

Effects of far-red light on tritrophic interactions between the two-spotted spider mite (*Tetranychus urticae*) and the predatory mite *Phytoseiulus persimilis* on tomato

Davy Meijer,^{a*}  Jaimie van der Vleut,^{a,b} Berhane T. Weldegergis,^a Thibault Costaz,^a Marcus Vinicius Alfenas Duarte,^b Apostolos Pekas,^b Joop J. A. van Loon^a and Marcel Dicke^a



Abstract

BACKGROUND: The use of light-emitting diode (LED) lights in horticulture allows growers to adjust the light spectrum to optimize crop production and quality. However, changes in light quality can also influence plant–arthropod interactions, with possible consequences for pest management. The addition of far-red light has been shown to interfere with plant immunity, thereby increasing plant susceptibility to biotic stress and increasing pest performance. Far-red light also influences plant emission of volatile organic compounds (VOCs) and might thus influence tritrophic interactions with biological control agents. We investigated how far-red light influences the VOC-mediated attraction of the predatory mite *Phytoseiulus persimilis* to tomato plants infested with *Tetranychus urticae*, and its ability to control *T. urticae* populations.

RESULTS: Far-red light significantly influences herbivore-induced VOC emissions of tomato plants, characterized by a change in relative abundance of terpenoids, but this did not influence the attraction of *P. persimilis* to herbivore-induced plants. Supplemental far-red light led to an increased population growth of *T. urticae* and increased numbers of *P. persimilis*. This resulted in a stronger suppression of *T. urticae* populations under supplemental far-red light, to similar *T. urticae* numbers as in control conditions without supplemental far-red light.

CONCLUSION: We conclude that supplemental far-red light can change herbivore-induced VOC emissions but does not interfere with the attraction of the predator *P. persimilis*. Moreover, far-red light stimulates biological control of spider mites in glass-house tomatoes due to increased population build-up of the biocontrol agent.

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1 INTRODUCTION

The development of light emitting diode (LED) technology and its ability to manipulate the spectral composition of the light has led to a surge of scientific research into the effects of light quality on plant development. Most research is focused on using LEDs to enhance crop production and quality in horticulture.^{1,2} However, using LEDs to influence plant chemistry and physiology can affect plant defences towards biotic stress and thereby influence the interactions between plants and arthropods.³ This provides both challenges and opportunities for pest management. On the one hand, optimizing the light environment for crop production could impair plant immune responses to biotic stress following the growth-defence trade-off.⁴ This is exemplified in tomato, where the addition of far-red (FR) light increased fruit size, while reducing resistance to a fungal pathogen.⁵ On the other hand, LEDs might

also provide opportunities to improve pest management. There is increasing attention for the possible application of LEDs to stimulate plant resistance to arthropod pests, but with varying success.^{6,7}

Other opportunities might lie in the application of LEDs to stimulate biological control. For example, raising the proportion of blue light in the spectrum increases the proportion of females in the parasitoid wasp *Aphidius ervi* Haliday (Hymenoptera, Braconidae) and

* Correspondence to: D Meijer, Laboratory of Entomology, Wageningen University, PO Box 16, NL-6700, AA Wageningen, The Netherlands, E-mail: davy.meijer@wur.nl

a Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands

b Biobest Group N.V., R&D Department, Westerlo, Belgium

increased parasitism rates on the pea aphid (*Acyrtosiphon pisum* Harris) (Hemiptera: Aphididae).⁸ Understanding how changes in light quality influence the interactions between crops, pests and their biocontrol agents is important for the proper application of LEDs in sustainable glasshouse production.

An interesting aspect to study in this regard is the ratio between red and far-red light (R:FR). In natural canopies, an increase in FR light (reduction of R:FR) is an indication of increased plant density and shading by neighbouring plants, and induces what is called the shade avoidance syndrome (SAS). Shade avoidance responses include morphological and physiological adaptations that increase the plant's survival in competitive environments, including early flowering and increased light interception.⁹ These responses can be beneficial for crop production because they can lead to shorter cropping cycles and increased whole-plant photosynthesis.¹⁰ However, shade avoidance responses also influence plant-arthropod interactions with both pests and beneficial arthropods.³ Expression of SAS often goes at the expense of plant immunity against biotic stress^{3,11,12} and results in increased performance of pest arthropods.^{7,13–15} The use of FR LEDs in horticulture can therefore be detrimental for crop health and could lead to pest outbreaks.

Changes in R:FR also influence the emission of volatile organic compounds (VOCs) and can thereby also indirectly influence plant-arthropod interactions.^{15,16} VOCs are an important aspect of plant ecology by which plants can mediate interactions with the surrounding environment.¹⁷ For example, VOCs play an important role in indirect plant defences through the attraction of natural enemies.¹⁸ Upon detection of herbivore feeding, plants emit herbivore-induced plant volatiles (HIPVs), a blend of VOCs that may provide information on herbivore species, density and life stage, and on the abiotic conditions that the plant is exposed to.^{19,20} The emission of these cues is an important aspect underlying the success of biological control in agricultural settings.²¹ Cortés *et al.*¹⁵ found that exposure to FR light altered the emission of VOCs in tomato, which led to increased attractiveness of these plants to the predatory bug *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). This indicates that plants may compensate for the reduced direct defences by enhancing the attraction of natural enemies of pests, and might therefore provide a role for FR light in stimulating biological control in glasshouses.

