Virus-infected melon plants emit volatiles that induce gene deregulation in neighboring healthy plants

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ABSTRACT

López-Berenguer, C., Donaire, L., González-Ibeas, D., Gómez-Aix, C., Truniger, V., Pechar, G.S. and Aranda, M.A. Virus-infected melon plants emit volatiles that induce gene deregulation in neighboring healthy plants. *Phytopathology*

It is well described that viral infections stimulate the emission of plant volatiles able to recruit viral vectors thereby promoting virus spread. In contrast, much less is known on the effects that emitted volatiles may have on the metabolism of healthy neighboring plants, which are potential targets for new infections through vector transmission. Watermelon mosaic virus (WMV) (genus *Potyvirus*, family *Potyviridae*) is an aphid-transmitted virus endemic in cucurbit crops worldwide. We have compared gene expression profiles of WMV infected melon plants with those of healthy or healthy-but-cohabited-with-infected plants. Pathogenesis-related (PR) and small heat shock protein encoding genes were deregulated in cohabited plants, and *PR* deregulation depended on the distance to the infected plant. The signaling was short distance in the experimental conditions used, and cohabiting had a moderate effect on the plant susceptibility to WMV. Static headspace experiments showed that benzaldehyde and γ -butyrolactone were significantly over-emitted by WMV-infected plants. Altogether, our data suggests that perception of a volatile signal encoded by WMV-infected tissues triggers a response to prepare healthy tissues or/and healthy neighboring plants for the incoming infections.

INTRODUCTION

As sessile organisms, one of the strategies that plants use to communicate and react to environmental cues is the production and sensing of volatile organic compounds (VOCs) (Dudareva et al. 2013; Pickett and Khan 2016). Changes of plant's volatile profiles have been reported after both abiotic and biotic stresses, and the role of VOCs in defenses activation is widely acknowledged (Dong et al. 2016; Ninkovic et al. 2019). Their function as chemical airborne signals extends to the plant itself, affecting neighboring plants and other interacting organisms at different trophic levels (Bouwmeester et al. 2019; Guerrieri 2016; Simpraga et al. 2016). Plant VOCs include a wide range of lipophilic molecules with a high vapor pressure and low molecular weight, biosynthesized from the secondary metabolism of the plant (Pichersky et al. 2006). Based

on their biosynthetic origin, plant VOCs could be classified into four major groups: terpenoids, phenylpropanoids/benzenoids, fatty acid derivatives and amino acid derivatives (Dudareva et al. 2013; Dudareva et al. 2006). Methyl salicylate (MeSa) and methyl jasmonate (MeJa) are volatile defense hormones that have been often described priming defenses in plants perceiving them, but other VOCs seem to have also this role, particularly those belonging to the green leaf volatile group (Hammerbacher et al. 2019).

Compared to other stressors, virus infection has received moderate attention regarding its ability to modify VOCs emission profiles and the effects that these alterations may have. Since plant virus spread depends predominantly on insect vectors, most studies focused on the effects that infection has on emitted VOCs and how these impact on vector behavior. In general terms, when viral infection occurs, host plants change their volatile profile inducing alterations in the interaction with vectors to promote virus spread. These alterations seem to be directly related to the nature of the viral transmission (Carr et al. 2019; Eigenbrode et al. 2018). Vector probing is optimal for transmission of non-persistently transmitted viruses, but persistent viruses need long feeding periods for vector uptake. Thus, infection by the non-persistently transmitted cucumber mosaic virus (CMV) induces a volatile signal to attract aphids quickly but also stimulates the synthesis of antifeedants that deter aphids after feeding (de Vos and Jander 2010; Groen et al. 2016; Mauck et al. 2012; Mauck et al. 2010). In contrast, infection by the persistently-transmitted potato leaf roll virus (PLRV) induces a volatile blend that not only attracts its aphid vector to infected leaves, but also acts as an aphid arrestant, thus promoting prolonged aphid feeding periods that favor PLRV transmission (Eigenbrode et al. 2002). Similar effects have been described for viruses transmitted by thrips (Mwando et al. 2018) and persistent-circulative geminiviruses transmitted by whiteflies (Feres et al. 2016), although exceptions to the 'attract and deter' rule for non-persistently transmitted viruses have also been reported (reviewed in Carr et al. 2019). In contrast, very little is known about the effects that emitted volatiles may have on the metabolism of healthy neighboring plants, which are potential targets for new infections through vector transmission (Yao et al. 2012). In the case of the mechanically transmitted tobacco mosaic virus (TMV), an early and groundbreaking work showed that tobacco plants infected with TMV emitted MeSa, and when healthy plants were exposed to volatiles emitted by TMV-infected plants, the expression of the Pathogen-Related 1 (PR1) gene was induced and plants showed increased resistance to TMV (Shulaev et al. 1997).

