0.706	**<0.000**	6.911 ± 2.560	15.028 ± 0.883	**0.022**
Asx	0.887 ± 0.040	3.089 ± 0.789	**0.040**	0.519 ± 0.275	2.196 ± 1.808	0.248	0.402 ± 0.270	0.856 ± 0.508	0.262	0.251 ± 0.041	n. d.	**0.009**
Gly	0.159 ± 0.047	n. d.	**0.028**	0.213 ± 0.036	0.736 ± 0.307	0.096	0.118 ± 0.080	0.396 ± 0.155	0.071	n. d.	0.021 ± 0.019	0.193
His†	0.354 ± 0.091	1.200 ± 0.189	**0.007**	0.142 ± 0.006	0.330 ± 0.181	0.215	0.203 ± 0.088	n. d.	*0.057*	0.085 ± 0.027	0.136 ± 0.007	0.073
Ala	0.659 ± 0.311	2.504 ± 1.760	0.208	0.485 ± 0.438	32.298 ± 26.819	0.176	0.533 ± 0.543	4.876 ± 7.160	0.404	0.195 ± 0.032	0.316 ± 0.164	0.328
Glx	0.564 ± 0.139	1.790 ± 0.344	**0.015**	0.488 ± 0.242	1.540 ± 0.945	0.188	0.246 ± 0. 276	0.584 ± 0.372	0.280	0.131 ± 0.045	n. d.	**0.037**
Total	**63.835 ± 3.202**	**242.150 ± 5.440**	**<0.000**	**30.658 ± 4.000**	**210.972 ± 82.420**	*0.063*	**26.842 ± 5.731**	**101.166 ± 12.981**	**0.004**	**18.519 ± 2.374**	**43.159 ± 8.464**	**0.029**

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the youngest leaf and Leaf 8, de oldest leaf.

n. d. – not detected, for PCoA, was taken as 0.0001; n. p. –not present, the plant have 7 leaves completed extended

** Essential amino acid*

† Semi-essential amino acid

Table S9b Free amino acids in the phloem of the leaves of control and PepGMV-infected plants of chili before colonization *T. vaporariorum* at 20 dpi.

	Leaf 5			Leaf 6			Leaf 8			Leaf 8		
AA	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	9.140 ± 3.819	18.562 ± 2.742	**0.03**	9.210 ± 3.601	13.313 ± 1.447	0.177	11.013 ± 0.956	14.733 ± 1.89	*0.057*	n. p.	n. p.	-
Pro	n. d.	0.080 ± 0.139	0.423	n. d.	0.074 ± 0.130	0.423	n. d.	7.076 ± 12.123	0.418	n. p.	n. p.	-
Tyr	n. d.	7.243 ± 3.409	0.067	n. d.	2.447 ± 0.236	**0.003**	n. d.	11.012 ± 6.762	0.106	n. p.	n. p.	-
Cys†	0.560 ± 0.049	1.216 ± 0.385	0.096	0.868 ± 0.069	0.967 ± 0.118	0.293	1.413 ± 0.760	2.404 ± 0.384	0.139	n. p.	n. p.	-
Thr*	n. d.	n. d.	-	n. d.	0.248 ± 0.198	0.162	n. d.	0.148 ± 0.053	**0.041**	n. p.	n. p.	-
Leu	0.006 ± 0.005	n. d.	0.184	n. d.	0.035 ± 0.032	0.194	n. d.	n. d.	-	n. p.	n. p.	-
Ile*	0.133 ± 0.102	0.374 ± 0.048	**0.038**	0.053 ± 0.092	0.200 ± 0.094	0.126	n. d.	n. d.	-	n. p.	n. p.	-
Phe*	0.012 ± 0.011	n. d.	0.196	n. d.	0.041 ± 0.043	0.235	n. d.	n. d.	-	n. p.	n. p.	-
Arg	n. d.	n. d.	-	n. d.	0.189 ± 0.011	**0.001**	n. d.	n. d.	-	n. p.	n. p.	-
Ser	n. d.	n. d.	-	n. d.	0.058 ± 0.102	0.423	n. d.	0.065 ± 0.011	0.422	n. p.	n. p.	-
Val*	n. d.	0.497 ± 0.129	**0.022**	n. d.	0.059 ± 0.041	0.13	n. d.	n. d.	-	n. p.	n. p.	-
Lys*	7.025 ± 1.359	13.784 ± 2.429	**0.022**	7.768 ± 1.748	9.108 ± 1.969	0.428	6.739 ± 3.293	11.712 ± 1.472	0.104	n. p.	n. p.	-
Asx	0.313 ± 0.042	n. d.	**0.006**	0.293 ± 0.067	n. d.	**0.017**	0.355 ± 0.064	0.506 ± 0.034	**0.036**	n. p.	n. p.	-
Gly	n. d.	n. d.	-	n. d.	0.139 ± 0.021	**0.008**	0.041 ± 0.038	0.136 ± 0.096	**0.045**	n. p.	n. p.	-
His†	0.063 ± 0.016	0.146 ± 0.028	**0.019**	0.136 ± 109	n. d.	0.163	0.108 ± 0.043	n. d.	**0.050**	n. p.	n. p.	-
Ala	0.118 ± 0.054	0.161 ± 0.144	0.672	0.110 ± 0.47	n. d.	*0.054*	0.203 ± 0.082	0.080 ± 0.139	0.276	n. p.	n. p.	-
Glx	0.114 ± 0.039	n. d.	**0.037**	0.074 ± 0.015	n. d.	**0.013**	0.128 ± 0.049	0.012 ± 0.022	**0.039**	n. p.	n. p.	-
Total	**17.485 ± 5.347**	**42.066 ± 7.748**	**0.014**	**18.516 ± 5.395**	**26.883 ± 2.672**	0.097	**20.003 ± 4.886**	**47.830 ± 19.760**	0.128	n. p.	n. p.	-

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the youngest leaf and Leaf 8, de oldest leaf.

n. d. – not detected, for PCoA, was taken as 0.0001; n. p. –not present, the plant have 7 leaves completed extended

** Essential amino acid*

† Semi-essential amino acid

Table S10a Free amino acids in the phloem of the leaves of control and PepGMV-infected plants of chili not colonized (control), with eggs of *T. vaporariorum*.

