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RESEARCH ARTICLE

Artificial exposure to herbivore-induced volatile organic compounds reduces herbivory and modulates gene expression in potato plants

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Abstract

1. Climate change accelerates pest development, posing a serious threat to agricultural yields worldwide. While the application of chemical pesticides can control pests, their overuse poses risks to human health and the environment and contributes to the emergence of pesticide-resistant populations. Within this context, the use of volatile organic compounds (VOCs) emerges as a promising tool for sustainable crop pest management.
2. Informed by and building on prior work identifying key candidate herbivore-induced volatiles, we investigated whether exposure to artificial VOC blends mimicking those induced by natural herbivore damage enhances potato (*Solanum tuberosum*) resistance to herbivory.
3. Plants were paired in cages and each cage was exposed to either VOC blends similar to those produced by undamaged (control) plants or VOC blends emulating those from plants damaged by *Spodoptera exigua* (induced). After 3 days of exposure, one plant per cage was removed and leaf tissue collected for gene expression analyses, while the remaining plants were subjected to an herbivory bioassay to assess resistance to *S. exigua* feeding. After assessing resistance, we again collected leaves for gene expression analyses in induced receivers.
4. Plants exposed to induced VOC blends exhibited a significant reduction in herbivory compared to those exposed to control blends. Molecular analyses indicated that *4CL* defensive gene expression increased to a greater extent in response to damage for plants previously exposed to the induced VOCs blend versus those exposed to control VOC blends, consistent with volatile-mediated priming of induced defences.
5. *Synthesis and applications*. These findings provide strong mechanistic evidence for VOCs mediating pest resistance and suggest that exposure to herbivore-induced, plant-derived VOCs can enhance pest resistance in potato crops. These results might offer novel information for developing ecologically sustainable

plant volatile-based strategies for boosting protection against pests in potato plantations.

KEYWORDS
induced resistance, plant–plant signalling, *Solanum tuberosum*, *Spodoptera exigua*, volatile organic compounds

1 | INTRODUCTION

Over the last four decades, research on more than 40 plant species, including various agricultural crops and forestry species (Karban, 2015; Ninkovic et al., 2021), has demonstrated that plants can perceive and respond to complex blends of volatile organic compounds (VOCs) emitted by attacked conspecific or heterospecific neighbouring plants (Heil & Karban, 2010; Karban, Yang, & Edwards, 2014). These responses include cases whereby VOC exposure primes or fully induces defences in 'receiver' plants that are exposed to VOCs released by attacked neighbouring 'emitter' plants (Martinez-Medina et al., 2016; Mauch-Mani et al., 2017), ultimately enhancing resistance—here defined as a reduction in herbivore damage or performance, or reduced pathogen infection or growth—in receiver plants (Karban, 2015; Moreira & Abdala-Roberts, 2019). Accordingly, VOCs act as cues shaping plant–enemy interactions within and among plants within patches (Heil & Karban, 2010; Kessler, 2018), and can have strong impacts on herbivores, pathogens and plant-associated food webs ultimately shaping plant performance and fitness.

With the long-standing push for alternative pest management strategies with low environmental impacts, the use of VOCs mediating plant resistance has gained increased attention over the last decade (Murali-Baskaran et al., 2022; Ninkovic et al., 2021; Pickett & Khan, 2016; Piesik et al., 2020; Turlings & Erb, 2018). For example, exposing plants to artificially produced VOCs has been proposed as a 'green vaccination' approach to managing crop pests and diseases (Luna, 2016; Stenberg et al., 2015; Turlings & Erb, 2018). This strategy is of particular interest to agriculture, as VOC-mediated priming in plants is less costly than full defensive response, potentially minimizing growth-defence trade-offs and therefore ultimately enhancing yield (Stenberg et al., 2015). For this, a priori ecological knowledge on candidate compounds followed by mechanistic studies manipulating VOC blends and detailed assessments of receiver induced responses are needed to unveil the mechanistic underpinnings of VOC-mediated crop resistance. Accordingly, several studies have promisingly demonstrated higher plant resistance following artificial VOC exposure (e.g. Erb et al., 2015; Frank et al., 2021; Karban, Wetzel, et al., 2014; Meents et al., 2019; Moreira et al., 2018). For example, in wheat (*Triticum aestivum*), exposure to methyl salicylate (MeSA) increased the accumulation of salicylic acid (SA), flavonoids and benzoxazinoids, resulting in elevated resistance to the grain aphid *Sitobion avenae* (Li et al., 2024). Similarly, in a greenhouse study, tomato (*Solanum lycopersicum*) plants exposed to (Z)-3-hexenyl propanoate exhibited reduced infestation by *Tuta absoluta* compared to unexposed plants (Pérez-Hedo et al., 2021). Both studies evaluated not only induced resistance but also molecular-level responses associated with plant defence priming and induction. Despite these findings, the functional role of specific volatile compounds or VOC induced blends and the molecular and biochemically level responses shaping induced resistance in receivers is often unknown, precluding a predictive understanding of VOC-mediated defensive responses in many species. This gap represents a critical barrier for elucidating the physiological and ecological basis for plant–plant signalling needed to harness VOC-mediated interactions for sustainable crop protection.