Watermelon mosaic virus (WMV) is a positive sense, single stranded RNA virus that belongs to the potyvirus group (Desbiez and Lecoq 2004; Purcifull et al. 1984). WMV is non-persistently transmitted by several aphid species and has a broad host range that includes 23 families of dicotyledonous species, including cucurbits and legumes (Castle et al. 1992; Gray et al. 1986; Hajizadeh et al. 2017; Lecoq and Pitrat 1985). It is widespread and causes high economic losses in cucurbit crops, including melon, watermelon, pumpkin and zucchini (Desbiez and Lecoq 2008; Grafton-Cardwell et al. 1996; Luis-Arteaga et al. 1998; Moreno et al. 2004). While performing transcriptomic analyses of susceptible and resistant WMV-infected plants (González-Ibeas et al. 2012), we obtained preliminary data on the potential modification of healthy plant's transcriptomes after cohabiting with WMV-infected plants. Here we show results that confirm these preliminary observations, and data from experiments designed to understand the nature and effects of the aerial signal responsible for these changes.

MATERIALS AND METHODS

Plant and virus material. Plant growing conditions. Melon (*Cucumis melo* L., cv Tendral verde tardío) seeds (Fitó seeds, Barcelona, Spain) were germinated in Petri dishes on two layers of humidified laboratory paper discs at 25 °C for 72 h. Then they were planted in 1.2 liter pots with a mixture of peat and coconut fiber (2:1) substrate (Projar, Valencia, Spain) and covered with a thin layer of vermiculite. They were fertilized once a week with a universal fertilizer (Compo, Münster, Germany). Depending on the design of each experiment, plants grew in climatic chambers or in a glasshouse. For the experiments of cohabitation (Fig. 1A), plants were grown in two identical cabinet-type growth chambers (MLR-35H, Sanyo, Osaka, Japan) under the same conditions (16h:8h, 25 °C:18 °C light: dark photoperiod) for 4 weeks after germination. We accommodated 6 plants per chamber shelf. One week after germination, for only one of the growth chambers, three plants were extracted from each shelf, inoculated with WMV as described below, and relocated into the chamber but to a random position on its shelf. Three weeks after, the second true leaf of three plants per shelf and treatment was harvested and used for microarray analysis; samples from each shelf and treatment were pooled together. For the experiments in methacrylate containers, these were kept in a walk-in growth chamber where conditions were 16:8 h, 25:18 °C light:dark photoperiod with relative humidity values of 65-60 %. Illumination was with fluorescent tubes (Philips