	Leaf 1			Leaf 2			Leaf 3			Leaf 4		
AA	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	n. d.	0.348 ± 0.154	*0.060*	n. d.	95.326 ± 44.509	0.066	n. d.	51.307 ± 22.511	*0.059*	n. d.	43.768 ± 12.537	**0.026**
Pro	1.694 ± 0.752	1.611 ± 0.645	0.892	18.533 ± 11.267	1.731 ± 0.719	0.122	21.179 ± 23.321	0.807 ± 0.432	0.269	25.497 ± 18.449	0.624 ± 0.177	0.145
Tyr	1.814 ± 0.685	12.389 ± 4.371	**0.049**	23.481 ± 17.790	19.210 ± 13.369	0.757	14.692 ± 14.017	4.425 ± 2.741	0.332	16.164 ± 9.314	6.148 ± 2.815	0.197
Cys†	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-
Thr*	0.022 ± 0.007	0.785 ± 0.267	**0.038**	0.031 ± 0.015	0.806 ± 0.324	*0.053*	0.105 ± 0.182	0.435 ± 0.305	0.199	0.015 ± 0.026	0.298 ± 0.102	**0.034**
Leu	0.059 ± 0.025	0.849 ± 0.343	*0.057*	0.578 ± 0.450	0.677 ± 0.481	0.807	0.306 ± 0.218	0.420 ± 0.189	0.533	0.193 ± 0.047	0.350 ± 0.089	0.072
Ile*	0.044 ± 0.015	0.655 ± 0.245	**0.049**	0.357 ± 0.194	0.642 ± 0.312	0.263	0.373 ± 0.408	0.324 ± 0.156	0.859	0.177 ± 0.082	0.276 ± 0.086	0.222
Phe*	0.046 ± 0.020	0.494 ± 0.173	**0.045**	0.481 ± 0.378	0.395 ± 0.283	0.771	0.256 ± 0.178	0.256 ± 0.145	0.995	0.169 ± 0.040	0.208 ± 0.075	0.489
Arg	0.058 ± 0.027	n. d.	0.066	0.277 ± 0.188	0.178 ± 0.158	0.525	0.377 ± 0.234	n. d.	0.108	0.292 ± ± 0.103	0.166 ± 0.040	0.155
Ser	0.084 ± 0.031	1.278 ± 0.229	**0.011**	0.628 ± 0.301	1.256 ± 0.705	0.260	0.598 ± 0.457	0.904 ± 0.954	0.652	0.317 ± 0.073	0.488 ± 0.291	0.417
Val*	12.310 ± 5.449	0.566 ± 0.212	*0.065*	124.377 ± 80.482	0.510 ± 0.234	0.117	67.806 ± 46.178	0.331 ± 0.196	0.127	57.622 ± 28.596	0.226 ± 0.056	0.074
Lys*	0.051 ± 0.007	2.128 ± 0.924	*0.060*	0.374 ± 0.185	1.893 ± 0.939	0.102	0.314 ± 0.254	1.019 ± 0.329	**0.046**	0.191 ± 0.070	0.889 ± 0.175	**0.012**
Asx	0.010 ± 0.018	1.930 ± 0.700	**0.042**	n. d.	1.975 ± 0.779	**0.048**	n. d.	0.885 ± 0.360	*0.051*	n. d.	0.808 ± 0.254	**0.031**
Gly	0.025 ± 0.026	1.296 ± 0.349	**0.024**	n. d.	1.232 ± 0.689	0.090	n. d.	0.780 ± 0.666	0.180	n. d.	0.509 ± 0.234	*0.064*
His†	n. d.	0.569 ± 0.199	**0.038**	n. d.	0.540 ± 0.294	0.086	n. d.	0.555 ± 0.588	0.244	n. d.	0.232 ± 0.059	**0.021**
Ala	0.011 ± 0.018	0.818 ± 0.309	**0.045**	n. d.	0.739 ± 0.293	**0.049**	n. d.	0.488 ± 0.386	0.160	n. d.	0.298 ± 0.113	**0.045**
Glx	0.014 ± 0.024	2.276 ± 1.077	0.068	n. d.	1.859 ± 0.848	*0.063*	n. d.	1.034 ± 0.393	**0.045**	n. d.	0.853 ± 0.203	**0.018**
Total	**16.241 ± 6.903**	**27.993 ± 9.978**	0.178	**169.118 ± 110.719**	**128.971 ± 53.141**	0.612	**106.007 ± 85.291**	**63.971 ± 27.988**	0.489	**100.638 ± 50.956**	**56.142 ± 17.218**	0.266

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the youngest leaf and Leaf 8, de oldest leaf.

n. d. – not detected, for PCoA, was taken as 0.0001; n. p. –not present, the plant have 7 leaves completed extended

** Essential amino acid*

† Semi-essential amino acid

Table S10b Free amino acids in the phloem of the leaves of control and PepGMV-infected plants of chili not colonized (control), with eggs of *T. vaporariorum*.

	Leaf 5			Leaf 6			Leaf 7			Leaf 8		
AA	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	n. d.	21.170 ± 8.597	*0.051*	3.559 ± 3.103	44.068 ± 4.419	**<0.000**	n. d.	49.951 ± 13.741	**0.024**	n. d.	40.0 ± 6.114	**0.008**
Pro	11.377 ± 7.937	0.382 ± 0.075	0.138	9.032 ± 3.415	0.495 ± 0.057	**0.049**	9.023 ± 7.750	0.627 ± 0.076	0.201	12.607 ± 6.409	0.712 ± 0.041	0.085
Tyr	8.113 ± 4.487	2.612 ± 1.073	0.162	7.748 ± 1.220	7.654 ± 1.883	0.946	8.216 ± 4.130	9.177 ± 3.808	0.782	12.549 ± 3.776	5.227 ± 0.200	0.078
Cys†	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-
Thr*	n. d.	0.164 ± 0.035	**0.015**	0.013 ± 0.023	0.248 ± 0.045	**0.004**	n. d.	0.291 ± 0.0	**0.010**	0.0402 ± 0.035	0.350 ± 0.000	**0.004**
Leu	0.086 ± 0.029	0.214 ± 0.059	**0.045**	0.140 ± 0.88	0.247 ± 0.005	0.170	0.110 ± 0.021	0.304 ± 0.129	0.117	0.173 ± 0.060	0.365 ± 0.047	**0.014**
Ile*	0.095 ± 0.034	0.166 ± 0.049	0.121	0.097 ± 0.043	0.265 ± 0.045	**0.009**	0.122 ± 0.050	0.282 ± 0.068	**0.034**	0.226 ± 0.055	0.369 ± 0.020	**0.034**
Phe*	0.076 ± 0.028	0.109 ± 0.040	0.307	0.111 ± 0.067	0.134 ± 0.12	0.614	0.073 ± 0.035	0.166 ± 0.101	0.246	0.111 ± 0.011	0.204 ± 0.034	**0.031**
Arg	0.116 ± 0.105	n. d.	0.195	0.184 ± 0.081	0.095 ± 0.164	0.460	0.146 ± 0.058	n. d.	**0.049**	0.210 ± 0.032	0.217 ± 0.188	0.957
Ser	0.157 ± 0.027	0.192 ± 0.058	0.411	0.190 ± 0.106	0.496 ± 0.037	**0.027**	0.151 ± 0.019	0.379 ± 0.189	0.170	0.212 ± 0.191	0.724 ± 0.117	**0.024**
Val*	32.204 ± 21.885	0.181 ± 0.046	0.127	14.846 ± 25.714	0.190 ± 0.010	0.428	38.195 ± 17.616	0.241 ± 0.020	*0.065*	50.956 ± 10.953	1.006 ± 1.048	**0.015**
Lys*	0.135 ± 0.021	0.651 ± 0.013	**<0.000**	0.135 ± 0.045	0.701 ± 0.070	**0.001**	0.149 ± 0.029	0.957 ± 0.053	**<0.000**	0.182 ± 0.061	1.125 ± 0.027	**<0.000**
Asx	n. d.	0.440 ± 0.104	**0.018**	n. d.	0.770 ± 0.073	**0.003**	n. d.	0.918 ± 0.205	**0.016**	n. d.	0.940 ± 0.038	**0.001**
Gly	n. d.	0.243 ± 0.066	**0.024**	0.018 ± 0.032	0.478 ± 0.044	**<0.000**	n. d.	0.441 ± 0.148	**0.036**	n. d.	0.749 ± 0.087	**0.004**
His†	n. d.	0.051 ± 0.088	0.423	n. d.	0.066 ± 0.114	0.423	n. d.	n. d.	-	n. d.	n. d.	-
Ala	n. d.	0.118 ± 0.104	0.188	n. d.	0.062 ± 0.108	0.423	n. d.	n. d.	-	n. d.	0.390 ± 0.016	**0.001**
Glx	n. d.	0.590 ± 0.034	**0.001**	n. d.	0.676 ± 0.062	**0.003**	n. d.	0.922 ± 0.077	**0.002**	n. d.	1.064 ± 0.022	**<0.000**
Total	**52.356 ± 34.180**	**27.284 ± 10.132**	0.331	**36.075 ± 23.335**	**56.646 ± 6.437**	0.263	**56.187 ± 27.503**	**64.658 ± 18.230**	0.683	**77.267 ± 20.395**	**53.442 ± 5.138**	0.174

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the youngest leaf and Leaf 8, de oldest leaf.

n. d. – not detected, for PCoA, was taken as 0.0001; n. p. –not present, the plant have 7 leaves completed extended

** Essential amino acid*

† Semi-essential amino acid

Table S11a Free amino acids in the phloem of the leaves of control and PepGMV-infected plants of chili colonized, with eggs of *T. vaporariorum*.