In this study, we investigated the mechanisms potentially underlying VOC-mediated enhancement of herbivory resistance in potato (*Solanum tuberosum* L., Solanaceae) plants by exposing them to ecologically relevant control and induced artificial VOC blends, and further measured defence-associated molecular-level responses associated with plant defence priming and induction. To this end, we exposed plants of a widely cultivated commercial potato variety to either a 'control' or 'induced' volatile blend using artificial emitters and then measured leaf damage by *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) as a proxy for induced resistance, and additionally measured defensive gene expression levels for plants before and after damage. The VOC blends used here to test artificial induced resistance were composed of 10 individual compounds previously found to correlate with enhanced resistance in receiver potato plants exposed to volatile blends emitted by plants damaged by *S. exigua* feeding (Martín-Cacheda et al., 2023a, 2023b; Vázquez-González et al., 2022, 2024). To investigate molecular changes potentially underlying induced resistance, we analysed the expression of nine genes associated with plant defences in damaged versus undamaged plants under each treatment to test for VOC-associated priming effects on plant defence induction. Accordingly, by testing for volatile blend-specific effects and assessing the molecular basis of defence-associated responses and associated resistance patterns, our approach allowed us to yield key insight into VOC-mediated interactions between potato plants, knowledge that can contribute to potential application of VOCs in sustainable crop management strategies.

2 | MATERIALS AND METHODS

2.1 | Study species

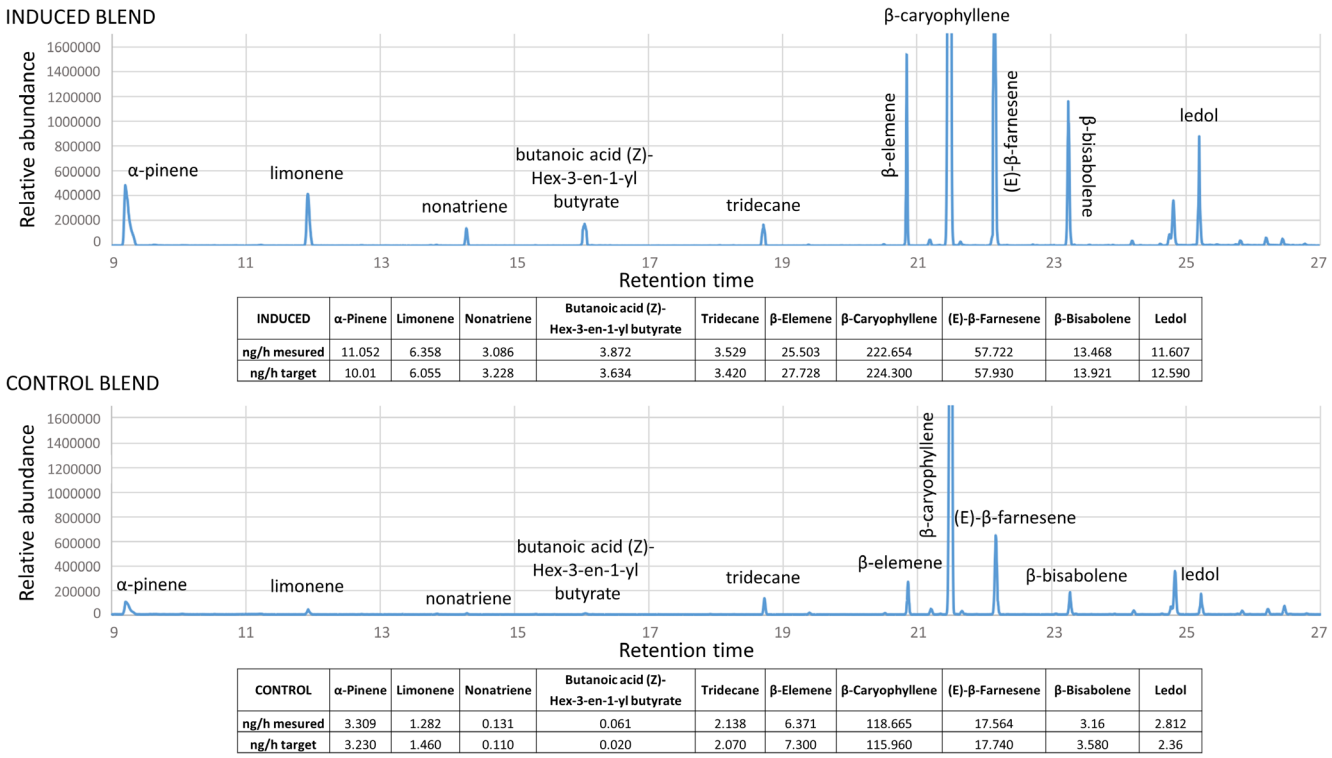
Solanum tuberosum is an herbaceous species that typically grows up to 60 cm in height and propagates through both seeds and tubers. It