In the present study we investigate the potential application of FR light for enhancing biological control. We address the effects of changes in R:FR on tomato HIPV emissions after herbivory by the two-spotted spider mite (*Tetranychus urticae* Koch) (Acari: Tetranychidae) and subsequent effects on the attraction of the specialist predator *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) in laboratory conditions. We included both a reduction and an increase in R:FR compared to sunlight levels because previous work has indicated that both changes in R:FR can influence arthropod attraction and that different arthropod species can respond differently to either an increase or decrease in R:FR.^{7,15,16,22} It is expected that lowering the R:FR will alter the HIPV composition and lead to the increased attraction of *P. persimilis*. Increasing R:FR is expected to have a weak or no effect on HIPV emission and predator attraction because increasing the R:FR did not affect the performance of *T. urticae*.⁷ We further studied the effects of FR light on the population development of *T. urticae* under glasshouse conditions, both in the presence and absence of *P. persimilis*. Supplementing FR light is expected to increase *T. urticae* population development. Suppression of the *T. urticae* population by *P. persimilis* is expected to be stronger under conditions of supplemental FR light because

the greater prey availability is expected to increase *P. persimilis* numbers.

2 MATERIALS AND METHODS

2.1 Plant and arthropod material

Seeds of tomato (*Solanum lycopersicum* cv. Moneymaker) were used in all experiments. Two-spotted spider mites (*T. urticae*) were obtained from the stock colony at the Laboratory of Entomology (Wageningen University, the Netherlands). The *T. urticae* strain has been maintained on tomato plants for at least 5 years and is considered to be adapted to this host plant. The predatory mites (*P. persimilis*) were obtained from a commercial strain of Biobest Biological Systems (Westerlo, Belgium).

2.2 Volatile-mediated attraction of predators

2.2.1 Experimental design

Laboratory experiments were performed to investigate the effects of R:FR on tomato HIPV emissions and the attraction of the predatory mite *P. persimilis*. Tomato seeds were sown in rockwool blocks (7.5 cm × 7.5 cm) soaked in Tomato 2.0 nutrient solution (Unifarm, Wageningen, the Netherlands). Two weeks after germination, the plants were transferred to a climate-controlled growth chamber (25/18 °C day/night, 70 ± 3% relative humidity and 16 h/8 h light/dark) and divided over three separate compartments with different light quality treatments. Each treatment consisted of 150 μmol m⁻² s⁻¹ white light (5700 K) supplemented with 60 μmol m⁻² s⁻¹ (+FR), 30 μmol m⁻² s⁻¹ (CL) or 0 μmol m⁻² s⁻¹ (-FR) of FR light (735 nm). The control light (CL) treatment was set at a R:FR of 1.2, which is equivalent to sunlight, and was reduced (+FR) or increased (-FR) to 0.6 and 8.2, respectively. Both white and FR light were provided by Dyna LED-modules (Heliospectra, Gothenburg, Sweden). The different compartments were separated with white reflective plastic to prevent light contamination between treatments. Plants were exposed to the light treatments for 1 week before being exposed to *T. urticae* spider mites (SM). Twenty-five female *T. urticae* adults were introduced on the two youngest fully developed leaves and were left to feed freely for 5 days before plants were used in further experiments. Other plants remained uninfested (U) for 5 days under the same conditions, resulting in six treatment combinations (+FR_{SM}, +FR_U, CL_{SM}, CL_U, -FR_{SM} and -FR_U). All laboratory experiments were performed at the Laboratory of Entomology at Wageningen University.

2.2.2 Y-tube olfactometer

The response of *P. persimilis* to plant volatiles was observed in a two-choice Y-tube olfactometer as described previously,²³ with some adjustments. The upper arms of the Y-shaped Pyrex tube were connected to 15 L glass jars containing the plants as odour sources. The rockwool base of the plants was wrapped in aluminium foil before placement into the jar. The plants were left to acclimate for 15 min before starting choice assays. Compressed air was filtered by active charcoal and passed through the jars at a regulated flow of 2 L min⁻¹ to carry the plant volatiles into the arms of the Y-tube. The glass jars were isolated in light-tight cabinets, each equipped with a Dyna LED module to provide plants with their respective light treatment throughout the choice assays while preventing visual detection by the predators. Predatory mites were individually released at the downwind arm on a Y-shaped metal wire that was placed in the centre of the Y-tube. Their choice for either odour source was recorded when they passed a mark halfway along the wire in one of the upper arms,

or no choice was recorded if they did not pass this mark within 10 min after their release. We tested ten or more predatory mites for each plant pair to obtain at least ten choices per plant pair. The position of the odour sources was switched after every five predatory mites to prevent positional bias. After each plant pair, the Y-tube system was flushed with clean air for 10 min before introducing a new plant pair. Spider-mite infested plants from the three light treatments (+FR_{SM}, CL_{SM} and -FR_{SM}) were compared pair-wise. Because *P. persimilis* can be infected with a bacterial pathogen, making them unresponsive to HIPVs,²⁴ choice assays between uninfested and infested plants of the control-light treatment (CL_U and CL_{SM}) were also performed as a positive control. Each pair-wise comparison was performed daily for a total of 12 days, resulting in 120 replicates of responsive predatory mites per pair-wise comparison.

2.2.3 Collection of headspace VOCs

Volatiles were collected from plants of all six treatment combinations. The rockwool base of plants was wrapped in aluminium foil before plants were placed in 15 L glass jars. They were left to acclimate for 15 min before starting the headspace collection. Air was filtered through active charcoal before reaching the jars and volatiles were collected by drawing air with a suction pump through a stainless-steel cartridge containing 200 mg of Tenax TA (20/35 mesh, CAMSCO, Houston, TX, USA) at 150 mL min⁻¹ for 2 h. During volatile collection, plants remained exposed to their respective light treatments. Volatile samples of empty rockwool blocks soaked with Tomato 2.0 nutrient solution and wrapped in aluminium foil were also collected to correct for background odours. The Tenax cartridges with VOC samples were dry purged under a stream of helium (50 mL min⁻¹) for 15 min to remove excess moisture.

