Journal of Economic Entomology, XX(XX), 2025, 1–9
https://doi.org/10.1093/jee/toaf134
Research

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Plant Resistance

Signals from aphid-infested neighboring plants modify aphid performance and host plant biochemistry

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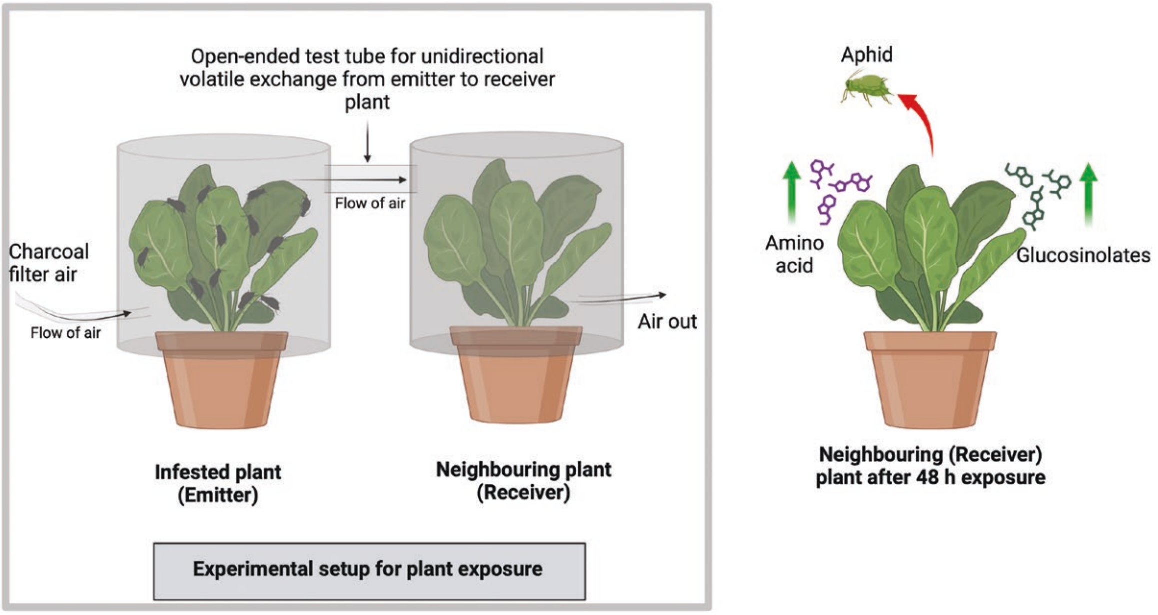
Subject Editor: Joe Louis

Received on 4 January 2025; revised on 29 March 2025; accepted on 12 May 2025

Plants release signals to communicate with their environment. When damaged by insect herbivores, plants emit herbivore-induced plant volatiles (HIPVs) that can affect pest performance, recruit biocontrol agents, and trigger defence responses in neighboring plants. While plant communication via HIPVs has been well-documented over the past few decades, key aspects, such as the specific biochemical mechanisms underlying defence activation in neighboring plants and the variation in response to herbivory, remain poorly understood. This study aims to investigate the effect of HIPVs released by aphid (*Brevicoryne brassicae*)-infested *Brassica napus* (emitter) plants on neighboring uninfested *conspecific* plants (receiver). After 48 h exposure, emitter plants were discarded, and receiver plants were utilized in bioassays to assess aphid performance (fecundity and survival), preference, as well as amino acid and glucosinolates contents of receiver host plants. Results revealed that receiver plants showed a significant reduction in aphid fecundity, although no significant effect on aphid survival was observed. Aphid feeding preference was significantly altered, with *B. brassicae* showing a reduced preference for receiver plants. Additionally, significant increases in some key amino acids and total concentrations were recorded in receiver plants. Glucosinolates (GSs) analysis showed a significant change in total GS content, with a significant increase in the individual GSs, glucoiberin (GIB), and glucoraphanin (GRA). Current findings provide evidence that receiver plants activate their defence mechanism against *B. brassicae* by altering amino acid and glucosinolate levels. This study highlights the potential of HIPVs to enhance sustainable pest management strategies.