Master TL-DW 840) providing $170\text{--}208\ \mu\text{mol m}^{-2}\text{ s}^{-1}$. The dimension of the methacrylate containers was $36 \times 36 \times 52\text{ cm}$; methacrylate panels of 10 mm width were used to build them. The number of plants and their stages varied among experiments and are specified in the corresponding Results sections. Experiments to study signaling distance were carried out in a glasshouse at the CEBAS-CSIC experimental station 'La Matanza' (Lat. 38.105885° ; Long. 1.034673°). These experiments were performed during March 2017, and meteorological conditions were $15.1\text{ }^\circ\text{C}$ average temperature with $32.9\text{ }^\circ\text{C}$ maximum and $6.1\text{ }^\circ\text{C}$ minimum; maximum and minimum relative humidity were 99 % and 9 %, respectively. The size of the glasshouse room was $3 \times 4\text{ m}$, with a minimum high of 2.5 m and a maximum of 3.7 m. Plants were placed right in the center of the room, on the floor. The plants used for this experiment were grown for 14 days after germination in walk-in growth chambers with the conditions described above, and after that time plants were moved to the glasshouse for the cohabitation experiment. When plants were 21 days old, one WMV-infected plant (see the Results section) of the same age was introduced to the chamber and kept in the middle of the healthy plants (see Results section for a diagram of the experiment) for 1 h; plants were sampled immediately, and samples were frozen in liquid N_2 as quick and efficiently as possible.

The viral isolate used was WMV-M116 (Diaz-Pendon et al. 2005). Mechanical inoculation was carried out according to standard protocols. Zucchini leaves (*Cucurbita pepo* cv. Negro Belleza; Fitó) infected with WMV-M116 and frozen at $-80\text{ }^\circ\text{C}$ were used as inoculum source. The leaves were ground in a sterile mortar together with phosphate buffer 30 mM pH 8.0 (1 g in 5 ml). Carborundum was sprinkled on the expanded cotyledons of 7 days melon plants after germination and the inoculum was applied by rubbing the cotyledons.

WMV detection by ELISA. To check the presence/absence of WMV, samples were analyzed by a double antibody sandwich enzyme-linked immunosorbent assay (ELISA). The protocol followed was the one described by the manufacturer of the WMV ELISA kit (DSMZ, Braunschweig, Germany). The samples were ground with 400 μl extraction buffer (1:20 w:v). Rabbit IgG was used as specific primary antibody, and rabbit conjugated IgG-AP was used as secondary antibody. For the enzymatic reaction, 4-nitrophenyl phosphate was used as substrate. Absorbance measurements were spectrophotometrically determined (Tecan Sunrise, Männedorf, Switzerland) at a wavelength of 405 nm.

Measurement of transcripts accumulation by RT-qPCR. RNA extraction was performed using Tri-Reagent (Sigma-Aldrich, St. Louis, MO, USA) following the instructions of the manufacturer. To reduce variability, each biological replicate corresponded to a mixture of 2-3 individual plants from the same treatment. To eliminate traces of genomic DNA, the total RNA obtained was treated with Turbo DNA-free kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's indications. RNA was quantified using a Nanodrop One (Thermo Fisher Scientific, Waltham, MA, USA). Each sample was normalized to a concentration of 250 ng μl^{-1} . cDNA synthesis was performed with Expand Reverse Transcriptase (Roche, Mannheim, Germany) following the manufacturer's instructions. Real time qPCR was carried out in a 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) with a SYBR Green PCR Master Mix reaction (Applied Biosystems, Foster City, CA, USA). The following PCR conditions were used for all reactions: 95 °C, 10 min (initial denaturation) and 40 two-step cycles: 95 °C, 15 s and 60 °C, 1 min. Finally, the dissociation curve was: 95 °C, 15 s and 60 °C, 1 min. Cyclophilin was used as an endogenous control. The sequences of all the primers used have been previously described (Gonzalez-Ibeas et al. 2011). Each biological sample had three technical replicates. The relative expression of the different genes was calculated according to the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001; Schmittgen and Livak 2008).