The collected volatiles were thermally released from the Tenax TA adsorbent using an Ultra 50:50 thermal desorption unit (Markes, Llantrisant, UK) at 250 °C for 10 min under a 20 mL min⁻¹ helium flow, while the volatiles were simultaneously re-collected in a thermally cooled universal solvent trap: Unity (Markes) at 0 °C. When desorption was completed, the volatile compounds were released from the cold trap by ballistic heating at 40 °C s⁻¹ to 280 °C, which was then kept for 10 min, while all the volatiles were transferred to a ZB-5 MS analytical column (30 m × 0.25 mm ID × 1 mm F.T.) with 10 m built-in guard column (Phenomenex, Torrance, CA, USA), placed inside the oven of a Thermo Trace GC Ultra (Thermo Fisher Scientific, Waltham, MA, USA) for further separation of the plant volatiles. The gas chromatography (GC) oven temperature was initially held at 40 °C for 2 min and was immediately raised at 6 °C min⁻¹ to a final temperature of 280 °C, where it was kept for 4 min under a constant helium flow of 1 mL min⁻¹. A Thermo Trace DSQ quadrupole mass spectrometer (Thermo Fisher Scientific) coupled to the GC was operated in an electron impact (EI) ionization mode at 70 eV in a full scan with a mass range of 35–400 amu at 4.70 scans s⁻¹. The mass spectrometry (MS) transfer line and ion source were set at 275 °C and 250 °C, respectively. Automated baseline correction, peak selection ($S/N > 3$) and alignments of all extracted mass signals of the raw data were processed following an untargeted metabolomic workflow using MetAlign software, producing detailed information on the relative abundance of mass signals representing the available metabolites.²⁵ This is followed by reconstruction of the extracted mass features into potential compounds using the MSclust software through data reduction by means of unsupervised clustering and extraction of putative metabolite mass spectra.²⁶

Tentative identification of volatile metabolites was based on comparison of the reconstructed mass spectra with those in the NIST 2008 and Wageningen Mass Spectral Database of Natural Products MS libraries, as well as experimentally obtained linear retention indices (LRIs).

2.3 Population dynamics of *T. urticae*

A glasshouse experiment was designed to study the effects of supplemental FR light on the population development of *T. urticae* and on the ability of the predatory mite *P. persimilis* to suppress *T. urticae* populations. Tomato seeds were sown in potting soil (Biobest) and grown under standard glasshouse conditions. Six weeks after germination, plants were transferred to another glasshouse compartment (22/15 °C, 70 ± 10% relative humidity and 16 h/8 h light/dark), which was divided into four corridors using white reflective sheets. All four corridors received 15 μmol m⁻² s⁻¹ broad-spectrum white light (5700 K) provided by Elix LED modules (Heliospectra) as a supplement to natural daylight. Two corridors were further supplemented with 20 μmol m⁻² s⁻¹ of FR light (Philips Greenpower LEDs, Eindhoven, the Netherlands), creating two light treatments with R:FR ratios of 1.2 (CL) and 0.6 (+FR), respectively (measured at midday). Each corridor contained two rows of eight plants, of which the first and last plants were border plants and not used for the experiment. All plants were placed at 50 cm distance from each other and from the plastic sheets. One row was used to study spider mite population growth in the absence of its predator, *P. persimilis* (-Pp), while the other row received predatory mites during the experiment (+Pp). This resulted in four treatment combinations based on supplementation with FR light and the presence of *P. persimilis* (CL/-Pp, CL/+Pp, +FR/-Pp, +FR/+Pp).

After 1 week of exposure to the light treatments, all experimental plants were infested with *T. urticae*. The second, fourth and sixth leaf (counted from the bottom) each received three adult female spider mites. After 24 h, the spider mites were checked and any dead spider mites were replaced. One week after infestation, the number of mobile spider mites (i.e. all life stages, excluding eggs) per leaf was counted using a magnifier glass. At the same timepoint, plants assigned to the +Pp treatment received one adult female *P. persimilis* on each of the three infested leaves. The number of mobile *T. urticae* per leaf was counted weekly for another 2 weeks. In the fourth week after the initial spider mite infestation, all infested leaves were removed per plant, soaked in a detergent solution (Green care®, Nivelles, Belgium) and rinsed above a 36 μm sieve to collect the mobile mites and eggs of both species. The number of mobile mites and eggs per plant was counted under a stereomicroscope.

2.4 Statistical analysis

Choice responses of *P. persimilis* in the Y-tube were analysed with a two-sided binomial test with $\alpha = 0.05$, for each pair-wise comparison separately. Data are presented as the total number of *P. persimilis* choosing either odour source in each pair-wise comparison.

The volatile emission data, expressed as peak heights, were imported into SIMCA-P 17 statistical software (Umetrics, Umeå, Sweden), followed by log-transformation, mean-centring and unit-variance scaling before being subjected to multivariate data analysis. Unsupervised principal component analysis (PCA) and/or supervised partial least squares-discriminant analysis (PLS-DA), and its extension orthogonal partial least squares-discriminant analysis (OPLS-DA) were used as tools to compare and correlate

treatment groups. The results of the analysis are visualized in score plots, which reveal the sample structure according to model components, and loading plots, which display the contribution of the variables (individual VOCs) to these components as well as the relationships among the variables. The R^2 and Q^2 metrics, which describe the explained variation within the data set and the predictability of the model, respectively, were calculated based on the averages of the seven-fold cross-validation. The R^2 and Q^2 values range between 0 and 1, and the closer these metrics are to 1, the higher the variance explained by the model and the more reliable the predictive power of the model. Significant differences in the total emission of individual VOCs between light treatments of either infested or uninfested plants were analysed using Kruskal–Wallis analysis. For pair-wise comparisons, VOCs with a variable importance in projection (VIP) score ≥ 1 were subjected to Mann–Whitney U analyses to test for differences in total emissions between treatments, following the pair-wise comparisons as performed in the Y-tube choice assays. VOCs with a VIP score ≥ 1 are considered to contribute most to the differences in volatile blends between treatments. These analyses were performed using SPSS version 21.2 (SPSS Inc., Chicago, IL, USA).