Keywords: plant–plant communication, herbivore-induced plant volatiles, aphid, *Brassica*, induced defence, conspecific plants

Graphical Abstract



Introduction

Plants possess remarkable abilities to sense and respond to their environment, employing a diverse array of chemical signals to communicate with neighboring organisms in both abiotic and biotic contexts (Karban 2021, Ninkovic, Markovic and Rensing 2021). These chemical signals play pivotal roles in shaping plant interactions, from facilitating mutualistic relationships with beneficial microbes to orchestrating defences against herbivores and pathogens (Stam et al. 2014, Ninkovic et al. 2019). Through intricate signaling pathways, plants detect changes in their surroundings and mount tailored responses to optimize their fitness and survival in dynamic ecosystems (Jump and Peñuelas 2005).

Brassica crops, members of the cruciferous plant family, are economically and nutritionally importance (Rakow 2004, Warwick 2011, Hasanuzzaman 2020). The annual global production of *Brassica* crops, measured in millions of tonnes, fulfills the dietary needs of millions of people worldwide (Greer et al. 2023). After soybean (*Glycine max* L.), *Brassica* is considered the second largest oilseed crop in the world (Gupta 2015). Additionally, *Brassica* plants, such as *Arabidopsis*, have long served as models for studying plant-insect interactions. However, these crops are highly susceptible to a variety of herbivorous insects, including aphids (Capinera 2012) such as the cabbage aphid (*Brevicoryne brassicae* L.) (Van Poecke 2007, Bhattacharya 2019, Ali 2023). The cabbage aphid is a common pest of *Brassica* crops that poses significant challenges to agricultural productivity (Hughes 1963, Pontoppidan et al. 2003). The *B. brassicae* feeds directly on plant sap, weakening the plant nutritionally and reducing its vigour, while also indirectly transmitting plant viruses (Ali et al. 2024).

In response to insect damage, plants adjust their defence systems and produce defence-related compounds such as secondary metabolites, including alkaloids, glucosinolates (GSs), and volatile organic compounds (Fürstenberg-Hägg et al. 2013, Ali et al. 2024). These volatile compounds, known as herbivore-induced plant volatiles (HIPVs), play a crucial role in plant communication and herbivore defence strategies. Upon herbivore attack, HIPVs signal neighboring plants, alerting them to herbivore presence (War et al. 2011). Some HIPVs act as priming agents, prompting nearby plants to strengthen their defences before a potential herbivore attack. However, not all volatile-mediated plant communication leads to priming. Certain HIPVs can directly deter herbivores, attract natural enemies of herbivores, or even alter interactions within plant-associated microbial communities (Takabayashi and Shiojiri 2019). The potential application of HIPVs in sustainable pest management strategies has garnered significant interest within the scientific community (Peñaflor and Bento 2013). By identifying and deploying specific HIPVs, researchers aim to develop strategies that enhance crop protection while reducing reliance on conventional pesticides. For example, synthetic or naturally derived HIPVs could be used to prime crop defences before pest outbreaks, attract natural enemies of herbivores, or interfere with pest host-finding behavior (Khan et al. 2008, Pickett and Khan 2016, Karban 2021). These approaches align with the principles of integrated pest management, offering environmentally friendly alternatives to chemical control.

While HIPV-mediated plant-plant communication has been studied, the specific mechanisms by which emitter plants influence neighboring receiver plants remain under investigation. Studies have demonstrated that HIPVs can prime defences, attract natural enemies, or alter plant physiology to reduce herbivore feeding (Takabayashi and Shiojiri 2019, Karban 2021). Recent research has further elucidated these processes in *Brassica* species. For example, (Kang et al. 2018) found that β-ocimene, a HIPV emitted by *Brassica rapa* L. subsp. *pekinensis*, enhances plant defences against *Myzus persicae* by increasing glucosinolate content and altering aphid feeding behavior. Similarly Peng et al. (2011) demonstrated that *Brassica oleracea* plants exposed to HIPVs from herbivore-damaged neighbors exhibited heightened defensive responses, including increased glucosinolate production. Pashalidou et al. (2020) further reported that HIPVs released by *B. rapa* in response to herbivory can prime defences in neighboring plants, enhancing resistance to subsequent insect attacks. Additionally have shown that aphid-induced HIPVs can modify plant–insect interactions by influencing both aphid performance and the recruitment of natural enemies (Catola et al. 2018, Moreira et al. 2018). These findings underscore