	Leaf 1			Leaf 2			Leaf 3			Leaf 4		
AA	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plan** (nmol g^{-1} FW)**t**	**P-value**
Met*	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-
Pro	1.627 ± 1.256	1.721 ± 2.260	0.954	0.923 ± 0.290	5.391 ± 1.531	**0.033**	1.222 ± 0.459	1.139 ± 0.342	0.816	1.472 ± 0.479	2.812 ± 3.166	0.541
Tyr	0.753 ± 0.473	0.248 ± 0.430	0.243	0.347 ± 0.114	0.000 ± 0	**0.034**	0.319 ± 0.084	2.153 ± 2.943	0.393	0.474 ± 0.426	0.429 ± 0.377	0.898
Cys†	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-
Thr*	0.088 ± 0.002	0.059 ± 0.102	0.670	0.070 ± 0.016	0.415 ± 0.137	**0.047**	0.078 ± 0.035	0.102 ± 0.078	0.659	0.110 ± 0.013	0.127 ± 0.138	0.857
Leu	0.026 ± 0.004	n. d.	**0.008**	0.025 ± 0.006	n. d.	**0.020**	0.028 ± 0.013	n. d.	*0.062*	0.035 ± 0.010	n. d.	**0.027**
Ile*	0.035 ± 0.004	n. d.	**0.004**	0.032 ± 0.007	n. d.	**0.015**	0.033 ± 0.012	n. d.	**0.039**	0.043 ± 0.006	n. d.	**0.007**
Phe*	0.056 ± 0.009	n. d.	**0.009**	0.052 ± 0.012	n. d.	**0.018**	0.051 ± 0.017	n. d.	**0.035**	0.057 ± 0.018	n. d.	**0.031**
Arg	0.053 ± 0.007	0.053 ± 0.093	0.989	0.045 ± 0.007	0.224 ± 0.101	0.092	0.044 ± 0.022	0.107 ± 0.081	0.308	0.069 ± 0.016	0.131 ± 0.142	0.532
Ser	0.784 ± 0.112	0.077 ± 0.134	**0.002**	0.548 ± 0.281	0.383 ± 0.134	0.430	0.536 ± 0.155	0.184 ± 0.067	**0.043**	0.903 ± 0.154	0.288 ± 0.301	*0.052*
Val*	0.854 ± 0.704	0.674 ± 1.092	0.824	0.731 ± 0.046	4.475 ± 1.338	**0.040**	1.063 ± 0.295	0.948 ± 0.878	0.845	0.719 ± 0.658	3.342 ± 3.625	0.336
Lys*	0.171 ± 0.011	1.027 ± 1.552	0.440	0.158 ± 0.019	0.380 ± 0.186	0.173	0.154 ± 0.045	0.370 ± 0.292	0.328	0.167 ± 0.037	0.340 ± 0.326	0.454
Asx	0.386 ± 0.123	0.285 ± 0.494	0.762	0.234 ± 0.062	1.947 ± 0.647	**0.043**	0.305 ± 0.160	1.140 ± 0.867	0.234	0.370 ± 0.022	1.158 ± 1.236	0.385
Gly	0.089 ± 0.019	0.484 ± 0.803	0.484	0.068 ± 0.022	2.364 ± 0.763	**0.035**	0.068 ± 0.017	1.276 ± 0.737	0.105	0.078 ± 0.034	1.818 ± 1.785	0.233
His†	0.091 ± 0.108	0.108 ± 0.151	0.879	0.085 ± 0.074	0.513 ± 0.048	**0.002**	0.059 ± 0.044	0.327 ± 0.243	0.194	0.073 ± 0.049	0.491 ± 0.453	0.250
Ala	0.566 ± 0.806	0.191 ± 0.331	0.516	0.072 ± 0.019	0.453 ± 0.211	0.088	n. d.	1.916 ± 2.775	0.354	0.122 ± 0.076	0.358 ± 0.397	0.413
Glx	0.263 ± 0.052	0.176 ± 0.304	0.669	0.218 ± 0.066	1.074 ± 0.547	0.111	0.212 ± 0.056	0.603 ± 0.318	0.163	0.284 ± 0.057	0.836 ± 0.844	0.374
Total	**5.843 ± 1.158**	**5.104 ± 5.632**	0.843	**3.608 ± 0.861**	**17.617 ± 3.842**	**0.020**	**4.172 ± 1.375**	**10.266 ± 7.260**	0.281	**4.974 ± 1.419**	**12.131 ± 12.704**	0.432

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the youngest leaf and Leaf 8, de oldest leaf.

n. d. – not detected, for PCoA, was taken as 0.0001; n. p. –not present, the plant have 7 leaves completed extended

** Essential amino acid*

† Semi-essential amino acid

Table S11b Free amino acids in the phloem of the leaves of control and PepGMV-infected plants of chili colonized, with eggs of *T. vaporariorum*.

	Leaf 5			Leaf 6			Leaf 7			Leaf 8		
AA	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-
Pro	1.066 ± 0.177	1.407 ± 0.540	0.391	2.598 ± 2.581	1.119 ± 0.521	0.427	0.788 ± 0.688	1.298 ± 0.688	0.437	1.544 ± 0.277	1.160 ± 1.482	0.700
Tyr	0.094 ± 0.162	0.747 ± 0.414	0.097	0.422 ± 0.370	0.410 ± 0.422	0.966	0.402 ± 0.402	1.770 ± 0.410	0.098	0.727 ± 0.529	2.326 ± 2.369	0.363
Cys†	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-
Thr*	0.094 ± 0.016	0.077 ± 0.049	0.620	0.221 ± 0.209	0.060 ± 0.022	0.313	0.089 ± 0.077	0.148 ± 0.077	0.338	0.154 ± 0.054	0.216 ± 0.110	0.450
Leu	0.038 ± 0.012	n. d.	**0.033**	0.086 ± 0.102	n. d.	0.284	0.030 ± 0.027	n. d.	0.191	0.071 ± 0.031	n. d.	*0.059*
Ile*	0.049 ± 0.013	n. d.	**0.024**	0.083 ± 0.082	n. d.	0.221	0.041 ± 0.035	n. d.	0.184	0.082 ± 0.033	n. d.	0.049
Phe*	0.053 ± 0.015	n. d.	**0.027**	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-
Arg	0.030 ± 0.026	0.081 ± 0.051	0.225	0.037 ± 0.063	0.063 ± 0.024	0.555	0.041 ± 0.036	0.154 ± 0.036	**0.046**	0.088 ± 0.006	0.114 ± 0.197	0.841
Ser	0.696 ± 0.056	0.140 ± 0.051	**<0.000**	1.638 ± 1.560	0.109 ± 0.040	0.231	0.616 ± 0.538	0.150 ± 0.538	0.272	1.007 ± 0.162	0.122 ± 0.041	**0.008**
Val*	0.917 ± 0.294	2.126 ± 0.910	0.138	1.385 ± 1.247	0.942 ± 0.940	0.650	0.882 ± 0.796	2.739 ± 0.796	0.306	1.352 ± 0.235	1.606 ± 1.520	0.801
Lys*	0.153 ± 0.045	0.255 ± 0.135	0.319	0.263 ± 0.248	0.198 ± 0.103	0.706	0.136 ± 0.122	0.351 ± 0.122	0.087	0.230 ± 0.130	0.398 ± 0.104	0.160
Asx	0.298 ± 0.036	0.914 ± 0.594	0.214	1.087 ± 1.018	0.512 ± 0.175	0.431	0.292 ± 0.252	0.670 ± 0.252	0.106	0.491 ± 0.154	0.712 ± 0.215	0.227
Gly	0.290 ± 0.389	1.174 ± 0.446	*0.062*	0.205 ± 0.231	0.932 ± 0.321	**0.038**	0.053 ± 0.046	1.462 ± 0.046	**0.006**	0.120 ± 0.010	1.162 ± 0.336	**0.033**
His†	0.042 ± 0.041	0.202 ± 0.072	**0.041**	0.046 ± 0.080	0.153 ± 0.053	0.134	0.041 ± 0.037	0.248 ± 0.037	**0.023**	0.085 ± 0.024	0.255 ± 0.079	*0.054*
Ala	0.069 ± 0.008	0.130 ± 0.046	0.145	0.276 ± 0.351	0.127 ± 0.045	0.539	0.065 ± 0.058	0.448 ± 0.058	0.237	0.118 ± 0.023	1.466 ± 0.498	**0.042**
Glx	0.241 ± 0.015	0.658 ± 0.490	0.278	0.852 ± 0.774	0.453 ± 0.129	0.467	0.229 ± 0.205	0.651 ± 0.205	**0.051**	0.333 ± 0.070	0.535 ± 0.125	0.089
Total	**4.130 ± 0.940**	**7.909 ± 3.517**	0.198	**9.199 ± 8.704**	**5.077 ± 1.525**	0.500	**3.704 ± 3.242**	**10.089 ± 3.242**	*0.055*	**6.405 ± 1.131**	**10.072 ± 1.310**	**0.022**