Spider mite population development over the first 3 weeks of the glasshouse experiment was analysed using generalized least squares (GLS) on log-transformed data, including a temporal autocorrelation structure of order 1 (AR-1) to account for the repeated measures, and a fixed variance structure between predator treatments to correct for heteroscedasticity. Light, predator presence, time and leaf number were included as explanatory variables. Data on the number of eggs and mobile stages of *T. urticae* counted after the destructive measurements in the fourth week were similarly analysed with GLS on log-transformed data, with a fixed variance structure between predator treatments to correct for heteroscedasticity. Light and predator presence were included as explanatory variables. The number of eggs and mobile stages of *P. persimilis* were analysed using Mann–Whitney U analysis. In all GLS analyses, the optimal models were selected by comparing the Akaike information criterion (AIC) values from the full models (i.e. including all interactions) to the minimal model (without interactions). The analyses were performed in R (version 4.2.0; R Core Team, 2020) using the 'nlme' and 'car' packages.^{27,28}

3 RESULTS

3.1 Y-tube choice assays

In the positive control, predatory mites showed a significant preference for volatiles from spider mite-infested plants over those from uninfested plants of the CL treatment (Fig. 1; Supporting Information, Table S1), indicating a responsive predator population. Predators did not distinguish between volatiles from CL_{SM} and +FR_{SM} plants or between volatiles from CL_{SM} and –FR_{SM} plants, indicating that changes in R:FR do not influence the attraction of *P. persimilis* to spider-mite infested plants. Predators did show a preference for volatiles from –FR_{SM} plants compared to those from +FR_{SM} plants.

3.2 Headspace VOC composition

Across the six different treatment combinations we identified 70 VOCs (Table S2). These compounds were detected in at least 50% of the samples from at least one of the treatment combinations. A multivariate analysis (OPLS-DA) including the uninfested plants from all three light treatments (+FR_U, CL_U and –FR_U) did not result in a reliable model (permutation test: $R^2 = 0.545$, $Q^2 = 0.028$), indicating that the composition of the emitted volatile blends does not differ significantly between treatments (Fig. 2(A,B)). A similar OPLS-DA on the spider mite-infested plants from all three light treatments (+FR_{SM}, CL_{SM} and –FR_{SM}) also resulted in an unreliable model (permutation test: $R^2 = 0.077$, $Q^2 = -0.031$), indicating that differences in R:FR do not influence HIPV emissions (Fig. 2(C,D)).

When comparing blend compositions pair-wise, matching the comparisons made for predator behaviour in the Y-tube choice assays, we did find significant separation between volatile blends that largely correspond to the observed behavioural responses. OPLS-DA on the blend compositions of CL_U and CL_{SM} plants show a clear, significant separation (permutation test: $R^2 = 0.988$, $Q^2 = 0.487$), where 17 compounds with a VIP score ≥ 1 contributed most to the separation (Supporting Information, Fig. S1). When comparing the emission rates of individual VOCs, four compounds showed significant differences between treatments (Table S3); with the monoterpene linalool, the sesquiterpene (*E*)- β -ionone and the green leaf volatile ester, (*Z*)-3-hexen-1-ol propanoate showing increased

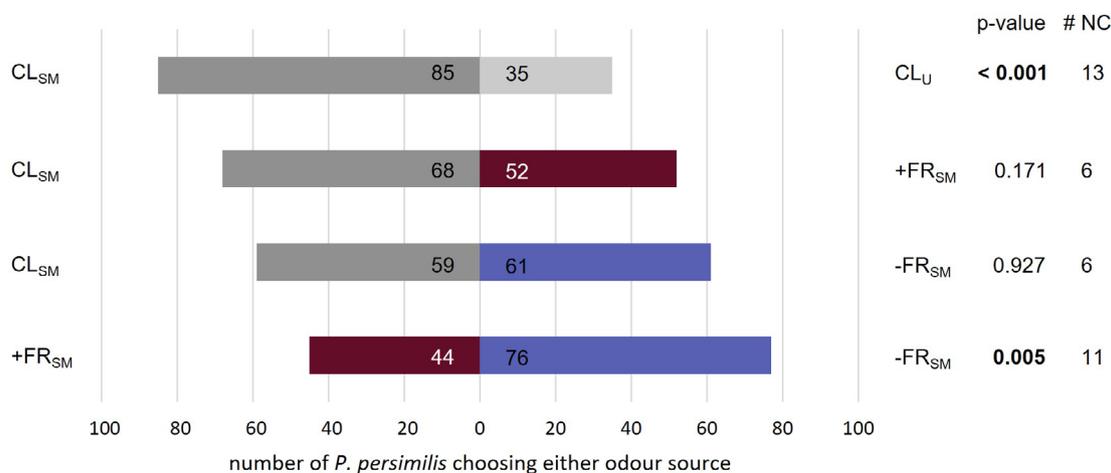


Figure 1. Total number of *Phytoseiulus persimilis* choosing either odour source in a Y-tube olfactometer for pair-wise comparisons between plants exposed to different R:FR (+FR = 0.6, CL = 1.2 and –FR = 8.2) and either infested with *Tetranychus urticae* spider mites (SM) or uninfested (U). The *P*-values in bold indicate statistically significant differences between treatments (two-sided binominal test). NC = no choice.