was domesticated approximately 8000 years ago in the central Andes of Peru and Bolivia (Hijmans & Spooner, 2001) and was introduced to Europe in the latter half of the 16th century. Today, it ranks among the most important food crops globally in terms of human consumption, with over 4000 edible varieties and an annual production exceeding 383 million tonnes (FAOSTAT, 2023). Along with substantial increases in production over recent decades (Devaux et al., 2020), the intensification of cultivation has also increased pest and disease pressures on this crop, generating considerable economic losses. Among the most economically damaging pests is the beet armyworm, *S. exigua*, a generalist herbivore that consumes leaves and tubers and can substantially reduce plant growth and yield (Brown & Dewhurst, 1975). Over the past two decades, this pest has caused severe outbreaks across multiple regions worldwide, and populations are increasingly developing resistance to pesticides (e.g. Zhang et al., 2014). Previous studies have demonstrated that *S. exigua* leaf herbivory significantly increases overall VOC release in potato plants and causes substantial shifts in VOC blend composition (Martín-Cacheda et al., 2023a, 2023b; Vázquez-González et al., 2022, 2024). These herbivore-induced modifications of VOC profiles are in turn associated with plant–plant signalling effects, with induced VOCs conferring increased herbivore resistance in nearby undamaged potato plants (Martín-Cacheda et al., 2023a, 2023b; Vázquez-González et al., 2022, 2024).

2.2 | Experimental design and procedures

In February 2024, to investigate the role of specific VOCs involved in plant–plant signalling, we prepared two artificial VOC blends. These blends were formulated to mimic the constitutive (control) and herbivore-induced emissions of potato plants, using 10 individual compounds previously identified as strongly induced after *S. exigua* damage (Martín-Cacheda et al., 2023a, 2023b; Vázquez-González et al., 2022, 2024), and structuring compositional changes in VOC blends associated with enhanced defence responses and resistance in receiver potato plants, namely: α-pinene, limonene, 1,3,7-nonatriene, 4,8-dimethyl-, (3E)- (referred to hereafter as nonatriene), butanoic acid, 3-hexenyl ester, (Z)-, tridecane, β-elemene, β-caryophyllene, (E)-β-farnesene, β-bisabolene and ledol (Figure 1). These compounds have also been found to increase defensive responses in several plant species such as maize, mule fat or batata (Meents et al., 2019; Moreira et al., 2018; Rasmann et al., 2005), and to repel or deter insect herbivores (Hu et al., 2021; Sendel et al., 2022).

Artificial volatile solutions mimicking emissions from either control or induced potato plants were prepared by mixing the 10 pure compounds (see Figure S1 for suppliers) to achieve similar abundances of each one relative to those present in VOC emissions released by damaged and undamaged potato plants in the above



INDUCED	α-Pinene	Limonene	Nonatriene	Butanoic acid (Z)-Hex-3-en-1-yl butyrate	Tridecane	β-Elemene	β-Caryophyllene	(E)-β-Farnesene	β-Bisabolene	Ledol
ng/h mesured	11.052	6.358	3.086	3.872	3.529	25.503	222.654	57.722	13.468	11.607
ng/h target	10.01	6.055	3.228	3.634	3.420	27.728	224.300	57.930	13.921	12.590

CONTROL	α-Pinene	Limonene	Nonatriene	Butanoic acid (Z)-Hex-3-en-1-yl butyrate	Tridecane	β-Elemene	β-Caryophyllene	(E)-β-Farnesene	β-Bisabolene	Ledol
ng/h mesured	3.309	1.282	0.131	0.061	2.138	6.371	118.665	17.564	3.16	2.812
ng/h target	3.230	1.460	0.110	0.020	2.070	7.300	115.960	17.740	3.580	2.36

FIGURE 1 Chromatographic profiles of individual volatile organic compounds (VOCs) used for each VOC emission treatment: Control (constitutive emissions) versus induced (herbivore damage *Spodoptera exigua* emissions) blends. Each profile is accompanied by a table showing the target concentrations of VOCs collected from plants in previous greenhouse experiments (Martín-Cacheda et al., 2023a, 2023b; Vázquez-González et al., 2022, 2024) and the synthetic (measured concentrations of compounds in the synthetic blend in the laboratory at back-check) concentrations, expressed in nanograms per hour (ng h^{-1}), normalized to a 20 ng of naphthalene internal standard (IS) peak.