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the youngest leaf and Leaf 8, de oldest leaf.

n. d. – not detected, for PCoA, was taken as 0.0001; n. p. –not present, the plant have 7 leaves completed extended

** Essential amino acid*

† Semi-essential amino acid

Table S12a Free amino acids in the phloem of the leaves of control and PepGMV-infected plants of chili not colonized (control), with nymphs of *T. vaporariorum*.

	Leaf 1			Leaf 2			Leaf 3			Leaf 4		
AA	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	6.613 ± 2.205	100.054 ± 13.427	**0.006**	4.466 ± 1.138	67.037 ± 26.058	*0.053*	19.464 ± 8.478	47.123 ± 11.055	**0.029**	13.345 ± 2.678	37.600 ± 5.220	**0.006**
Pro	8.010 ± 2.250	2.081 ± 0.112	**0.045**	8.557 ± 3.730	3.288 ± 1..314	0.122	40.952 ± 10.210	1.844 ± 0.600	**0.022**	11.888 ± 6.563	2.103 ± 0.872	0.120
Tyr	4.297 ± 0.475	65.866 ± 7.001	**0.004**	4.252 ± 1.121	36.306 ± 14.790	*0.063*	21.652 ± 6.984	34.528 ± 11.577	0.190	10.454 ± 3.473	52.825 ± 38.609	0.197
Cys†	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-
Thr*	n. d.	1.663 ± 0.012	**<0.000**	n. d.	1.606 ± 0.697	*0.057*	n. d.	0.946 ± 0.367	**0.047**	n. d.	0.770 ± 0.096	**0.005**
Leu	n. d.	n. d.	-	n. d.	0.484 ± 0.200	*0.052*	n. d.	0.257 ± 0.070	**0.023**	n. d.	0.205 ± 0.026	**0.005**
Ile*	n. d.	n. d.	-	n. d.	0.403 ± 0.167	*0.052*	n. d.	0.223 ± 0.062	**0.025**	n. d.	0.174 ± 0.026	**0.007**
Phe*	n. d.	0.246 ± 0.054	**0.016**	n. d.	0.351 ± 0.211	0.102	n. d.	0.149 ± 0.057	**0.045**	n. d.	0.113 ± 0.024	**0.015**
Arg	n. d.	n. d.	-	n. d.	0.314 ± 0.122	**0.047**	n. d.	0.158 ± 0.049	**0.030**	n. d.	0.157 ± 0.021	**0.006**
Ser	n. d.	1.473 ± 0.099	**0.002**	n. d.	1.631 ± 1.289	0.160	n. d.	0.385 ± 0.242	0.110	n. d.	0.289 ± 0.067	**0.018**
Val*	n. d.	2.043 ± 0.271	**0.006**	n. d.	0.642 ± 0.277	*0.057*	n. d.	0.343 ± 0.103	**0.020**	n. d.	6.739 ± 11.190	0.406
Lys*	n. d.	17.007 ± 10.578	0.108	n. d.	7.011 ± 2.756	**0.048**	n. d.	3.155 ± 1.493	0.067	n. d.	2.733 ± 0.557	**0.014**
Asx	n. d.	1.398 ± 0.062	**0.001**	n. d.	0.552 ± 0.499	0.195	n. d.	1.534 ± 0.543	**0.039**	n. d.	1.113 ± 0.127	**0.004**
Gly	n. d.	1.183 ± 0.103	**0.003**	n. d.	1.459 ± 0.938	0.115	n. d.	0.498 ± 0.173	**0.038**	n. d.	0.443 ± 0.089	**0.013**
His†	n. d.	1.260 ± 0.144	**0.004**	n. d.	0.490 ± 0.225	*0.064*	n. d.	0.266 ± 0.079	**0.028**	n. d.	0.204 ± 0.013	**0.001**
Ala	n. d.	0.623 ± 0.068	**0.004**	n. d.	1.175 ± 0.632	0.084	n. d.	0.461 ± 0.164	**0.040**	n. d.	0.402 ± 0.083	**0.014**
Glx	n. d.	1.985 ± 0.077	**<0.000**	n. d.	1.357 ± 0.460	**0.036**	n. d.	1.225 ± 0.415	**0.036**	n. d.	0.883 ± 0.082	**0.003**
Total	**18.919 ± 4.811**	**196.881 ± 21.378**	**0.003**	**17.275 ± 5.968**	**124.108 ± 48.228**	*0.060*	**82.068 ± 23.734**	**93.095 ± 25.907**	0.616	**35.688 ± 7.382**	**106.752 ± 46.108**	0.113

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the youngest leaf and Leaf 8, de oldest leaf.

n. d. – not detected, for PCoA, was taken as 0.0001; n. p. –not present, the plant have 7 leaves completed extended

** Essential amino acid*

† Semi-essential amino acid

Table S12b Free amino acids in the phloem of the leaves of control and PepGMV-infected plants of chili not colonized (control) with nymphs of *T. vaporariorum*.