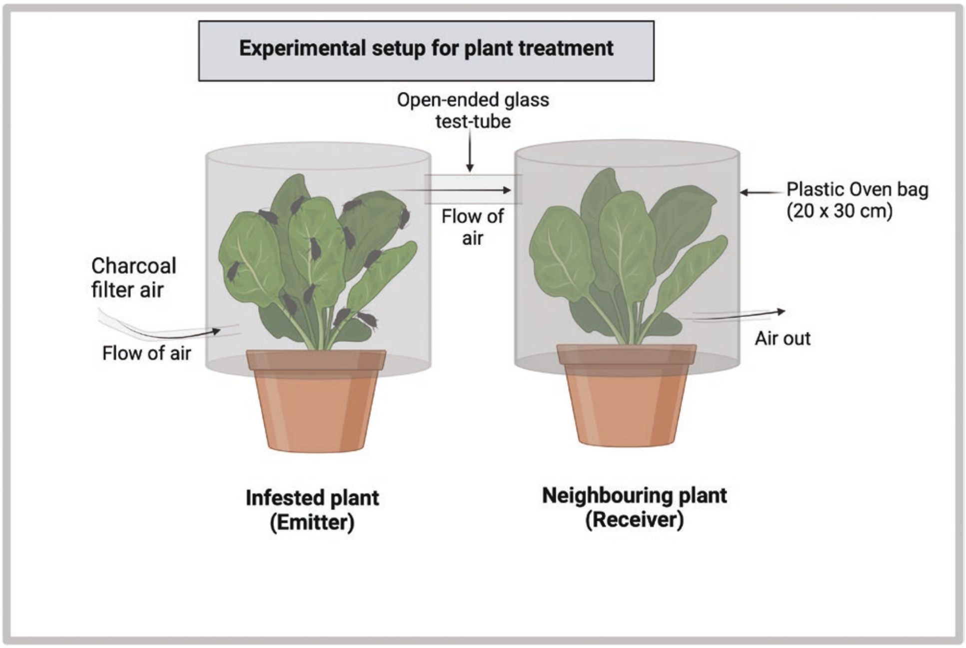


Figure 1. Experimental setup for plant treatment to investigate the effect of herbivore-induced plant volatiles (HIPVs) from *Brevicoryne brassicae*-infested *Brassica napus* (emitter) on neighboring uninfested plants (receiver). Airflow was regulated using a charcoal-filtered inlet system, ensuring unidirectional volatile transfer through a sterilized, open-ended glass test tube. Emitter plants were either infested with 100 adult aphids 24 h before the experiment or left uninfested (control). Receiver plants were exposed to volatiles for 48 h before subsequent bioassays.

the complex roles of HIPVs in shaping ecological interactions and highlight the need for further exploration. In this study, we seek to address this gap in knowledge by investigating the influence of aphid-induced volatiles on neighboring *Brassica* plants, particularly focusing on amino acids (AAs) and GSs, which have been less explored in the context of interplant communication. Our research aims to shed light on the dynamics of interplant communication and their implications for plant defence strategies against the insect pest *B. brassicae*.

The aim of this study is to investigate the impact of volatile compounds released by aphid-infested *Brassica* plants (emitter), on neighboring uninfested *Brassica* plants (receiver). To assess this, we evaluated the performance and behavior of adult *B. brassicae* on receiver plants to determine any defensive or attractive effects induced by the emitted volatiles. Additionally, we analyzed the effect of these volatiles on the AAs and GSs content of receiver plants, providing insights into potential biochemical responses triggered by the presence of nearby infested plants. While this research primarily seeks to understand the physiological effects of HIPVs on neighboring plants, it may also provide insights into whether these volatile-mediated defences could contribute to broader pest management strategies.

Materials and Methods

Insect and Plant

A stock colony of the cabbage aphid, *B. brassicae* (Hemiptera: Aphididae), was maintained on *Brassica napus* Linnaeus in a Bugdorm cage (60 × 60 × 60 cm) under controlled environmental conditions (24 ± 2 °C, 35 ± 5% RH, 16L: 8D photoperiod). The seeds of *B. napus* were grown individually in 11 cm pots under constant environmental conditions in a growth chamber (LPH-214-S, NK system, Japan) at 20 °C, 35 ± 5% RH, 16L: 8D photoperiod. All experiments were conducted with plants at Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie growth stage (BBCH) growth stage 14 (Meier 1997, Ali et al. 2021).