mentioned studies (Martín-Cacheda et al., 2023a, 2023b; Vázquez-González et al., 2022). Specifically, we calculated target concentrations as the mean concentration of each compound released by damaged (induced) and undamaged (control) plants under greenhouse conditions based on data from Martín-Cacheda et al. (2023a, 2023b) and Vázquez-González et al. (2022) (see Figure 1). Under field conditions, VOC emission profiles of individual plants of the same species, genotype and environmental context are inherently variable and never identical. Accordingly, our artificial blends were designed to reflect this natural heterogeneity by using average emission quantities and relative proportions, rather than assuming a fixed or an 'idealized' chemical composition. Dichloromethane was used as solvent (Honeywell Riedel-de Haën ≥99.5% (GC), CAS#75-09-2). Quality and effectiveness of the two blend types were preliminarily assessed (Figure 1) using the same preparative process and GC–MS analytical method from previous studies on potato–potato communication (Martín-Cacheda et al., 2023a, 2023b, 2024; Vázquez-González et al., 2022, 2024). Briefly, volatile dispensers (i.e. artificial emitters) were placed individually in glass chambers, and their VOC emissions were collected for 2 h on charcoal adsorbent tubes (SKC sorbent tube filled with Anasorb CSC coconut-shell charcoal) using a Sidekick 224-52MTX pump set to 0.25 L min^{-1}. Charcoal filters were then eluted with 150 µL of dichloromethane (CAS#75-09-2, Merck, Dietikon, Switzerland) containing naphthalene as an internal standard (CAS#91-20-3, 20 ng in 1 µL dichloromethane). For each sample, 1.5 µL of the extract was injected into an Agilent 7890B gas chromatograph (GC) coupled to an Agilent 5977B mass-selective detector, equipped with a 30 m × 0.25 mm × 0.25 µm HP-5MS fused-silica column (Agilent, Santa Clara, CA, USA). The GC was run in pulsed splitless mode (injector at 250°C, injection pressure 15 psi) with helium as the carrier gas. The oven program consisted of an initial hold at 40°C for 3.5 min, followed by a ramp of 5°C min^{-1} to 230°C and a subsequent 3-min post-run hold at 250°C, with a constant helium flow of 0.9 mL min^{-1}. The transfer line temperature was 280°C. In the mass spectrometer (electron impact mode), spectra were acquired over a 33–350 m/z range, with source and quadrupole temperatures set to 230°C and 150°C, respectively. Volatile compounds were identified using pure standards and by matching mass spectra with entries in the NIST library (NIST MS Search version 2.3). Emission rates of individual VOCs were quantified from normalized peak areas and reported as nanograms per hour (ng h^{-1}). For each compound, normalized peak area was obtained by dividing its integrated peak area by that of the internal standard (Abdala-Roberts et al., 2022). Consequently, the emission values for single VOCs represent naphthalene-equivalent nanograms of compound released per hour by the experimental dispensers. Chromatography glass vial (2 mL) with a capillary glass tube (2 mm internal diameter, 20 mm length) inserted through the cap PTFE/silicone septum was used as VOCs dispenser (Erb et al., 2015; Moreira et al., 2018). After validation, artificial blends were kept at −80°C until use.

In May 2024, we planted individual tubers of a single potato cultivar (*Solanum tuberosum* L. cv. Desiree) into 4-L pots filled with a peat-based potting substrate (Gramoflor GmbH & Co. KG Produktion, Vechta, Germany with a basal nutrient level of: 50–300 mg/L N, 80–300 of mg/L P_2O_3 and 80–400 mg/L K_2O). Plants grew in a glasshouse with controlled light (10 h photoperiod, PAR 725 ± 19 µmol m^{-2} s^{-1}) and temperature regimes (10°C at night, 25°C by day) and relative humidity (54 ± 0.96%) and were uniformly watered twice a week to keep the substrate consistently moist. At 3 weeks post-planting, we chose *S. tuberosum* individuals of comparable size, grouped them into pairs, and to prevent VOC cross-contamination between treatments, we placed each pair inside plastic cages measuring 37.5 × 37.5 × 96.5 cm. Each cage had two frontal mesh-covered openings to allow airflow. Within cages, plants were spaced 20 cm to prevent direct contact, while adjacent cages were kept 2 m apart to avoid VOC cross-signalling between replicates. Plant pairs were randomly assigned to one of two artificial VOC induction treatments: control (constitutive) or induced VOC blend (Figure 2). In total, 90 cages were used (45 per VOC blend group). Volatile exposure consisted in placing artificial emitters containing either control or induced VOC blend in pure DCM (1 mL), as designed and tested previously. Then, this setup released VOCs achieving target emission rates (ng h^{-1}) for each compound, similar to those found for control and herbivore-induced emissions in previous works (see above; Figure 1). Each artificial emitter was placed on the base of a pot (the same 4-L pots used for planting) positioned equidistantly between the two plants. The vial base was aligned with the base of the plants, while the tip of the vial was aligned with the lower portion of the foliage (Figure 2). Plants were exposed to the VOC treatments for three consecutive days, in line with the time period during which receiver plants were exposed to emitter-plant VOCs in previous studies (in *S. exigua* damaged and undamaged control plants, Martín-Cacheda et al., 2023a, 2023b; Vázquez-González et al., 2022, 2024). After exposure, emitter vials were removed and one of the two receiver plants (hereafter referred to as the undamaged plant) was removed from each cage for leaf collection for gene expression analyses (see ahead).

2.3 | Bioassay testing induced resistance in receiver plants

The same day that artificial emitters were removed, we performed a bioassay on the remaining receiver plants to determine whether prior exposure to induced VOC blends increased their resistance to herbivory. For this assay, a single third-instar *S. exigua* larva was gently placed, using a fine paintbrush, on each of two fully expanded leaves per plant. Each selected leaf was enclosed in a mesh bag to prevent caterpillars from moving between leaves on the same plant or to neighbouring plants. After a 48-h feeding period, we excised the leaves and captured images with an Honor 90 Lite smartphone (equipped with a triple camera system: 100 MP main camera, a 5 MP ultra-wide-angle camera, and 2 MP macro camera, with 10× digital zoom). We quantified the proportion of leaf area consumed using the BioLeaf – Foliar Analysis™ smartphone application (Machado et al., 2016).