	Leaf 5			Leaf 6			Leaf 7			Leaf 8		
AA	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	11.176 ± 2.123	27.222 ± 0.844	**0.002**	15.570 ± 13.953	22.234 ± 5.109	0.503	4.921 ± 8.523	28.789 ± 3.283	**0.028**	11.655 ± 10.134	36.478 ± 4.851	**0.034**
Pro	17.184 ± 8.480	0.552 ± 0.362	0.077	35.416 ± 17.590	1.156 ± 0.160	0.078	42.271 ± 24.864	1.768 ± 0.725	0.106	15.290 ± 12.245	1.420 ± 0.343	0.189
Tyr	13.007 ± 4.745	6.169 ± 8.236	0.296	25.428 ± 14.241	22.204 ± 5.830	0.744	39.673 ± 24.559	24.192 ± 6.802	0.390	38.521 ± 2.322	24.603 ± 1.820	**0.002**
Cys†	n. d.	n. d.	-	n. d.	0.126 ± 0.113	0.193	n. d.	0.290 ± 0.121	*0.053*	n. d.	n. d.	-
Thr*	n. d.	0.213 ± 0.234	0.256	n. d.	0.559 ± 0.157	**0.025**	n. d.	0.990 ± 0.346	**0.038**	n. d.	0.807 ± 0.171	**0.015**
Leu	n. d.	0.134 ± 0.008	**0.001**	n. d.	0.291 ± 0.145	0.074	n. d.	0.598 ± 0.273	*0.063*	0.048 ± 0.018	0.293 ± 0.254	0.235
Ile*	n. d.	0.080 ± 0.013	**0.009**	n. d.	0.275 ± 0.141	0.078	n. d.	0.462 ± 0.105	**0.017**	0.027 ± 0.019	0.348 ± 0.302	0.206
Phe*	n. d.	0.101 ± 0.034	**0.035**	n. d.	0.284 ± 0.168	0.100	0.057 ± 0.098	0.992 ± 0.486	0.074	n. d.	1.806 ± 0.451	**0.020**
Arg	n. d.	n. d.	-	n. d.	0.257 ± 0.128	0.074	n. d.	0.396 ± 0.064	**0.009**	n. d.	0.341 ± 0.295	0.184
Ser	n. d.	0.155 ± 0.019	**0.005**	n. d.	0.378 ± 0.060	**0.008**	n. d.	0.473 ± 0.089	**0.012**	n. d.	0.631 ± 0.140	**0.016**
Val*	n. d.	11.921 ± 10.207	0.180	0.028 ± 0.018	0.461 ± 0.203	*0.065*	n. d.	0.655 ± 0.156	**0.018**	11.043 ± 19.052	7.283 ± 12.085	0.790
Lys*	n. d.	0.580 ± 0.856	0.361	n. d.	2.498 ± 1.987	0.161	0.076 ± 0.071	1.552 ± 0.967	0.118	0.116 ± 0.037	1.337 ± 0.611	0.074
Asx	n. d.	0.861 ± 0.044	**0.001**	0.432 ± 0.374	0.862 ± 0.167	0.174	0.645 ± 0.149	1.114 ± 0.224	**0.047**	1.152 ± 0.160	1.217 ± 0.038	0.557
Gly	n. d.	0.287 ± 0.015	**0.001**	n. d.	0.439 ± 0.084	**0.012**	n. d.	0.563 ± 0.059	**0.004**	n. d.	0.713 ± 0.085	**0.005**
His†	n. d.	0.144 ± 0.014	**0.003**	n. d.	0.237 ± 0.096	*0.051*	n. d.	0.327 ± 0.029	**0.003**	n. d.	0.421 ± 0.026	**0.001**
Ala	n. d.	0.412 ± 0.183	*0.060*	n. d.	0.338 ± 0.081	**0.019**	n. d.	0.520 ± 0.157	**0.029**	n. d.	0.501 ± 0.073	**0.007**
Glx	n. d.	0.617 ± 0.051	**0.002**	n. d.	0.859 ± 0.304	**0.039**	n. d.	1.050 ± 0.195	**0.011**	n. d.	1.242 ± 0.259	**0.014**
Total	**41.367 ± 15.079**	**49.447 ± 2.346**	0.452	**76.874 ± 45.582**	**53.458 ± 14.587**	0.473	**87.654 ± 43.228**	**64.730 ± 8.746**	0.457	**77.850 ± 18.435**	**79.442 ± 9.378**	0.902

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the youngest leaf and Leaf 8, de oldest leaf.
n. d. – not detected, for PCoA, was taken as 0.0001; n. p. –not present, the plant have 7 leaves completed extended
** Essential amino acid*
† Semi-essential amino acid

Table S13a Free amino acids in the phloem of the leaves of control and PepGMV-infected plants of chili colonized, with nymphs of *T. vaporariorum*.

	Leaf 1			Leaf 2			Leaf 3			Leaf 4		
AA	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plan** (nmol g^{-1} FW)**t**	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	n. d.	n. d.	-	n. d.	0.004 ± 0.008	0.423	n. d.	n. d.	-	n. d.	n. d.	-
Pro	0.442 ± 0.016	0.778 ± 0.210	0.108	0.343 ± 0.116	1.068 ± 0.143	**0.003**	0.462 ± 0.160	0.931 ± 0.212	**0.042**	0.951 ± 0.670	0.992 ± 0.617	0.942
Tyr	0.661 ± 0.475	1.546 ± 1.273	0.354	0.913 ± 0.425	3.199 ± 0.170	**0.005**	2.252 ± 0.704	2.773 ± 0.644	0.399	1.640 ± 1.189	1.875 ± 0.966	0.804
Cys†	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-
Thr*	0.026 ± 0.008	0.006 ± 0.000	**0.046**	0.0269 ± 0.016	0.095 ± 0.156	0.526	0.004 ± 0.000	0.004 ± 0.000	0.608	0.046 ± 0.036	0.085 ± 0.044	0.296
Leu	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-
Ile*	n. d.	n. d.	.	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-
Phe*	n. d.	0.080 ± 0.004	**0.001**	n. d.	0.038 ± 0.022	0.092	n. d.	n. d.	-	n. d.	n. d.	-
Arg	0.028 ± 0.008	n. d.	**0.025**	0.008 ± 0.014	0.004 ± 0.008	0.719	n. d.	n. d.	-	n. d.	0.088 ± 0.045	0.076
Ser	0.038 ± 0.016	0.043 ± 0.014	0.702	0.043 ± 0.043	0.542 ± 0.076	**0.002**	0.056 ± 0.052	0.298 ± 0.068	**0.009**	0.096 ± 0.070	0.068 ± 0.044	0.591
Val*	0.829 ± 0.724	1.811 ± 0.285	0.130	0.801 ± 0.311	2.277 ± 0.203	**0.004**	1.286 ± 0.176	1.450 ± 0.306	0.475	0.612 ± 0.626	0.861 ± 0.752	0.683
Lys*	0.168 ± 0.089	0.297 ± 0.235	0.448	0.181 ± 0.049	0.893 ± 0.445	0.107	0.165 ± 0.010	0.501 ± 0.153	**0.062**	0.287 ± 0.197	0.204 ± 0.098	0.566
Asx	0.197 ± 0.143	0.248 ± 0.127	0.668	0.065 ± 0.029	0.254 ± 0.025	**0.001**	0.165 ± 0.003	0.381 ± 0.067	**0.030**	0.251 ± 0.197	0.234 ± 0.052	0.895
Gly	0.397 ± 0.299	0.390 ± 0.140	0.974	0.182 ± 0.126	0.191 ± 0.021	0.911	0.269 ± 0.147	n. d.	0.087	0.351 ± 0.315	0.231 ± 0.167	0.598
His†	0.100 ± 0.066	0.102 ± 0.036	0.960	0.043 ± 0.017	0.112 ± 0.012	**0.006**	0.059 ± 0.014	n. d.	**0.019**	0.120 ± 0.106	0.166 ± 0.110	0.632
Ala	0.126 ± 0.098	0.059 ± 0.001	0.356	0.202 ± 0.154	0.072 ± 0.010	0.282	0.049 ± 0.015	0.177 ± 0.063	0.066	0.261 ± 0.308	0.335 ± 0.479	0.834
Glx	0.188 ± 0.125	0.240 ± 0.076	0.576	0.092 ± 0.040	0.233 ± 0.030	**0.010**	0.166 ± 0.109	0.106 ± 0.095	0.513	0.267 ± 0.142	0.176 ± 0.127	0.455
Total	**3.200 ± 0.809**	**5.599 ± 2.366**	0.215	**2.899 ± 1.119**	**8.998 ± 0.889**	**0.002**	**4.933 ± 0.480**	**6.621 ± 1.479**	0.178	**4.882 ± 2.736**	**5.316 ± 1.558**	0.826

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the youngest leaf and Leaf 8, de oldest leaf.

n. d. – not detected, for PCoA, was taken as 0.0001; n. p. –not present, the plant have 7 leaves completed extended

** Essential amino acid*

† Semi-essential amino acid

Table S13b Free amino acids in the phloem of the leaves of control and PepGMV-infected plants of chili colonized, with nymphs of *T. vaporariorum*.