Plant Treatment

To investigate the effect of plant signals released from *B. brassicae*-infested plants on neighboring uninfested plants, we employed a controlled airflow system to facilitate unidirectional volatile transfer (Fig. 1). The setup consisted of emitter (*B. napus*) plants (infested) and enclosed alongside receiver plants (uninfested) within separate sterilized chambers. Each emitter and receiver plant was individually enclosed in a sterilized oven bag (35 × 40 cm) to prevent external contamination while maintaining controlled airflow. A sterilized, open-ended glass test tube (3 cm diameter × 15 cm length) connected the two chambers, ensuring the transmission of plant signals in one direction. To regulate volatile transfer, purified air was introduced into the emitter chamber through a charcoal-filtered inlet system at a flow rate of 600 ml/min. The outlet airflow was maintained at 400 ml/min, creating a slight positive pressure gradient in the bag that ensured continuous HIPV movement from the emitter to the receiver plant while preventing backflow. The infested (emitter) plants were inoculated with 100 adult *B. brassicae* aphids 24 h prior to exposure to induce HIPV release (Beckendorf et al. 2008). Receiver plants remained uninfested but were exposed to volatiles from the emitter for 48 h under controlled environmental conditions ((20 ± 5 °C, 40 ± 5 % RH, 16L: 8D photoperiod)). For control group plants, uninfested emitter plants were used, while all other conditions, including airflow regulation and enclosure, remained identical. Following the 48-h exposure period, emitter plants were removed, and receiver plants were used in all bioassays. These included assessments of aphid performance, feeding preference, amino acid content, and glucosinolate profiles to determine the impact of HIPV exposure on aphid and receiver plants.

Aphid Performance

The evaluation of *B. brassicae* performance on receiver plants was conducted using clip-cages (25 mm d). To investigate the effect of volatile compounds released from infested and uninfested *Brassica* plants on receiver plant defence against *B. brassicae*, observations were recorded after 48 h of the start of the experiment. Two clip-cages containing 10 alate *B. brassicae* were attached to the lower (abaxial) surface of leaves (second and third oldest) of each plant for 48 h. The plants were placed in an acclimatization room at constant laboratory conditions (20 ± 5 °C, 40 ± 10 % RH, 16L: 8D h photoperiod). Each plant represented one replication and 10 replicates were performed. The number of survived aphids and produced nymphs were recorded after 48 h.

Feeding Preference

To study the feeding preferences of *B. brassicae*, choice tests were conducted. Circular leaf discs (2 cm in diameter; six discs in total, with three from control plants and three from plants exposed to infested plants volatiles) were placed together in the same Petri dish. Each dish contained a moist filter paper at the base to maintain the turgidity of the leaf discs, allowing *B. brassicae* to choose between the two treatments. Twenty adult apterous aphids were positioned in the center of each Petri dish using a paintbrush. The Petri dishes were then covered with black paper to prevent any phototactic responses and kept in a growth chamber at 20 °C (Kumar et al. 2011). A total of 20 replicates were performed, and the number of aphids on each leaf disc was recorded after 24 h.

Amino Acid Analysis

The methodology was adapted from Ahmed et al. (2022). To extract free amino acids, 100 mg of fresh leaf tissue was ground in a 0.1 M HCl solution using a glass mortar and pestle. Samples were collected from receiver plants that were either exposed to volatiles from infested emitter plants or uninfested emitter plants. All receiver plants used in the study were free of aphids. The second and third oldest leaves were selected for analysis. Analysis was conducted using the LTQ-XL linear ion trap mass spectrometer (LC-MS; Thermo Fisher Scientific). Liquid chromatographic separations were performed using the XTerra MS C_{18} Column (12.5 nm pore size, 5 µm, 4.6 × 150 mm); obtained from Waters (Milford, MA, USA). A three-step gradient elution was employed with mobile phase A (5% acetonitrile and 0.1% formic acid) and mobile phase B (100% acetonitrile). The spray voltage was set at 4.5 kV, and the ion transfer capillary temperature was maintained at 320 °C. Masses of precursors and products for each amino acid were referenced from a study conducted by Cao et al. (2014). Quantification was performed using an external standard amino acid mixture of known concentrations (AAS18; Sigma-Aldrich, Co.), supplemented with cysteine, tryptophan, asparagine, and glutamine. Ten replicates were conducted for both groups.

Glucosinolate Analysis

Samples were collected from receiver plant leaves (plants exposed to uninfested and infested emitter plants). All receiver plants were free of aphids, and the second and third oldest leaves were used for analysis. To deactivate myrosinase activity in fresh leaves (100 mg), they were immersed in a 1.5-ml centrifuge tube and boiled in water for 2 to 3 min. Subsequently, the leaves were ground in 1 ml of Milli Q water using a glass mortar and pestle. The resulting mixture was centrifuged at 12,000 g and 4 °C for 20 min. Glucosinolates present in the supernatant were quantified using the LTQ-XL linear ion trap mass spectrometer (Ahmed et al. 2022). The relative concentration of glucosinolates was determined using a standard curve generated with 2-propenyl glucosinolate (sinigrin) (Ahmed et al. 2022). Ten replicates were conducted.