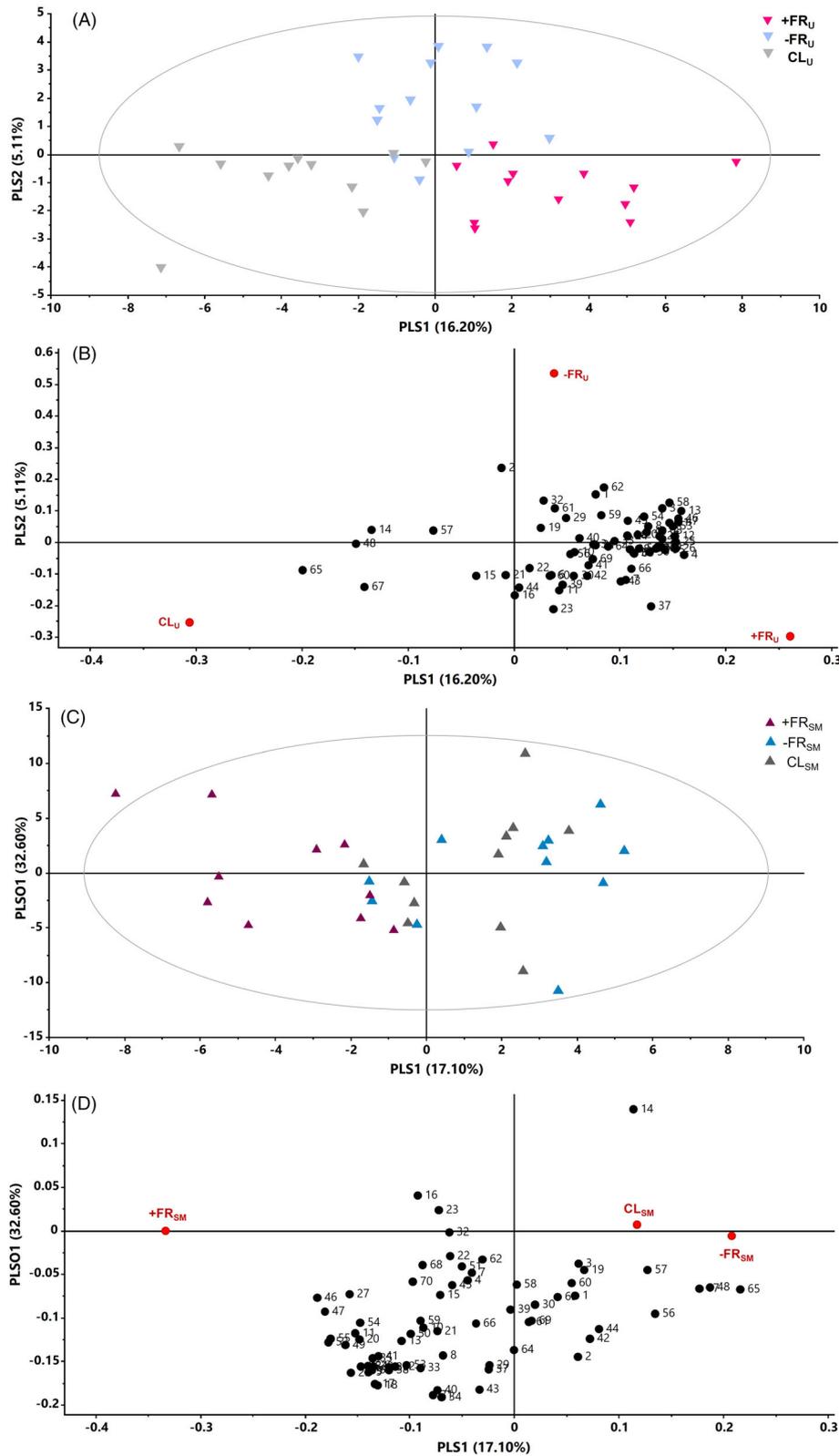


Figure 2. Effects of exposure to different R:FR on the blend of volatile organic compounds (VOCs) collected from the headspace of tomato (*Solanum lycopersicum* cv Moneymaker) plants. (A) Multivariate comparison using orthogonal projection to latent structures-discriminant analysis (OPLS-DA) between uninfested plants exposed to different R:FR (+FR_U = 0.6, *n* = 12; CL_U = 1.2, *n* = 11; -FR_U = 8.2, *n* = 13) based on the relative quantitative results of the volatiles displayed in a two-dimensional score plot. (B) Loading plot indicating the contribution of each VOC to the separation between uninfested plants exposed to different R:FR. (C) Same analysis as described for (A) but now comparing plants infested with *Tetranychus urticae* spider mites and exposed to different R:FR (+FR_{SM} *n* = 10; CL_{SM} *n* = 11; -FR_{SM} *n* = 11). (D) Loading plot indicating the contribution of each VOC to the separation between spider mite infested plants exposed to different R:FR. For VOC identification: numbers in loading plots correspond to the numbers in Supporting Information, Table S2.