Collection and analysis of volatile organic compounds. For static headspace analysis (Tholl et al. 2006), the plants were enclosed in a methacrylate showcase sealed with inert material with a maximum capacity of 8-10 melon plants of 21 days after germination (see Plant Growing section). Plants remained enclosed in the container for 1 h, during this time the conditions of temperature and concentration of CO₂ and O₂ remained constant (data not shown). VOCs sampling was carried out using solid phase microextraction (SPME) fibers that allow detection limits of the order of parts per million. Following the manufacturer's recommendations in our experimental conditions, we used SPME fibers coated with 100 μm polydimethylsiloxane as adsorbent (SPME portable field sampler, Supelco, Bellefonte, PA, USA). After 1 h of incubation, the VOCs adsorbed by the fibers were transferred to a gas chromatograph coupled to a mass spectrometer (GC-MS Agilent 7890A GC, Agilent Technologies, Santa Clara, CA, USA) with an ionization detector (Agilent 5975C series inert MSD with triple axis detector, Agilent Technologies, Santa

Clara, CA, USA). The compounds were separated by a capillary column of 30 m $\times$ 0.25 mm. The carrier gas was Helium with a flow of 1 ml min⁻¹. After each manual injection, the SPME fiber remained for 5 min in the injector in splitless mode to ensure complete desorption of all analytes at a temperature of 270 °C. The column oven temperature was 40 °C for 2 min and then increased from 40 °C to 270 °C for 20 min (15 °C min⁻¹). The mass spectrometer (MS) worked in electronic impact ionization mode with an ionization energy of 70 eV. The acquisition was carried out in full-scan mode which consisted in sweeping between two masses (30 to 350 m z⁻¹) with a ratio of 5 scan s⁻¹. The data obtained was processed with the MSD ChemStation program (Agilent Technologies, Santa Clara, CA, USA). The compounds were tentatively identified with the NIST (National Institute of Standards and Technology) mass spectra database considering similarity values above 70 % and then by comparison with the fragmentation patterns and retention times of the pure standard compounds.

RESULTS

Gene expression deregulation after cohabitation of healthy melon with WMV-infected plants. To assess if gene deregulation takes place in healthy melon plants that cohabited with WMV-infected plants, an experiment was carried out using two identical vertical climatic chambers (Fig. 1A): In one chamber, WMV-infected plants were distributed on each of its three shelves, whereas no infected plants were introduced in the other chamber. The number of plants in both chambers was the same. Fifteen days after inoculation, Mock-inoculated plants from both chambers as well as WMV-infected plants were sampled separately for each shelf, their RNAs were extracted and used to measure the accumulation of four transcripts encoding proteins related to the stress response: A pathogenesis-related protein PR1-like (PR1), a glucan endo-1,3-beta-glucosidase (PR2), a PR4-like protein with a chitin recognition domain (PR4) and a 17.5 kDa class I heat shock protein (HSP17.5). The average transcript accumulation differed significantly among samples from the same treatment but placed in different chamber shelves. For instance, PR transcripts tended to over-accumulate in healthy/cohabited plants from the bottom shelf whereas their expression remained unchanged with respect to non-cohabited plants for plants in the top shelves (Fig. 1B). In general terms, cohabited plants in the bottom shelf were responding similarly than WMV-infected plants, with up-regulation of the PR genes and down-regulation of HSP17.5. The amplitude of the deregulation was

much larger for the PR genes (Fig. 1B); thus, we chose to use their transcript accumulation as marker for further experiments.

Gene expression deregulation depends on the distance to the WMV-infected plant.

A possible explanation of the above results could involve VOCs emission into the atmosphere by the WMV-infected melon plants; when nearby non-infected plants perceive these VOCs, a defensive response equivalent to the priming effect described in other experimental systems (Baldwin et al. 2006; Simpraga et al. 2016) may be triggered. If this were the case, one would expect that the response of the cohabiting plants would depend on their distance to the VOCs emitting plants (Giron-Calva et al. 2012; Heil and Adame-Alvarez 2010). To analyze this aspect, a new experiment was designed in which plants were placed on a horizontal surface at increasing distances from a WMV-infected plant within a closed glasshouse compartment (Fig. 2A). After 1 h of cohabiting, the plants were sampled and the accumulation of PR1, PR2 and PR4 transcripts was estimated. For PR1 and PR4, plants separated more than 40 and 50 cm from the infected plant, respectively, did not show significant changes in the accumulation of the transcripts considered (Fig. 2B), while plants closer to the infected one showed upregulation inversely proportional to the distance (Fig. 2B, C). For PR2, the plant nearest to the infected one showed a moderate increase in expression, which was dampened for plants at 40 and 50 cm (Fig. 2B, C). These results support the above hypothesis; the expression of the selected marker genes seemed to depend on the distance to the infected plant and, therefore, on the dose of the supposed volatile signal. In addition, the signaling seems to be short distance, since the effect was not noticeable beyond 50 cm under the conditions used.