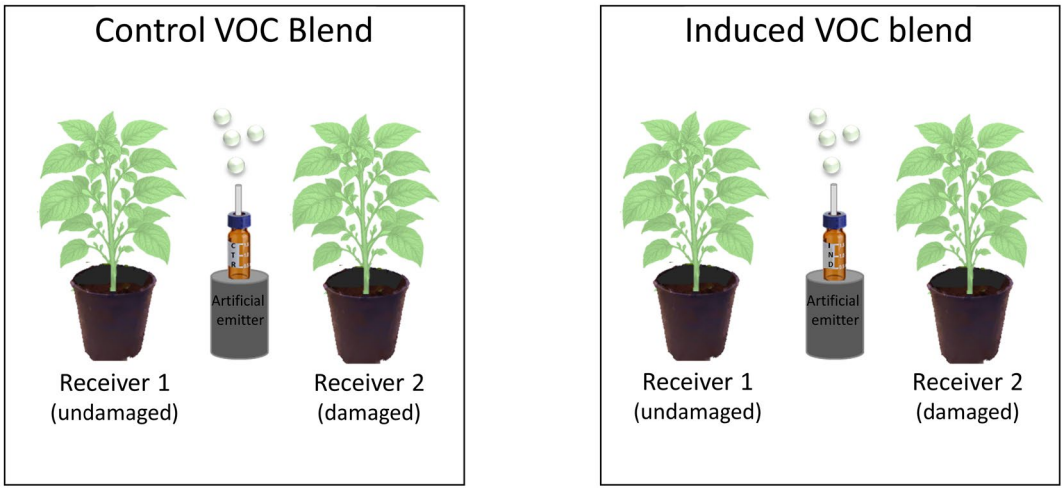


FIGURE 2 Schematic representation of the experimental design used to test the effects of volatile organic compound (VOC) emission treatment on induced resistance and gene expression in potato (*Solanum tuberosum*) plants ($N = 45$). Plants were grouped in pairs (two plants per cage), and a single artificial emitter (control: Constitutive emissions, or induced: *Spodoptera exigua* damage emissions; specific differences between blends in Figure 1) was placed between them.

2.4 | Effect of artificial induction treatment on gene expression levels

To test for gene expression changes associated with plant defence in response to the VOC treatment, we collected three leaves (leaf positions 2–4, counting downward from the apical meristem) per plant from undamaged plants removed from each cage after the 3 days of exposure to artificial emitters. Leaves were immediately immersed in liquid nitrogen and stored at −80°C until subsequent RNA extraction. In addition, to evaluate whether the VOC treatment affected defence induction in receiver plants (i.e. VOC priming effects on receiver induction) we collected three leaves (undamaged leaves from the same position as the undamaged plants) from remaining receiver plants after 48 h of *S. exigua* feeding (at the same time leaf sampling for herbivory measurements was conducted, see above) and processed them following the same procedure as for undamaged plants. Due to limitations imposed by the availability of materials for molecular analyses, we randomly selected a subset of 30 cages (same ones for both leaf sampling time points), 15 per treatment group (Moreira et al., 2021).

Leaf material was first flash-frozen and finely powdered in a mortar with liquid nitrogen. Total RNA was then isolated on a Maxwell® RSC Instrument (Promega, Madison, WI, USA) using the Maxwell® RSC Plant RNA Kit (Promega, Madison, WI), following the supplier's protocol. RNA extractions were performed as recommended, with the modification that Fruit Mate™ (TaKaRa, Shiga, Japan) was mixed 1:1 with the homogenization solution to enhance RNA purity. For first-strand cDNA synthesis, we used 1 μg of total RNA and the GoScript™ Reverse Transcription System (Promega, Madison, WI, USA), applying the manufacturer's instructions. The resulting cDNA was subsequently diluted 1:2 (v:v) with nuclease-free water prior to quantitative PCR. We analysed the expression of 10 genes (one reference gene and nine defence-associated targets) on a 7500 Real-Time PCR System (Applied Biosystems) using iTaq Universal SYBR Green Supermix (Bio-Rad). Among the target genes, three encoded terpene biosynthetic enzymes (Dudareva et al., 2013; Zhou & Pichersky, 2020): farnesyl pyrophosphate synthase 1-like (*FPPS*), a sesquiterpene precursor; solanesyl diphosphate synthase 3 (*SPPS*), a mono- and diterpene precursor; and lipoxygenase (*LOX*), a key enzyme in the biosynthesis of green leaf volatiles (GLVs) (Table 1). The remaining six genes are associated with major signalling pathways that are involved in the local and systemic defence responses of plants against herbivores and pathogens (Chen et al., 2009; Turner et al., 2002; Wang et al., 2002). These comprised ethylene response factor 1 (*ERF1*) and S-adenosylmethionine synthase 2 (*ADOMET*), both associated with the ethylene (ET) pathway; phenylalanine ammonia-lyase-like (*PAL*), 4-coumarate-CoA ligase 2 (*4CL*) and isochorismate synthase chloroplastic-like (*ICS*), linked to the SA pathway; and allene oxide synthase (*AOS*), involved in the jasmonic acid (JA) pathway (Table 1). Primers were either taken from previously published studies or designed with PRIMER3 (Rozen & Skaletsky, 1999) using sequences retrieved from the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Primer sequences, target genes and annealing temperatures are provided in Table 1. Real-time amplification efficiency for each gene was derived from standard curve slopes with LinRegPCR v12.17 (Ruijter et al., 2009; Tuomi et al., 2010). Candidate housekeeping genes were evaluated with BestKeeper, and *RPN7* was chosen as the reference gene for data normalization (Pfaffl et al., 2004). Relative transcript levels were then calculated following the efficiency-corrected Pfaffl (2001) method for RT-PCR.