	Leaf 5			Leaf 6			Leaf 7			Leaf 8		
AA	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**	**Control plant** (nmol g^{-1} FW)	**Infected plant** (nmol g^{-1} FW)	**P-value**
Met*	0.009 ± 0.009	n. d.	0.246	0.027 ± 0.019	n. d.	0.141	n. d.	n. d.	-	n. d.	n. d.	-
Pro	0.549 ± 0.117	0.758 ± 0.410	0.481	0.536 ± 0.311	0.586 ± 0.712	0.919	0.576 ± 0.175	0.431 ± 0.400	0.607	1.166 ± 1.082	0.610 ± 0.597	0.491
Tyr	2.044 ± 1.569	2.160 ± 0.361	0.911	1.267 ± 1.436	0.880 ± 0.991	0.722	3.052 ± 0.602	1.337 ± 1.429	0.162	3.180 ± 2.312	1.910 ± 2.111	0.521
Cys†	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-	n. d.	n. d.	-
Thr*	0.106 ± 0.060	0.004 ± 0.000	0.097	0.115 ± 0.152	n. d.	0.321	0.075 ± 0.011	n. d.	**0.007**	0.188 ± 0.136	n. d.	0.139
Leu	0.022 ± 0.031	n. d.	0.342	0.050 ± 0.069	n. d.	0.340	0.019 ± 0.021	n. d.	0.265	0.091 ± 0.079	n. d.	0.186
Ile*	0.026 ± 0.032	n. d.	0.286	0.055 ± 0.061	n. d.	0.258	0.030 ± 0.027	n. d.	0.194	0.113 ± 0.093	n. d.	0.171
Phe*	0.120 ± 0.159	n. d.	0.320	0.226 ± 0.079	n. d.	**0.038**	0.048 ± 0.043	n. d.	0.193	0.152 ± 0.090	n. d.	0.100
Arg	0.023 ± 0.022	0.005 ± 0.001	0.288	0.048 ± 0.050	n. d.	0.236	0.034 ± 0.003	n. d.	**0.004**	0.063 ± 0.026	n. d.	*0.053*
Ser	0.303 ± 0.129	0.044 ± 0.024	0.069	0.338 ± 0.319	0.037 ± 0.040	0.243	0.373 ± 0.107	0.155 ± 0.187	0.173	1.056 ± 0.857	0.220 ± 0.276	0.228
Val*	1.265 ± 0.623	1.594 ± 0.206	0.462	1.060 ± 0.850	0.803 ± 0.907	0.738	1.824 ± 0.340	1.394 ± 1.104	0.576	2.251 ± 1.283	1.972 ± 1.661	0.825
Lys*	0.292 ± 0.157	0.164 ± 0.028	0.292	0.259 ± 0.294	0.170 ± 0.086	0.659	0.295 ± 0.032	0.254 ± 0.150	0.689	0.403 ± 0.174	0.355 ± 0.231	0.786
Asx	0.191 ± 0.095	0.228 ± 0.061	0.601	0.183 ± 0.204	0.131 ± 0.177	0.757	0.197 ± 0.120	0.146 ± 0.084	0.581	0.306 ± 0.193	0.203 ± 0.129	0.492
Gly	0.072 ± 0.032	0.375 ± 0.145	*0.063*	0.058 ± 0.051	0.233 ± 0.328	0.455	0.079 ± 0.014	0.145 ± 0.050	0.139	0.142 ± 0.045	0.202 ± 0.080	0.339
His†	0.016 ± 0.014	0.109 ± 0.030	**0.020**	0.037 ± 0.038	0.067 ± 0.080	0.601	0.042 ± 0.013	0.010 ± 0.001	*0.052*	0.075 ± 0.049	0.014 ± 0.001	0.164
Ala	0.056 ± 0.027	0.110 ± 0.086	0.393	0.052 ± 0.042	0.098 ± 0.122	0.590	0.068 ± 0.005	0.059 ± 0.025	0.570	0.127 ± 0.075	0.081 ± 0.039	0.422
Glx	0.172 ± 0.148	0.236 ± 0.063	0.548	0.242 ± 0.219	0.139 ± 0.149	0.542	0.278 ± 0.100	0.181 ± 0.094	0.290	0.397 ± 0.236	0.251 ± 0.143	0.420
Total	**5.266 ± 2.794**	**5.786 ± 0.541**	0.779	**4.554 ± 0.4037**	**3.145 ± 3.540**	0.673	**6.991 ± 0.960**	**4.112 ± 3.472**	0.285	**9.710 ± 5.988**	**5.817 ± 5.208**	0.444

Asx = Asp, Asn; Glx = Glu, Gln. Each value represents the mean ± SD, n = 3 independent samples (each pooled from 6 plants), t-student test. The p-values with statistical significance are in bold. Leaf 1 is the youngest leaf and Leaf 8, de oldest leaf.

n. d. – not detected, for PCoA, was taken as 0.0001; n. p. –not present, the plant have 7 leaves completed extended

** Essential amino acid*

† Semi-essential amino acid

Table S14. ANOVA of virus load in leaf tissue of control and infected plants colonized and not infested by *T. vaporariorum*

	Df	Sum Sq	Mean Sq	F value	P value
Time	2	52233696	26116848	11.7	**0.0003**
Virus	1	59622777	59622777	26.6	**0.0000**
Whitefly	1	1130296	1130296	0.5	0.4840
Time:Virus	2	51129965	25564983	11.4	**0.0003**
Time:Whitefly	2	3676042	1838021	0.8	0.4516
Virus:Whitefly	1	33207	33207	0.015	0.9041
Virus:Time:Whitefly	1	3669401	3669401	1.638	0.2123
Residuals	25	55989531	2239581		

The p-values with statistical significance are in bold

Annexes 2

Published articles related to this work:

Ángeles-López, YI, Rivera-Bustamante, R, Heil, M. **Submitted**. Fatal Attraction of Non-Vector Impairs Fitness of Manipulating Plant Virus.

Ángeles-López, YI, Rivera-Bustamante, R, Heil, M. **In press**. Colonization by Phloem-Feeding Herbivore Overrides Effects of Plant Virus on Amino Acid Composition in Phloem of Chili Plants. *Journal of Chemical Ecology*. DOI: 10.1007/s10886-016-0747-2.

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RAPID COMMUNICATION

Colonization by Phloem-Feeding Herbivore Overrides Effects of Plant Virus on Amino Acid Composition in Phloem of Chili Plants

Yesenia Ithaí Ángeles-López[1] · **Rafael F. Rivera-Bustamante**[1] · **Martin Heil**[1]

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Abstract The 'adaptive host manipulation' hypothesis predicts that parasites can enhance their transmission rates via manipulation of their host's phenotype. For example, many plant pathogens alter the nutritional quality of their host for herbivores that serve as their vectors. However, herbivores, including non-vectors, might cause additional alterations in the plant phenotype. Here, we studied changes in the amino acid (AA) content in the phloem of chilli (*Capsicum annuum*) plants infected with *Pepper golden mosaic virus* (PepGMV) upon subsequent colonization with a non-vector, the phloem-feeding whitefly (*Trialeurodes vaporariorum*). Virus infection alone caused an almost 30-fold increase in overall phloem AAs, but colonization by *T. vaporariorum* completely reversed this effect. At the level of individual AAs, contents of proline, tyrosine, and valine increased, and histidine and alanine decreased in PepGMV -infected as compared to control plants, whereas colonization by *T. vaporariorum* caused decreased contents of proline, tyrosine, and valine, and increased contents of histidine and alanine. Overall, the colonization by the whitefly had much stronger effects on phloem AA composition than virus infection. We conclude that the phloem composition of a virus-infected host plant can rapidly change upon arrival of an herbivore and that these changes need to be monitored to predict the nutritional quality of the plant in the long run.