Gene expression deregulation associates with moderate effects on plant susceptibility to WMV. Changes in PR gene expression were also noticed when Mock-inoculated plants cohabited with WMV-infected plants in confined methacrylate containers. In this experiment, we placed four plants inside a methacrylate container and in the middle of these an infected or a Mock-inoculated plant. Cohabited and non-cohabited plants were sampled and the samples from each treatment pooled together; thus, a replicate consisted of the pooled samples from a single container, and there were three replicates per treatment. As in previous experiments, cohabitation for 1 h with a WMV-infected plant induced the up-regulation of PR1, PR2 and PR4 expression (Fig. 3).

We next used this experimental setup to study if these changes associate with any effect on the plant's susceptibility to the virus. After 1 h of cohabitation with WMV-infected plants in methacrylate containers as before, healthy plants were mechanically inoculated with WMV and grown in the glasshouse until symptom display. In order to identify subtle changes in the plant's susceptibility, we used concentrated inoculum, where infection efficiencies were close to 90 % in untreated controls, but also a 1:5 dilution of the concentrated inoculum. Cohabited plants inoculated with concentrated inoculum showed infection rates similar to control plants, whereas cohabited plants inoculated with diluted inoculum showed a rate of infection (53 %) lower than non-cohabited controls (91 %) (Table 1), suggesting that, indeed, cohabiting alters the plant susceptibility to WMV.

Static headspace analysis of volatile emission by WMV-infected plants. We then carried out a static headspace experiment to identify VOCs differentially emitted by infected plants. To maximize the possibility of identifying differentially emitted VOCs, eight WMV-infected plants and 8 healthy plants were confined in independent containers (Fig. 4A). For 1 h they were incubated with SPME fibers. After that time, volatiles adsorbed on the fiber were analyzed by GC-MS. In total, 122 compounds with a defined mass standard were identified in the NIST database (www.nist.gov/srd/nist-standard-reference-database-1a-v17). Seventy-eight and 52 emitted volatile compounds were detected for infected and healthy plants, respectively, with 48 compounds in common for both treatments (Supplementary Table 1). Only benzaldehyde and γ -butyrolactone showed significant differences when comparing infected vs healthy plants headspaces (Fig. 4B). Benzaldehyde has the highest volatility among the identified compounds, with a vapor pressure value of 133 Pa and a boiling point of 178.8 °C, compared to γ -butyrolactone, with vapor a pressure value of 60 Pa and a boiling point of 204 °C (Gindulyte et al. 2018). Both have a relative vapor density greater than that of the air (3.7 benzaldehyde and 3 γ -butyrolactone), which makes them heavier than the air and, therefore, with a tendency to be in the lower areas of the environment. The fact that only 2 volatile compounds have significant differences between the 2 treatments suggested a non-complex volatile signal.