2.5 | Statistical analyses

We used a generalized linear mixed model (GLMM) with a beta distribution and logit link function to evaluate the effect of VOC

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TABLE 1 Primer and gene information for qPCR analysis.

Abbreviation	Gene name	Pathway	5′-3′ sequence forward	5′-3′ sequence reverse	Accession number*	Annealing T[a]	References
RPN7	26S proteasome regulatory subunit, putative	Housekeeping	TTGGGGTGTCTGAGGATTTC	CATTCTTTGCATCAGGACGA	TC203637	56	Castro-Quezada et al. (2013)
FPPS	Farnesyl pyrophosphate synthase 1-like	Sesquiterpenes	GGCCAGAGCCTTATTATGTT	TTCTCTCCTGCAAGTGTTGT	XM_006344841.2	56	Moreira et al. (2021)
SPPS	Solanesyl diphosphate synthase 3	Mono and diterpenes	TACTGGATGATGCTGACACA	ACAACAGTTGCCAGAAGAGA	XM_006344841.2	56	Moreira et al. (2021)
LOX	Lipoxygenase	Green leaf volatiles	TTGCGGTGAATGACGTTGGTGTTC	GTGTCCCGGAAATGAGGATA	X79107.1	60	Moreira et al. (2021)
ERF1	Ethylene response factor ERF1	Ethylene	CAATCGGAGATTGACCACCT	ACCCTCCGCTGTATCAAATG	JN125860.1	56	Moreira et al. (2021)
ADOMET	S-Adenosylmethionine synthase 2	Ethylene	TTTGTCATTGGTGGACCTCA	GGATCCTTCCCAGAGAAAGC	XM_006349799.2	56	Moreira et al. (2021)
PAL	Phenylalanine ammonia-lyase-like	Salicylic acid	TTGCACAAGTTGCATCCATT	CACCAGCTCTTGCACTTTCA	XM_015303730.1	56	Cheng et al. (2019)
4CL	4-Coumarate-CoA ligase 2	Salicylic acid	TGCACAACAAGTTGATGGAG	CAATGCACAGAGCAAAACAG	NM_001318639.1	56	Moreira et al. (2021)
ICS	Isochorismate synthase, chloroplastic like	Salicylic acid	GTGCCAATTGAAGAGCAGAT	GAGAAGAAGCTCCGTTGATG	XM_006354731.2	58	Moreira et al. (2021)
AOS	Allene oxide synthase	Jasmonic acid	CCTTCTTTCTTCTCGTCGTG	TAAACGCAGCTTGATCATTG	AY135640.1	56	Moreira et al. (2021)

*Accession numbers of EST and reference sequence were obtained from NCBI.

emission treatment (fixed factor with two levels: control vs. induced blend) on the proportion of leaf area removed by *S. exigua* caterpillars. In addition, we used GLMMs to test the effects of VOC emission treatment (two levels: control vs. induced blend), damage status (two levels: undamaged and damaged), and their interaction (all fixed factors) on the relative expression of each target gene. Response distributions were selected based on data characteristics: Gamma distributions with a log link were used for continuous, strictly positive and right-skewed variables (*FPPS*, *LOX*, *ADOMET*, *PAL*, *4CL* and *AOS*), whereas Gaussian distributions with an identity link were used for approximately normally distributed responses (*SPPS*, *ERF1* and *ICS*). We log-transformed the latter response variables to improve the normality and homoscedasticity of the residuals. To handle the non-independence of plants sharing a cage, we modelled cage identity as a random effect in every model.