Keywords Adaptive host manipulation hypothesis · Amino acids · Herbivory · Begomovirus · Whitefly

Introduction

Host manipulation – defined as the expression of a host phenotype that is under the genetic control of a parasite and serves to enhance parasite transmission rates – is responsible for the 'fatal attraction' of *Toxoplasma*-infected prey to their predators, or of vector insects to the mammalian or plant host of the parasites that they transmit (Weinersmith and Faulkes 2014). For example, plant viruses frequently are reported to alter the nutritional quality of their host, thus attracting their vectors (Mauck et al. 2012). However, many pathogens require extended feeding by the herbivores on their host plant (Mauck et al. 2012), and the presence and feeding activity of herbivores might cause further alterations to the quality of the plant. This is particularly true for phloem-feeders such as whiteflies, which must establish particularly intimate associations with their host plant, where they cause multiple alterations to its defensive status (Ángeles-López et al. 2012; Luan et al. 2013; Yang et al. 2011). Vice-versa, the infection of tobacco (*Nicotiana tabacum*) plants with the begomovirus, Tomato yellow leaf curl China virus, enhanced the nutrient assimilation by whiteflies (*Bemisia tabaci*) on these plants (Wang et al. 2012).

We argue that a more complete understanding of the effects of tritrophic interactions among plants, pathogens, and herbivores on each of the involved species requires that studies go beyond the phenotypic characterization of an infected plant as it is faced by the arriving herbivore, and that such studies also should consider the effects of further species, such as non-vectors. As a first step towards this goal, we monitored the amino acid (AA) content in the phloem of chilli (*Capsicum*

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✉ Martin Heil
mheil@ira.cinvestav.mx

[1] Departamento de Ingeniería Genética, CINVESTAV-Irapuato, Km. 9.6 Libramiento Norte, 36821, Irapuato, Guanajuato, Mexico

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annuum) plants infected with *Pepper golden mosaic virus* (PepGMV) before and after the colonization of these plants by phloem-feeding whiteflies, *Trialeurodes vaporariorum*, and compared these values to plants that were colonized only by *T. vaporariorum*. This species of whitefly does not vector PepGMV [(Jones 2003) and ICTVdB Management (2009) 00.029.0.03.033. Pepper golden mosaic virus. URL: http://ictvdb.bio-mirror.cn/ICTVdB/00.029.0.03.033.htm, access date 9.7.2015]. Phloem composition in virus-infected plants changed dramatically upon the arrival of the whitefly, and was much more affected by *T. vaporariorum* than by PepGMV. We conclude that monitoring the established tritrophic system, i.e., the plant that is infected with the pathogen and fed upon by a herbivore, is required to obtain a more complete understanding of the nutritional quality of pathogen-infected plants for herbivores.

Methods and Materials

Plants and Insects Seeds of *Capsicum annuum* L. var. Sonora Anaheim (Seminis®) were planted in pots of 350 cm^3 with sterile soil mixture [3 parts Sunshine Mix 3™ (SunGro Horticulture, Bellevue, WA, USA), 1 part loam, 2 parts mulch, 1 part vermiculite (SunGro Horticulture) and 1 part perlite (Termolita S.A., Nuevo León, México)] and cultivated in greenhouses under a natural light regime as described previously (Ángeles-López et al. 2012). The colony of *Trialeurodes vaporariorum* was maintained on tomato plants (*Solanum lycopersicum*, cv. Río Fuego, obtained from Cal-Oro, Vegetable Seeds, United Genetics, Inc., Gilroy, USA) in a growth room (3.2 m [width] × 4.2 m [length] × 3 m [height]), under controlled conditions of light (≈300 μmol^{-1} m^2 s^{-1}) and photoperiod of 16 h L/8 h D, at 28 °C.

Virus Inoculation The two plasmids that contain the genome (components A and B) of PepGMV (Tamaulipas isolate, Geminiviridae) had been prepared as described in (Garzón-Tiznado et al. 1993) and were inoculated into chili plants with four extended leaves by using a low-pressure ballistic device, as described earlier (Carrillo-Tripp et al. 2007). Control plants were mock-inoculated with the carrier mix with no PepGMV plasmids.

Effect of *T. vaporariorum* on PepGMV-Infected Plants Chili plants show the most severe symptoms at 20 d post infection (20 dpi) with PepGMV (Carrillo-Tripp et al. 2007). Each six mock-inoculated or PepGMV-infected plants at 20 dpi were placed in mesh cages (2 × 1 × 1 m) in the greenhouse. Into each cage, 500 whiteflies were introduced and allowed to oviposit for 7 d. Then, the adults were removed and the eggs were allowed to develop until the first stage of the nymph. Because infection levels and disease severity in PepGMV-infected chili plants depend on leaf age (authors' personal observations, see also Carillo-Tripp et al. 2007), samples of phloem were collected from six plants per treatment separately from each of the eight youngest leaves per plant, at 20 dpi, at the egg stage (27 dpi) and at the first nymphal stage (32 dpi), as described previously (Deeken et al. 2008). Then, 300 μl of the phloem exudate were mixed with 500 μl of acetonitrile (HPLC degree; Baker, México), maintained over 12 h at 4 °C, purified, injected in a HPLC (Agilent Technologies 1200 Series, Waldbronn, Germany) equipped with a Pico-Tag® column (3.9 × 150 mm [C18], Particle 3 μm; Waters, Tauton, MA, USA) and analyzed at 254 nm. The AA standards (Sigma-Aldrich) for identification and quantification were run under the same conditions. The standard represented as Asx was use for the quantification of aspartic acid and asparagine; the standard Glx was used to quantify glutamic acid and glutamine. The entire experiment was repeated three times.

Heatmap Construction The data on AA contents were transformed to relative units (controls: samples from mock-inoculated, whitefly-free plants taken on the same day as the samples from the respective experimental plants). The relative changes were expressed on a logarithmic scale, and are visualized in color code (dark blue to intense red), using 'heatmap3' (Zhao et al. 2014).

Results

Virus-infection caused an almost 30-fold increase in the overall AA content in the phloem of *C. annuum* plants, but this pattern was completely reversed upon arrival of the whitefly, *T. vaporariorum* (Fig. 1a, Supplementary Tables S1–5). Amino acids such as arginine, cysteine, proline, serine, tyrosine, and valine exhibited up to 40 times higher contents in the phloem of PepGMV-infected as compared to mock-inoculated plants at 20 dpi, whereas PepGMV-infected plants contained less alanine, aspartic acid/asparagine, glutamic acid/glutamine, and histidine (Table S1). By contrast, all the last mentioned AAs (alanine, aspartic acid/asparagine, glutamic acid/glutamine, glycine, and histidine) increased their content in PepGMV-infected plants upon colonization by *T. vaporariorum*. This effect was seen already at 27 dpi (egg stage, adults removed, see Tables S2,3) and maintained stable until 32 dpi (1st-instar nymphs, Tables S4,5). By contrast, the contents of isoleucine, leucine, phenylalanine, proline, tyrosine, and valine dropped up to 30 times below control levels when plants had been colonized by ovipositing whiteflies (Fig. 1a). This latter effect was relieved after removal of the adults, since plants carrying only 1st-instar nymphs (32 dpi) did not exhibit any strong reduction in the contents of isoleucine, leucine, phenylalanine, or valine. Nevertheless,