Statistical Analysis

The complete datasets were separately subjected to normality (Shapiro-Wilk test) and homoscedasticity (Levene test) tests. Since there was not a consistent data normality and variance homogeneity, statistical analyses were performed using a generalized linear model (GLMs) with Poisson distribution. The package *car* facilitated employment of Anova()function to generate likelihood ratio test results. The figures were generated using the package *ggplot2*. Additional packages such *as dplyr, ggpubr, tidyr,* and *ggforce* were employed for data manipulation and visualization purposes. All evaluations regarding statistics and visualization were performed in R (v4.4.1) statistical environment (R Development Core Team 2024).

Results

Aphid Performance and Preference Parameters

The fecundity of *B. brassicae* was significantly lower on *B. napus* plants exposed to the conspecific plants infested with *B. brassicae* compared to control plants ($\chi^2 = 22.08$, $P < 0.001$, Fig. 2A) while its survival did not differ between exposed and control plants ($\chi^2 = 2.85$, $P = 0.091$, Fig. 2B). The preference of *B. brassicae* also was significantly lower on *B. napus* plants exposed to the conspecific plants infested with *B. brassicae* compared to control plants ($\chi^2 = 13.05$, $P < 0.001$, Fig. 2C).

Amino Acid Content

The contents of the amino acids, alanine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, phenylalanine, proline, serine, threonine, tryptophan as well as the total amino acid were consistently higher in *B. napus* plants exposed to *B. brassicae*-infested host plants ($P < 0.05$, Table 1, Fig. 3A).

Glucosinolate Content

The content of glucoiberin, glucoraphanin as well as the total glucosinolate was significantly higher in *B. napus* plants exposed to *B. brassicae*-infested conspecific host plants ($P < 0.05$, Table 2, Fig. 3B).

Discussion

While our study primarily focuses on plant-plant communication, the findings contribute to a broader understanding of how HIPVs influence aphid performance and plant biochemical responses, which may have potential implications for pest management. By demonstrating that volatile compounds released by aphid-infested *B. napus* plants can enhance the defence system of neighboring uninfested plants, we highlight how interplant signaling can influence herbivore interactions. However, the direct applicability of these findings in pest management remains an open question. In agricultural systems, HIPV-mediated defences might be leveraged in specific scenarios, such as intercropping strategies where HIPV-emitting plants enhance the resistance of susceptible crops. Nevertheless, additional research is required to assess whether these effects are consistent under field conditions and whether they can be effectively incorporated into crop protection strategies.

The reduction in aphid performance and altered feeding preferences observed in our study indicate that HIPVs can effectively enhance resistance in neighboring plants through volatile-mediated interactions. Several factors may contribute to these results. For instance, HIPVs could trigger the production of secondary metabolites such as glucosinolates and other defensive chemicals in the receiver plants (Zukalová and Vasak 2002, Ali et al. 2024). These compounds are known to have anti-herbivore properties, which can reduce aphid fecundity and survival (Zukalová and Vasak 2002, Pastorczyk and Bednarek 2016). Additionally, exposure to HIPVs may lead to the induction of physical defence mechanisms in plants. Previous studies have reported the enhancement of physical defences, such as increased trichome density, strengthening of cell walls, and the deposition of lignin and callose, which make it more difficult for aphids to feed and reproduce. These responses have been observed in plants in response to insect feeding, including *Pieris rapae* L., *Trichoplusia ni* Hübner and *B. brassicae* (Traw and Dawson 2002, Broekgaarden et al. 2011). Furthermore, HIPVs could alter the nutritional quality of the plant tissues, either by reducing the levels of essential nutrients or by increasing the concentrations of deterrent compounds, thereby negatively impacting aphid performance. Previous studies showed that in response to insect attacks plants adjust their AA content (Golan et al. 2017, Florencio-Ortiz et al. 2018). Increased free amino acids (AAs) can increase aphid preference and probing in the short term, suggesting that changes in amino acid profiles could influence aphid feeding behavior and infestation rates (Mauck, De Moraes and Mescher 2014). Additionally, HIPVs prime the receiver plants that may interfere with the olfactory signals that aphids use to locate suitable host plants, leading to altered feeding preferences and reduced infestation rates (Markovic et al. 2019). These combined effects underscore the multifaceted nature of plant defence mechanisms activated by HIPVs. However, while these findings advance our understanding of HIPV-mediated plant responses, they do not directly demonstrate a viable pest control strategy. Future research should investigate whether HIPV-induced defences persist long enough to be useful in pest management and whether they can be consistently induced under field conditions.