All statistical analyses were performed in R version 4.5.1 (R Core Team, 2023). The GLMMs for proportion of leaf damage and gene expression were implemented using the *glmmTMB* and *glmer* functions from the *glmmTMB* and the lme4 packages, respectively (Bates et al., 2015; Brooks et al., 2017). Model fit and residual diagnostics were evaluated using the *DHARMa* package (Hartig, 2024). *DHARMa* diagnostics for the beta-regression model for proportion of damage indicated approximately uniform residuals (KS: $D = 0.066$, $p = 0.42$) and no evidence of over- or underdispersion ($p = 0.32$). A slight excess of simulation outliers was detected (5 of 180 observations, $p = 0.015$), concentrated at very low damage values (under the herbivore-induced volatile blend treatment), but a refitted model excluding these observations produced nearly identical fixed-effect estimates (*T* effect −0.236 vs. −0.230) and similar ANOVA results ($\chi^2_{1,176} = 7.10$, $p = 0.008$ vs. $\chi^2_{1,171} = 8.73$, $p = 0.003$), such that all observations were retained. Diagnostic plots from *DHARMa* for both models are provided in Supporting Information (Figures S1 and S2). *DHARMa* residual diagnostics for the Gamma (*FPPS*, *LOX*, *ADOMET*, *PAL*, *4CL* and *AOS*) and Gaussian (*SPPS*, *ERF1* and *ICS*) GLMMs for gene expression indicated approximately uniform scaled residuals, no evidence of over- or underdispersion, and no extreme outliers. Diagnostic plots from *DHARMa* for all models are provided in Supporting Information (Figures S1 and S2).

To test fixed effects, we used Wald χ^2 tests obtained from the Anova() function (Fox & Weisberg, 2019), using Type II tests for models without interactions and Type III tests for models including interactions. We report back-transformed estimated marginal means (EMMs) accompanied by their 95% confidence intervals (95% CIs) as descriptive statistics using the format: EMM [2.5% lower CI, 97.5% upper CI]. When significant interactions were detected, we conducted post hoc pairwise contrasts using the *emmeans* function implemented in the *emmeans* package (Lenth, 2024). Compact letter displays (CLDs) were derived from Tukey-adjusted pairwise comparisons of EMMs for visual summarization of group differences. For the beta GLMM with logit link, pairwise contrasts were additionally computed on the original response scale using *regrid*(), allowing effect sizes to be expressed as differences between predicted values under specific treatment combinations (Lenth, 2024). For Gamma (log link) and Gaussian models with log-transformed responses, results are presented on the original response scale, ensuring direct interpretability of EMMs and comparisons.

2.6 | Ethics statement

This research did not require approval by an animal ethics committee because it involved only experimental work on plants and non-regulated invertebrate herbivores and therefore did not fall under national or institutional animal experimentation regulations.

3 | RESULTS

3.1 | Effects of VOC emission treatment on plant resistance

The VOC emission treatment significantly affected the proportion of leaf area consumed by *S. exigua* within damaged potato plants ($\chi^2_{1,176} = 7.10$, $p = 0.008$). Specifically, predicted marginal means showed a lower proportion of leaf damage in plants exposed to induced VOC emissions (0.063 [0.056, 0.071]) compared to those exposed to control VOC emissions (0.078 [0.069, 0.088]). This corresponds to an absolute difference of −0.015 [−0.020, −0.010] ($p < 0.001$). Relative to the control condition, this difference represents an approximately 19% reduction in damage (Figure 3).

3.2 | Effects of VOC emission treatment on plant gene expression levels

The VOC emissions treatment significantly altered the expression of two genes linked to induced resistance responses—*PAL* and *4CL*—and involved in the SA pathway; all other genes showed no effect of VOC emission treatment (Table 2). Specifically, we found that *PAL* expression was 9% lower for plants exposed to induced VOC emissions (1.14 [1.13, 1.15]) compared to those exposed to control emissions ($p < 0.001$; Figure 4f). In contrast, *4CL* expression was markedly 40% higher under induced emissions (1.61 [1.58, 1.64]) compared to control emissions (1.15 [1.14, 1.16]) ($p < 0.001$; Figure 4g). Damage status also had a significant effect on both genes, with expression levels of *PAL* and *4CL* being 333% and 872% higher, respectively, for damaged plants (*PAL*: 2.48 [2.46, 2.5]; *4CL*: 4.25 [4.20, 4.30]) relative to undamaged (*PAL*: 0.57 [0.57, 0.58]; *4CL*: 0.44 [0.43, 0.45]) plants. All other genes except *ERF1* (ethylene pathway) and *ICS* (SA pathway) showed significant effects of the damage treatment with percent increases ranging from 66% to 466% (Table 2, and see Figure S1 for EMMs and their 95% CIs), except for *SPPS*, a monoterpene precursor gene, which decreased by 25% in damaged plants (0.87 [0.72, 1.05]) compared to undamaged plants (1.16 [0.96, 1.39]) (Figure 4; Figure S1). Furthermore, we found that *PAL* and *4CL* were the only genes with a significant interaction between VOC emission