DISCUSSION

In this work we have described that healthy melon plants experience gene expression alterations after cohabiting with WMV-infected plants. This was observed in a range of experimental settings, including cabinet-like growth chambers, small methacrylate containers and medium size glasshouse rooms, for cohabiting periods that ranged from 1 h to weeks, although the amplitude of these alterations seemed to vary largely with conditions. This was evident from the very first experiment done and described here; gene deregulation in plants positioned on the bottom shelf of a cabinet-like growth chamber was deeper than those of plants in medium and top shelves, as evidenced by RT-qPCR data. Also, gene deregulation in plants increasingly distant from a WMV-infected plant in a glasshouse room decreased with distance. Thus, our data strongly suggested that there is a dose effect on whatever the signal is that healthy plants perceive. Also, the experiment in the vertical cabinet-like growth chamber suggested that the signaling molecules tended to accumulate to the bottom of confined spaces, denoting that they are likely denser than the air. This hypothesis fitted well with our findings on the nature of volatiles differentially emitted by WMV-infected plants. The volatile emission of healthy and WMV-infected plants was analyzed using static headspace containers. Two differentially emitted volatile compounds were identified, benzaldehyde and γ -butyrolactone. Healthy plants emitted both volatiles but to a lesser extent than WMV-infected plants. Both benzaldehyde and γ -butyrolactone are heavier than the air and, therefore, with a tendency to be in the lower areas of closed environments, including for instance the bottom part of a vertical growth chamber. γ -butyrolactone has the simplest structure of molecules in the family of butyrolactones (4-carbon lactones); broad biological activity has been described for it, being a component of the aroma of flowers (Raman et al. 2017), present in essential vegetable oils (Cao et al. 2016) and produced by microorganisms like endophytic fungi (Ibrahim and Asfour 2018) and bacteria (Takano 2006). Nevertheless, the difference in emitted VOCs between healthy and WMV-infected plants was largest for benzaldehyde. Benzaldehyde is an important intermediary in the non β -oxidative route of salicylic acid to produce benzoic acid (Dudareva et al. 2013; Muhlemann et al. 2014; Widhalm and Dudareva 2015) and is actively emitted to the environment by an ABC-type transporter (Adebesin et al. 2017). Involvement of benzaldehyde has been described in flower-pollinator (Blight et al. 1997; El-Sayed et al. 2018; Verdonk et al. 2003) and other plant-insect (Deng et al. 2004; He et al. 2017; Wei et al. 2017) interactions. To our knowledge, neither γ -butyrolactone nor benzaldehyde have been described as VOCs priming defense responses in healthy plants (see for a review Hammerbacher et al. 2019) except in one

case. Ribnicky et al. (1998) showed that tobacco leaves, when infected with TMV, emit small amounts of volatile benzaldehyde, even if basal endogenous benzaldehyde levels did not change after TMV infection. Moreover, these authors showed that exogenous application of volatile benzaldehyde was very effective in increasing tissue content of salicylic acid, PR1 mRNA and TMV resistance (Ribnicky et al. 1998). The same team previously showed that tobacco plants infected with TMV emitted MeSA, and when healthy plants were exposed to volatiles emitted by TMV-infected plants, the expression of the PR1 gene and TMV increased resistance was induced (Shulaev et al. 1997). In our case, we did not observe changes in MeSA accumulation (data not shown) or emission after WMV infection, but we observed an increase in benzaldehyde accumulation both as endogenous (data not shown) and emitted volatile. Thus, our observations further support benzaldehyde as an important molecule in signaling and priming defenses after plant virus infections; our data also suggest that at least in melon benzaldehyde and MeSA may have uncoupled biosynthesis routes and different functions.

The effects that volatile perception have on the plant's physiology and ecology are always difficult to identify or even predict. On the one hand, our data showed that WMV inoculations using diluted inoculum resulted in a smaller ratio of infected plants for those that cohabited with WMV-infected plants than for those that did not. Therefore, it seems that volatile perception may be priming a defense reaction in neighboring plants to prepare them against incoming infections. This makes sense in epidemiological terms, as often, for aphid-transmitted viruses, secondary infection foci appear distant from the primary foci and scattered around field plots. On the other hand, our experiment on the effect of cohabitation with distance showed that signaling is short distance, since plants separated as little as 40 cm no longer showed PR gene deregulation. This argues more in favor of communication of distant parts of the plant via volatile signaling. Again, this makes sense considering the architecture of the melon plant, which is a liana whose stems creep along the ground, and also the nature of the volatile compounds that we have identified as differentially emitted by infected plants, both of them denser than the air. In addition, both possibilities, plant to plant and within plant communication do not need to exclude each other. In conclusion, our data suggest that perception of a volatile signal precisely encoded by WMV-infected tissues triggers a response to prepare healthy tissues or/and healthy neighboring plants for the incoming infections.