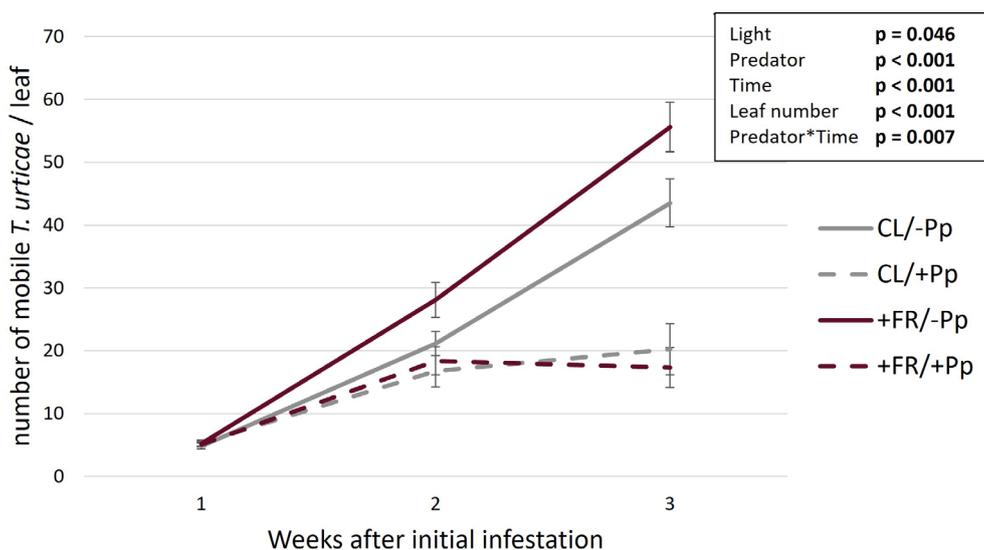


Figure 3. Average number (\pm standing error) of mobile *Tetranychus urticae* spider mites per leaf on plants exposed to control light conditions (CL; R:FR = 1.2) or control light supplemented with far-red light (+FR; R:FR = 0.6), either in the presence (+Pp) or absence (-Pp) of the predatory mite *Phytoseiulus persimilis*. Analyzed with generalized least squares (Supporting Information, Table S3).

emission in CL_{SM} samples, and the homoterpene (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) (isomer I) showing decreased emission in CL_{SM} compared to CL_U.

We also found reliable OPLS-DA models showing clear separation between the volatile blends of plants exposed to the +FR_{SM} and CL_{SM} treatments (permutation test: $R^2 = 0.802$, $Q^2 = 0.455$) and between plants exposed to the +FR_{SM} and -FR_{SM} treatments (permutation test: $R^2 = 0.710$, $Q^2 = 0.423$) (Fig. S2). Thirty-three compounds with a VIP ≥ 1 contributed most to the separation between +FR_{SM} and CL_{SM}, of which 16 compounds showed significant differences between treatments (Table S4). Six sesquiterpenes, six monoterpenes and the aldehyde (*E,E*)-2,4-hexadienal showed increased emissions in +FR_{SM} samples, while emissions of both isomers of the homoterpene TMTT and the sesquiterpene α -copaene were significantly reduced compared to CL_{SM} samples. Similarly, 34 compounds contributed most to the separation between +FR_{SM} and -FR_{SM}, with 19 compounds showing

significant differences in total emissions (Table S5). The emission of five sesquiterpenes and 11 monoterpenes increased in +FR_{SM} samples, while the homoterpene TMTT and the sesquiterpene α -copaene were significantly reduced compared to -FR_{SM} samples. No significant separation between the volatile blends of CL_{SM} and -FR_{SM} was found (Fig. S3). Only the emission of the aldehyde (*E,E*)-2,4-hexadienal was significantly increased in -FR_{SM}, as it was not detected in CL_{SM} samples ($P = 0.003$). These results indicate that supplemental FR light influences the emission of HIPVs in response to spider mites, but this has no consequences for the attraction of the predatory mite *P. persimilis* (Fig. 1).

3.3 Glasshouse population dynamics

The population development of *T. urticae* was significantly affected by all fixed factors included in the model, i.e. supplemental FR light ($P = 0.046$), predator presence ($P < 0.001$), time ($P < 0.001$) and leaf number ($P = 0.007$) (Fig. 3; Table S6). Supplemental FR light

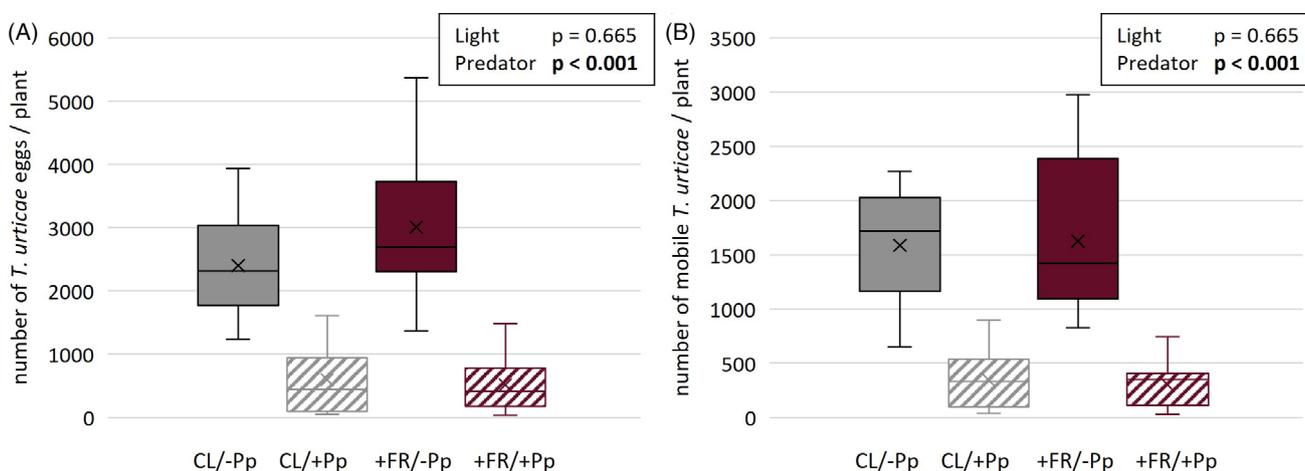


Figure 4. Boxplots representing the number of eggs (A) and mobile stages (B) of *Tetranychus urticae* per plant on plants exposed to control light conditions (CL; R:FR = 1.2) or control light supplemented with far-red light (+FR; R:FR = 0.6), either in the presence (+Pp) or absence (-Pp) of the predatory mite *Phytoseiulus persimilis*. Data was collected 4 weeks after initial infestation. Analyzed with generalized least squares (Supporting Information, Table S4). CL/-Pp, $n = 10$; CL/+Pp, $n = 9$; +FR/-Pp, $n = 11$; +FR/+Pp, $n = 11$.