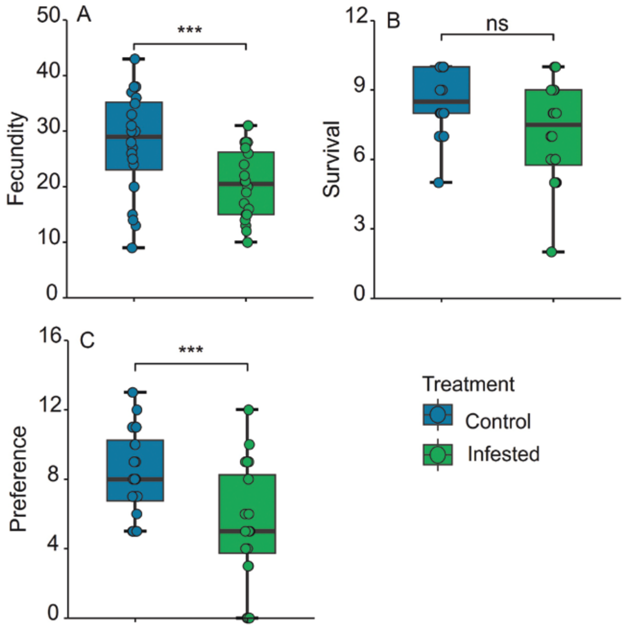


Figure 2. Box plots for the fecundity (A), survival (B) and preference (C) potential (as count data) of *Brevicoryne brassicae* over *Brassica napus* host plants exposed to either neighboring healthy *B. napus* host plants or *B. brassicae*-infested *B. napus* host plants. Circles with corresponding color demonstrate data points. The triple asterisks (*** = $P < 0.001$) indicate a significant difference between treatments while ns depict non-significant treatment effect. The lower and upper box boundaries delineate 25th (below) and 75th (above) percentiles, respectively, and the thick horizontal lines inside the boxes are the median values. The lower and upper error bars depict lowest and highest whiskers respectively.

The significant increase in total amino acid concentration in receiver plants, following exposure to HIPVs from aphid-infested emitter plants, underscores the complexity of plant responses to biotic stress signals. The 14% increase in total amino acids indicates that the receiver plants are reallocating their metabolic resources to bolster their defensive capabilities. This reallocation is likely a strategic

Table 1. Amino acid content (mean ± sem, nmol/g FW) in *Brassica napus* host plants exposed to healthy (control) and *Brevicoryne brassicae*-infested *B. napus* plants. The likelihood ratio test results of the generalized linear model with poisson distribution reveals treatment effects

Compound	Control	Infested	χ^2	*P*	Level
Alanine	122.4 ± 8.86	149.9 ± 2.41	27.82	<0.001	***
Arginine	21.1 ± 0.57	19.6 ± 1.45	0.55	0.457	
Asparagine	38.4 ± 2.02	43.9 ± 2.35	3.67	0.551	
Aspartic acid	345 ± 1.38	373.9 ± 5.26	11.62	<0.001	***
Cystine	16.7 ± 1.48	17.2 ± 1.20	0.07	0.786	
Glutamic acid	916 ± 3.87	1038 ± 28.79	76.22	<0.001	***
Glycine	307.1 ± 3.26	349.3 ± 3.91	27.15	<0.001	***
Histidine	19.1 ± 1.02	15.8 ± 1.22	3.13	0.077	
Isoleucine	30.9 ± 1.49	45.5 ± 1.45	28.07	<0.001	***
Leucine	41.2 ± 2.43	65.9 ± 1.27	57.48	<0.001	***
Lysine	26.4 ± 1.37	29.4 ± 1.13	1.62	0.204	
Methionine	8.3 ± 0.91	6.3 ± 0.70	2.75	0.097	
Phenylalanine	29.4 ± 2.00	48.3 ± 1.14	46.44	<0.001	***
Proline	53.1 ± 1.15	64.2 ± 1.33	10.52	0.001	**
Serine	86.8 ± 1.94	99 ± 2.10	8.02	0.005	**
Threonine	152.9 ± 2.11	186.3 ± 2.61	32.94	<0.001	***
Tryptophan	19.8 ± 1.12	24.9 ± 2.01	5.83	0.016	*
Tyrosine	36 ± 2.83	33.4 ± 1.27	0.97	0.324	
Valine	69 ± 3.18	64.9 ± 1.85	1.26	0.263	
Total	2340.0 ± 14.73	2676.0 ± 35.60	225.41	<0.001	***