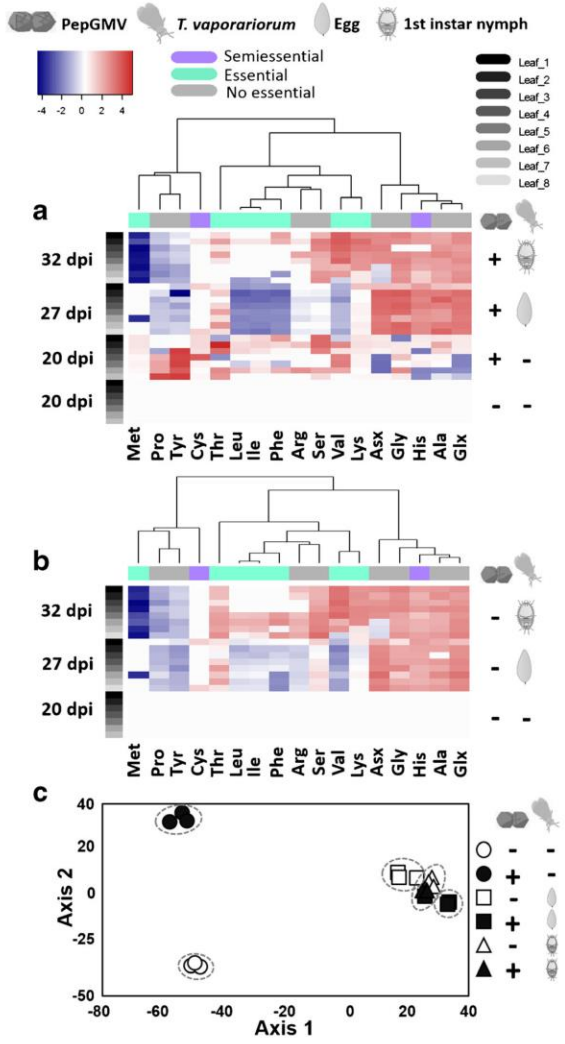

Fig. 1 Amino acid profiles in phloem of control and virus (PepGMV)-infected chili plants after the colonization by the whitefly, *Trialeurodes vaporariorum*. Values in the heatmaps (Panels **a, b**) are expressed in comparison to controls (mock-inoculated and free of whiteflies) on a logarithmic scale, and are color-coded (dark blue to intense red), separately for each leaf position. Panel **a** depicts amino acid contents in PepGMV-infected plants before and after colonization by *T. vaporariorum*. Panel **b** depicts amino acid contents in mock-inoculated plants before and after colonization by *T. vaporariorum*. The dendrograms above the heatmaps represent the similarity among the different profiles expressed as Euclidian distances and were calculated using **UPGMA** (**U**nweighted **P**air **G**roup **M**ethod with **A**rithmetic Mean). Panel **c** represents the result of a PCoA, which was used as an alternative method to quantify the similarities among the AA profiles. Leaf 1 represents the youngest, leaf 8 the oldest leaf. Sample size $N = 3$ independent samples, each representing a pool of six plants. Each relative value as represented in the heat map is based on the mean of the three replicates

the presence of nymphs had particularly strong effects on methionine, proline, and tryosine: three amino acids that exhibited enhanced contents in response to PepGMV alone, but strongly (10–45-fold) decreased contents at 32 dpi, that is, when the PepGMV-infected plant carried 1st-instar nymphs.

The overall patterns in phloem AA contents were very similar in mock-inoculated plants that were colonized by *T. vaporariorum* (Fig. 1b). For example, plants at 27 dpi (carrying eggs, adults removed) exhibited reduced contents of isoleucine, leucine, phenylalanine, proline, tyrosine, and valine as compared to controls, whereas contents of alanine, aspartic acid/asparagine, glutamic acid/glutamine, glycine, and histidine all were enhanced as compared to mock-inoculated and whitefly-free plants. Even more strikingly, the contents of valine (lower than controls during the egg stage) increased over control levels in plants carrying 1st-instar nymphs, and methionine, proline, and tryosine again exhibited strong decreases when plants shifted from carrying eggs to carrying 1st-instar nymphs (Fig. 1b, Tables S2–5).

A principal coordinate analysis (PCoA) confirmed that the AA contents of virus-infected and mock-inoculated, whitefly-free plants were clearly different from each other (Fig. 1c). However, the differences were much smaller when the plants were colonized by the whiteflies, both in the stage of eggs as well as when the plants carried 1st-instar nymphs. Virus-infected and control plants still exhibited some differences, but overall, the AA profiles of plants that were colonized by whiteflies grouped together in the PCoA, independently of their status of infection with PepGMV (Fig. 1c).

Discussion

Many herbivores choose virus-infected plants as hosts because such plants are characterized by suppressed anti-herbivore defenses and enhanced nutritional quality, or might simply allow for a more efficient nutrient uptake by the insect (Ángeles-López et al. 2012; Mauck et al. 2012; Wang et al. 2012; Yang et al. 2011). However, the plant phenotype that the insect originally encounters might differ strongly from the phenotype that the plants expresses when it is carrying both the virus and the herbivore. Our study revealed strong alterations in the phloem AA composition of virus (PepGMV)-infected chili plants once whiteflies started to colonize these plants. Infection with PepGMV alone led to an overall increase in the AA content of the phloem, which is in line with earlier studies on several plant-pathogen interactions (Mauck et al. 2012), although other studies demonstrate that virus infection does not necessarily enhance phloem AAs (Wang et al. 2012).

Interestingly, valine generally is considered as essential and was one of the AAs that showed the strongest quantitative increases in PepGMV-infected plants, but valine content

dropped dramatically once *T. vaporariorum* was colonizing these plants. Similarly, isoleucine, leucine, and phenylalanine are considered essential, and they were strongly reduced at 27 dpi, that is, seven days after whiteflies started to feed and oviposit on these plants. In general, the effect of the whitefly, *T. vaporariorum*, turned out to be much stronger than any virus-induced alteration, as evidenced by the observation that overall patterns in phloem AA composition were very similar between mock-inoculated and PepGMV-infected plants once these plants became colonized by the whiteflies (Fig. 1). It is tempting to speculate that the whiteflies quickly deplete the phloem of those AAs that are most important for their nutrition and thereby completely override the PepGMV-induced effects.

The observed changes in the nutritional quality of virus-infected plants lacked robustness to the colonization by whiteflies. This observation raises doubts concerning the validity of a generalized interpretation of such changes as the outcome of a manipulation effect. Alternative explanations for an increase in the phloem AA content of an infected plant include: (i) a mobilization of protein-bound amino acids to rescue them from the infected tissue; (ii) the allocation of amino acids from other parts of the plant to the infected tissue to enable the local synthesis of pathogenesis-related proteins and other N-containing compounds; (iii) a sink in the infected leaves that is created by an increased need for amino acids, owing to the synthesis of viral proteins; or (iv) a manipulation that the pathogen has evolved to attract its vector. The first two mechanisms would be under the control of the plant; the other two mechanisms would be under the control of the virus; however, only the last mechanism would represent a classical host manipulation that has been selected to enhance transmission of the pathogen.

Further studies will be required to find the mechanistic explanation of the effects of the virus, PepGMV, the whitefly, *T. vaporariorum*, or both, on the AA composition in the phloem of chili plants. Most importantly, our results demonstrate that colonization by a phloem-feeding insect can override the effects of virus infection on the phenotype of a host plant. We conclude that such changes need to be considered if we aim to understand the long-term effects of virus infection on the nutritional quality of plants for herbivores.

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