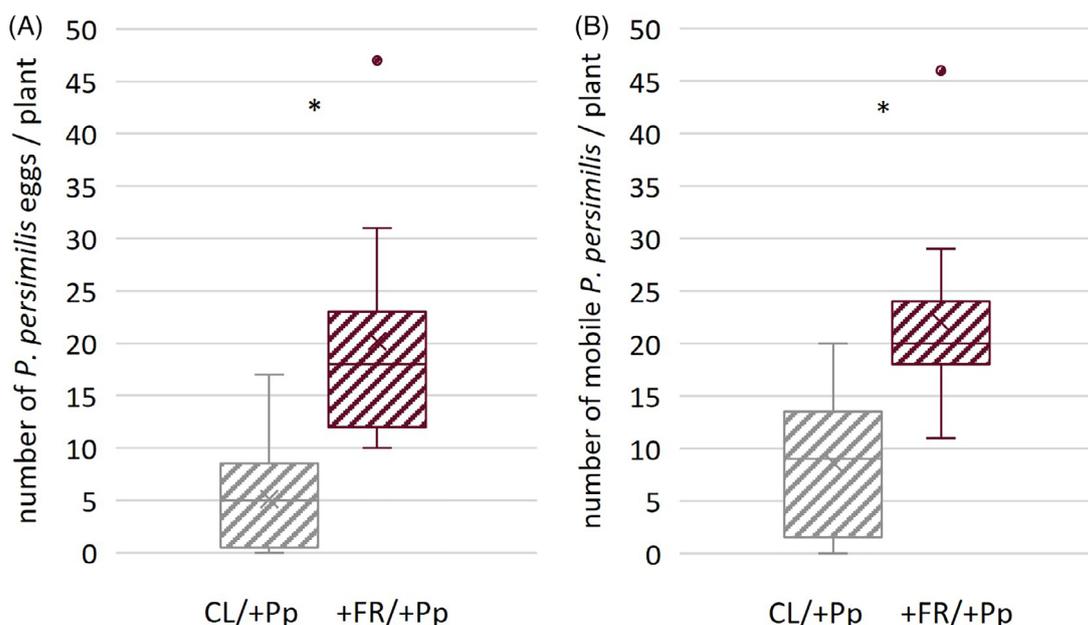


Figure 5. Boxplots representing the number eggs (A) and mobile mites (B) of *Phytoseiulus persimilis* per plant on plants exposed to control light conditions (CL; R:FR = 1.2) or control light supplemented with far-red light (+FR; R:FR = 0.6). Data was collected 4 weeks after initial infestation. An asterisk (*) indicates significant differences between treatments (Mann–Whitney U). CL/+Pp, $n = 9$; +FR/+Pp, $n = 11$.

significantly increased spider mite population size, but this effect was only apparent in the absence of predators. The presence of *P. persimilis* strongly reduced the number of spider mites. The number of spider mites significantly increased over time, and there was a significant interaction effect between time and predator presence ($P < 0.001$), indicating that the effect of *P. persimilis* increased over time. The significant effect of leaf number resulted from lower total numbers of *T. urticae* on older leaves compared to younger leaves, without interacting effects of light and predator presence (Fig. S4). In the destructive measurements of week 4, there was a significantly smaller number of *T. urticae* eggs ($P < 0.001$) and mobile stages ($P < 0.001$) in the presence of *P. persimilis* compared to the plants without predators (Fig. 4; Table S7). There was no significant effect of light on the number of spider mite eggs ($P = 0.665$) and mobile stages ($P = 0.665$). The number of *P. persimilis* eggs ($P < 0.001$) and mobile stages ($P < 0.001$) was significantly higher under supplemental FR light (Fig. 5). These results support the hypothesis that supplemental FR light can boost predator populations through higher prey availability.

4 DISCUSSION

The attraction of natural enemies through the emission of HIPVs is a form of indirect defence that has become less efficient in glasshouse tomatoes due to domestication.²¹ The possible role of FR light in stimulating indirect defence¹⁵ therefore provides opportunities to enhance biological control. In this study we aimed to identify how changes in the R:FR influence the volatile-mediated attraction of *P. persimilis* predatory mites towards plants infested with the two-spotted spider mite (*T. urticae*). Our results indicate that changes in R:FR can influence the HIPV emission of spider mite-infested tomato plants, but it does not affect the attraction of *P. persimilis* to the HIPV blend when compared to control light.

Previous studies have also shown significant effects of supplemental FR light on the VOC emissions of tomato and *Arabidopsis*

thaliana. Those changes were associated with clear behavioural responses of arthropods.^{15,16} Cortés et al.¹⁵ showed that exposure to supplemental FR light increased the attraction of the mirid predator *M. pygmaeus*. In the study by Cortés et al.¹⁵ and the current study, the FR-induced changes in VOC composition are mainly characterized by a change in the relative abundances of terpenoids, with some compounds showing increased emissions while others are reduced. Terpenoids play an important role in the attraction of the predatory mite *P. persimilis*,^{29–31} but play a minor role in the attraction of mirid predators such as *M. pygmaeus*.³² These differences between predator species might also explain the differences in observed behavioural responses.