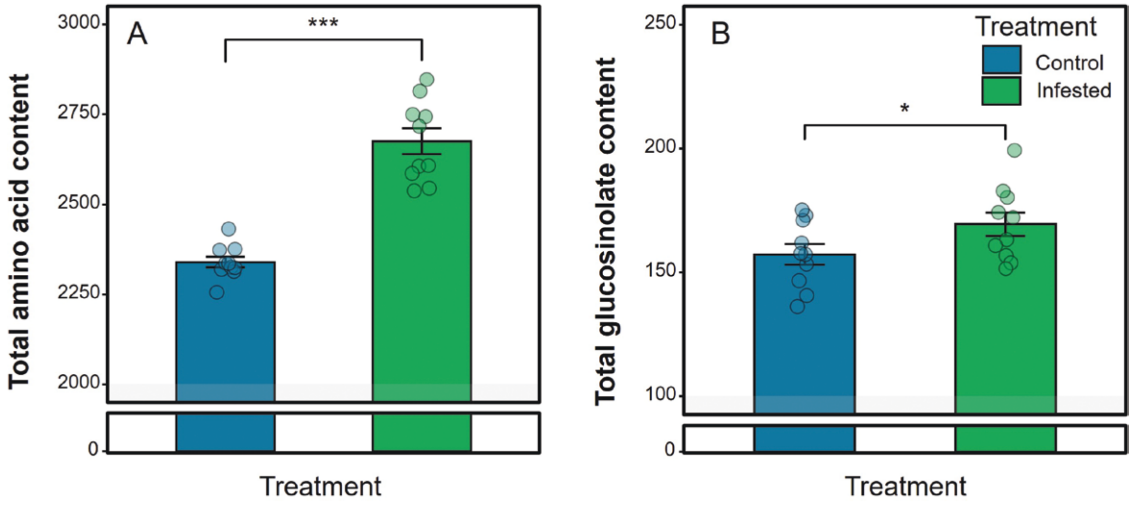


Figure 3. Bar plots demonstrating A) total amino acid content (mean ± sem, nmol/g FW) and B) total glucosinolate content (mean ± sem, nmol/g FW) from *Brassica napus* plants either exposed to healthy *B. napus* plants or *Brevicoryne brassicae*-infested *B. napus* plants. Individual data points are represented by circles filled with respective bar color which correspond to those in the common legend in B. The asterisks above each pair of comparison demonstrate the level of significance test (* = P < 0.05, *** = P < 0.001). Breaks in axis y facilitate presentation of error bars and data points.

Table 2. Glucosinolate contents (mean ± sem, nmol/g FW) in *Brassica napus* host plants exposed to healthy (control) and *Brevicoryne brassicae*-infested *B. napus* plants. The likelihood ratio test results of the generalized linear model with poisson distribution reveals treatment effect.

Compound	Control	Infested	χ^2	*P*	Level
Glucobrassicin	17.4 ± 10	16.2 ± 2.05	0.43	0.513	
Glucoiberin	0 ± 0	0.4 ± 0.22	5.55	0.019	*
Glucoraphanin	0 ± 0	2.7 ± 1.11	37.43	<0.001	***
4-Hydroxyglucobrassicin	6.8 ± 0.73	8.6 ± 1.25	2.11	0.147	
4-Methoxyglucobrassicin	30.8 ± 1.85	32.3 ± 2.03	0.36	0.550	
Neoglucobrassicin	79.3 ± 3.18	86.8 ± 3.90	3.39	0.066	
Progoitrin	23 ± 1.25	22.5 ± 1.54	0.06	0.815	
Total	157.3 ± 4.24	170.5 ± 4.93	4.55	0.033	*