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TABLES**Table 1.** Analysis of melon susceptibility to WMV after cohabitating plants with WMV-infected plants.

Priming¹	Inoculum²	Ratio³ (Infected / total)	%
Control	Concentrated	14/16	87
Cohabited	Concentrated	31/32	96
Control	Diluted	22/24	91
Cohabited	Diluted	17/32	53

¹Prior to inoculation, primed plants were kept for 1 h of cohabitation with WMV-infected plants in methacrylate containers.

²The viral isolate was WMV-M116 (Diaz-Pendon et al. 2005). Mechanical inoculation was carried out according to standard protocols. Zucchini leaves infected with WMV-M116 and frozen at -80 °C were used as inoculum source, and two inoculum concentrations were used: 1 g in 5 ml and 1 g in 25 ml of phosphate buffer 30 mM pH 8.0 for concentrated and diluted inocula, respectively.

³Plants were checked for WMV infection at 2 weeks after inoculation by a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) using a WMV ELISA kit (DSMZ, Braunschweig, Germany).

FIGURES

Fig. 1. Alteration of the expression of pathogen defense related genes in healthy melon plants cohabited with WMV-infected plants. **(A)** Experimental design of cohabitation in controlled growth chambers. The numbers correspond to the positions of the plants inside the growth chamber, being 1 the plants grown in the upper shelf, 2 the plants grown in the central shelf, and 3 the plants grown in the lower shelf. **(B)** Determination by qRT-PCR of the expression levels of 4 selected genes related to the pathogen response: *PR1* (Pathogenesis-related protein PR-1-like), *PR2* (Glucan endo-1,3-beta-glucosidase), *PR4* (Pathogenesis-related protein PR-4-like) and *HSP17.5* (17.5 kDa class I heat shock protein). Relative transcript expression levels for each sample are shown. Error bars represent standard error.

Fig. 2. Volatile signal effects with distance. **(A)** Experimental design to analyze expression changes in healthy plants located at different distances (cm) from a WMV-infected plant. Plants located at 140 cm were considered as controls. **(B)** Selected PR gene transcripts expression levels as determined by qRT-PCR. The distances represented on the X-axis are in cm. The Y axis shows the expression values determined by the $2^{-\Delta\Delta C_t}$ method. The endogenous control was cyclophilin and the reference samples are the controls (distance 140 cm). The error bars represent the standard error. **(C)** Linear regression analysis of PR gene expression in a distance up to 40 cm.

Fig. 3. Validation of the static headspace system for plant-plant signaling experiments. Selected PR gene transcripts expression levels as determined by qRT-PCR. The Y axis shows the expression values quantified by the $2^{-\Delta\Delta C_t}$ method. The endogenous control was cyclophilin and the reference samples were melon plants cohabited with a mock-inoculated plant. The error bars represent the standard error.

Fig 4. Volatile emission by melon WMV-infected or mock plants. **(A)** Experimental design of the static headspace system to measure the volatiles emitted. The WMV-infected or mock plants were kept inside a container during 1 h and the volatiles of the environment were adsorbed with SPME fibers. **(B)** Area percentage of emitted volatile compounds. Only benzaldehyde and γ -butyrolactone showed significant differences with p-values of 0.03 and 0.08 respectively (t-test) when comparing WMV-infected *vs* mock plants headspaces.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Volatile compounds with a defined mass standard identified in the NIST database (www.nist.gov/srd/nist-standard-reference-database-1a-v17) emitted by healthy and WMV-infected melon plants.