It should also be noted that both Cortés et al.¹⁵ and Kegge et al.¹⁶ simulated herbivory by spraying with methyl jasmonate (MeJA), the volatile ester of the stress hormone jasmonic acid (JA). Although MeJA application can mimic responses to true herbivory to some extent, there are distinctive differences between both types of responses,^{33,34} also in VOC emissions.³⁵ MeJA application might influence a limited range of JA-inducible volatile compounds and provide a more general stress signal, while herbivores induce a more complex VOC blend,^{35,36} thereby adding to the specificity of HIPV signals. For example, it has been shown that *P. persimilis* is more attracted to spider mite-infested plants compared to plants treated with JA.³⁶ It is worthwhile to investigate whether FR light differentially influences MeJA- and herbivore-induced VOC emissions, and how this influences the attraction of predatory arthropods in multiple predator–prey systems.

Several VOCs that are known to be important for the attraction of *P. persimilis* are the terpenoids linalool, (*E*)- β -ocimene, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and TMTT.^{31,37–39} The effects of supplemental FR light on the emission of these compounds is consistent between the data of Cortés et al.¹⁵ and the current results. The emission of TMTT is significantly reduced by exposure to supplemental FR light in spider mite-infested plants,

while the emission of linalool and DMNT is not significantly affected (Table S2). The emission of (*E*)- β -ocimene gradually increases with decreasing R:FR in both spider mite-infested and uninfested plants (Table S2). Other consistent effects of supplemental FR light between studies are the increased emission of α -caryophyllene and β -caryophyllene (Table S2).¹⁵ β -Caryophyllene is a sesquiterpene known to be involved in the attraction of natural enemies of herbivores.⁴⁰ Whether and how these VOCs contribute to the differential responses between predator species remains to be studied.

In the current study we found a significant preference of *P. persimilis* towards $-FR_{SM}$ plants compared to $+FR_{SM}$ plants, but no significant preference between CL_{SM} and $+FR_{SM}$ plants, even though we found separation between the volatile blends in both comparisons. The effect of $+FR_{SM}$ on the emission of individual compounds also shows large similarities between both comparisons. In both cases, $+FR_{SM}$ caused an increase in the emission of the monoterpenes (*E*)-*m*-mentha-4,8-diene and anetofuran and the sesquiterpenes α -caryophyllene, β -caryophyllene, bicyclo-elemene, and δ -elemene, while reducing the emission of the homoterpene TMTT and the sesquiterpene α -copaene, when compared to both CL_{SM} and $-FR_{SM}$. However, when compared in a three-way comparison between $+FR_{SM}$ (R:FR = 0.6), CL_{SM} (R:FR = 1.2) and $-FR_{SM}$ (R:FR = 8.2), the differences between $+FR_{SM}$ and CL_{SM} are less pronounced than the differences between $+FR_{SM}$ and $-FR_{SM}$ (Table S2), which correlates to the behavioural observations. This may indicate that the gradual increase in FR light might also cause a gradual response in VOC emissions of individual compounds. Although natural enemies of herbivores respond to the blend of HIPVs instead of individual compounds,^{38,41,42} changes in the relative abundance of individual compounds within the blend can change the attractiveness of the entire blend.^{18,31,38} For example, the homoterpene TMTT is not attractive to *P. persimilis* as a pure compound, but is an important determinant for prey localization when present in volatile blends.³⁸ It is, therefore, interesting to investigate whether and how different levels of FR light regulate the emission of VOCs and how this influences predator attraction.

Although low R:FR conditions do not result in an increased attraction of *P. persimilis*, our data show that there is still potential for the use of supplemental FR light to benefit crop production. FR light has the potential to shorten crop cycles and increase fruit yield under glasshouse conditions,¹⁰ but the downside of using FR light is the inhibition of plant immune responses and the increased susceptibility to pests and pathogens.³ We have previously shown that the performance of several herbivore species, including *T. urticae*, is significantly increased under low R:FR conditions.⁷ In the current study, we assessed the effects of supplemental FR light on *T. urticae* population development and the ability of *P. persimilis* to control spider mite populations in glasshouse conditions. We found that, although spider mite populations develop faster when plants are grown under $+FR$ conditions, *P. persimilis* can still adequately suppress *T. urticae* numbers to similar levels as in CL conditions. This indicates that the suppression of *T. urticae* populations is stronger under $+FR$ conditions. The explanation seems relatively straightforward: the inhibition of plant defences by FR light leads to increased plant quality for herbivores and consequently to increased egg production by *T. urticae*.⁷ The higher prey availability consequently stimulates the population growth of its predator, *P. persimilis*. This is confirmed by the increased number of *P. persimilis* eggs and mites found on plants exposed to $+FR$.

We conclude that the supplementation of FR light significantly changes the HIPV emissions of tomato plants infested with *T. urticae* spider mites, but does not influence the attraction of the predatory mite *P. persimilis*. Moreover, when *P. persimilis* is applied to infested plants, the higher availability of prey under supplemental FR conditions can lead to a rapid population increase of the biocontrol agent, leading to stronger suppression of the pest. However, when not adequately responded to, the increased performance and population growth of *T. urticae* under supplemental FR light can lead to serious problems for crop health. It is therefore important that infestations are detected early, before reaching economically damaging densities.

We recommend that future studies continue to address the relation between FR light and HIPV emission in multiple predator-prey interactions, using true herbivory. Especially the effects on *M. pygmaeus* invite further research due to its omnivorous nature. Plant feeding by *M. pygmaeus* can induce plant defences against pests and pathogens, but can also lead to economic crop damage when densities are too high.^{43,44} Changes in the plant's defensive status induced by FR light might change *M. pygmaeus* feeding behaviour between plants and arthropod prey and therefore its efficiency as biological control agent. Understanding how plant responses to supplemental FR light interact with tritrophic interactions is required to ensure efficient biological control in glasshouses when light conditions are manipulated to influence crop growth and fruit set.

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CONFLICT OF INTEREST

The authors declare to have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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