response to anticipated herbivore attack, optimizing the plant's readiness to withstand future damage (Zhou et al. 2015, Karasov et al. 2017). Specifically, the significant increases in threonine, glutamate, and glycine concentrations suggest targeted metabolic adjustments. Threonine is known to play a crucial role in tolerance against stress, which might contribute to the strengthening of plant tissues or the synthesis of defensive proteins (Muthuramalingam et al. 2018, Zaynab et al. 2019). Glutamate is a key molecule in nitrogen metabolism and serves as a precursor for the synthesis of other amino acids and defensive compounds (Liao et al. 2022). Its increase could enhance the plant's overall metabolic flexibility and capacity to generate various defensive molecules (Qiu et al. 2020). Glycine, involved in the synthesis of glutathione and other essential metabolites, might contribute to the plant's antioxidant defences, protecting it from oxidative stress induced by herbivore attacks (Alves et al. 2019, Li et al. 2020). These findings align with existing literature that highlights the role of amino acids in plant defence (Huang et al. 2011, Rojas et al. 2014, Trovato et al. 2021). For instance, increased amino acid levels in plants could act as signaling molecules, mediating the plant's response to herbivore-induced stress (Häusler et al. 2014, Liao et al. 2022). Increased amino acid concentrations in plants could also facilitate aphid preference and probing, providing further evidence of the complex interplay between plant defence and herbivore behavior (Mauck et al. 2014). In conclusion, the observed alterations in amino acid profiles in response to priming effect in receiver plant signify a strategic metabolic shift aimed at enhancing the plant's defensive state. This adaptive response not only prepares the plant for potential herbivore attacks but also exemplifies the complex ways in which plants communicate and react to environmental signals through metabolic reprogramming.

The analysis of GSs revealed a significant difference in total glucosinolate content between control and exposed plants, with specific glucosinolates, such as GIB and GRA, showing significant increases. This suggests that the changes in glucosinolate profiles are driven by the selective upregulation of specific compounds rather than a broad activation of the entire biosynthetic pathway. Instead, the plant appears to selectively enhance specific glucosinolates, which may offer more targeted defensive benefits against aphid herbivory. Glucoraphanin, a well-known precursor to sulforaphane, has been documented to possess strong anti-herbivory properties, deterring pests, and reducing their performance (Beekwilder et al. 2008, Ishida et al. 2014, Jeschke et al. 2017). Similarly, GIB is known for its role in the plant's defence system, it often accumulates in the wounded plant parts and plays a role in signaling pathways that activate further defensive responses (Villarreal-García et al. 2016, Bischoff 2021). Increased glucosinolate concentrations upon herbivore infestation, such as those observed in response to *M. persicae* infestation, can significantly reduce aphid performance, further supporting our findings (Kang et al. 2018). These findings align with the concept of 'induced defence', where plants tailor their defensive strategies based on the specific nature of the threat. By selectively increasing GIB and GRA, the receiver plants may be optimizing their defences to specifically counter the future challenges posed by *B. brassicae* (Ali et al. 2024). This targeted increase could be a result of complex signaling networks that discern the type of herbivore attack and accordingly modulate the plant's metabolic pathways to produce the most effective deterrents. The differential response in glucosinolate production also mirrors observations in related studies (Mewis et al. 2006, Kuśnierczyk et al. 2007, Agerbirk et al. 2009). Moreover, studies have demonstrated that the upregulation of particular glucosinolates can significantly impair aphid reproduction and survival, further supporting our findings (Nouri-Ganbalani et al. 2018, Palial et al. 2018). In summary, the significant increase in specific GSs like GIB and GRA in receiver plants highlights a strategic, targeted enhancement of chemical defences in response to HIPVs released by neighboring emitter plants. This selective biosynthesis likely contributes to a more efficient and effective defence mechanism, underscoring the complexity of plant-insect interactions and the potential for utilizing such insights in developing refined pest management strategies.

In conclusion, our study provides compelling evidence that plant-plant communication via HIPVs can significantly prime the neighboring uninfested plants against aphid *B. brassicae*. By demonstrating reduced aphid performance, altered feeding preferences, and metabolic adjustments in response to volatiles released by neighboring, our findings underscore the potential for utilizing natural plant signaling mechanisms in sustainable pest management strategies. The observed increases in specific amino acids and glucosinolates further illustrate how plants can strategically modify their chemical profiles to strengthen defences and deter herbivores. However, while our study highlights the promising role of HIPVs in priming plant defence in neighboring plant, it also acknowledges certain limitations. The scope of our research was confined to a specific pest-plant interaction and a limited set of parameters, which may not fully capture the complexity of plant responses across different species or environmental conditions. Future research should explore the broader applicability of HIPV-induced defences, including their effectiveness in diverse agricultural settings and against various pest species. Additionally, investigations into the underlying signaling pathways and genetic mechanisms responsible for these defensive responses could provide deeper insights into optimizing plant defence strategies. Overall, our study contributes valuable knowledge to the field of plant–insect interactions and offers a foundation for developing more refined, environmentally friendly pest management practices that harness the natural capabilities of plants.

Acknowledgements

The authors extend their appreciation to the University Higher Education Fund and the Research Support Program for Central Labs at King Khalid University (CL/PRI/A/4).

Author Contributions

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Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could influence this paper.

Data Availability

The data that support our findings of this study are available from the corresponding author upon reasonable request.

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