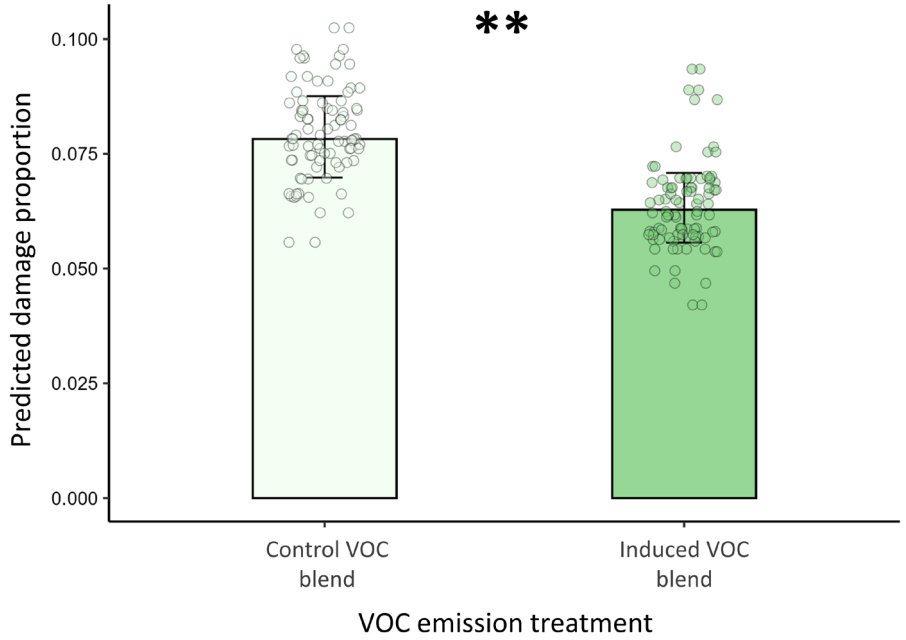

FIGURE 3 Effects of volatile organic compound (VOC) emission treatment (control: Constitutive emissions; induced: Emissions from *Spodoptera exigua*-damaged plants) on the predicted damage proportion (i.e. leaf area removed by *S. exigua* caterpillars) in potato (*Solanum tuberosum*) plants. Bars represent predicted marginal means (EMMs) on the response scale with 95% CIs, derived from the GLMM ($N = 45$). Data points are also shown. Asterisks indicate significant (**$p < 0.01$) differences between induction treatments.

TABLE 2 Effects of volatile organic compound (VOC) emission treatment (exposure to control vs. induced VOC blend), damage status (undamaged vs. damaged by *Spodoptera exigua*), and their interaction, on relative expression levels of defence-associated genes in potato (*Solanum tuberosum*) plants.

		VOC emission (*V*)		Damage (*D*)		*V* × *D*	
Gene	$DF_{num, den}$	χ^2	*p*	χ^2	*p*	χ^2	*p*
FPPS	1, 54	3.40	0.065	32.41	**<0.001**	2.40	0.121
SPPS	1, 54	0.03	0.857	5.13	**0.024**	0.77	0.390
LOX	1, 54	12.19	0.139	5.29	**0.023**	1.28	0.258
ERF1	1, 54	1.23	0.267	0.74	0.389	0.36	0.547
ADOMET	1, 54	0.19	0.665	11.74	**<0.001**	0.01	0.932
PAL	1, 54	1737.27	**<0.001**	223,796.76	**<0.001**	725.39	**<0.001**
4CL	1, 54	7318.78	**<0.001**	215,363.05	**<0.001**	839.77	**<0.001**
ICS	1, 54	0.01	0.926	0.04	0.851	0.15	0.695
AOS	1, 54	1.32	0.251	13.7	**<0.001**	1.37	0.241

Note: General linear mixed models (GLMMs) were used to analyse the expression of: (a) farnesyl pyrophosphate synthase 1-like (*FPPS*), (b) solanesyl diphosphate synthase 3 (*SPPS*), (c) lipoxygenase (*LOX*), (d) ethylene response factor 1 (*ERF1*), (e) S-adenosylmethionine synthase 2 (*ADOMET*), (f) phenylalanine ammonia-lyase-like (*PAL*), (g) 4-coumarate-CoA ligase 2 (*4CL*), (h) isochorismate synthase chloroplastic-like (*ICS*) and (i) allene oxide synthase (*AOS*). Cage ID was included as a random factor to account for non-independence between plant pairs ($N = 15$). χ^2 values, degrees of freedom and associated significance levels (*p*) are shown. Significant *p*-values ($p < 0.05$) are highlighted in bold.

treatment and damage status (Table 2; Figure 4f,g), again showing contrasting trends. For *PAL*, the increase in relative expression in response to damage was relatively greater (350%) for plants exposed to control emissions than for those exposed to induced emissions (314%) (control emissions—undamaged: 0.59 [0.58, 0.59], damaged: 2.65 [2.63, 2.67]; induced emissions—undamaged: 0.56 [0.55, 0.57], damaged: 2.32 [2.30, 2.34]) (Figure 4f). In contrast, *4CL* expression in response to damage (control: *Z* ratio = 464.07, $p < 0.0001$; induced: *Z* ratio = 348.99, $p < 0.0001$) was markedly greater for plants exposed to induced emissions (940%) compared to those exposed to control emissions (813%) (control emissions—undamaged: 0.38 [0.38, 0.39], damaged: 3.46 [3.43, 3.50]; induced—undamaged: 0.50 [0.49, 0.51], damaged: 5.20 [5.13, 5.28]) (Figure 4g). Although the interaction was not significant, *FFPS* and *AOS* also showed trends for stronger increase in expression in response to damage under induced relative to control VOCs emissions (Figure 4a,i).

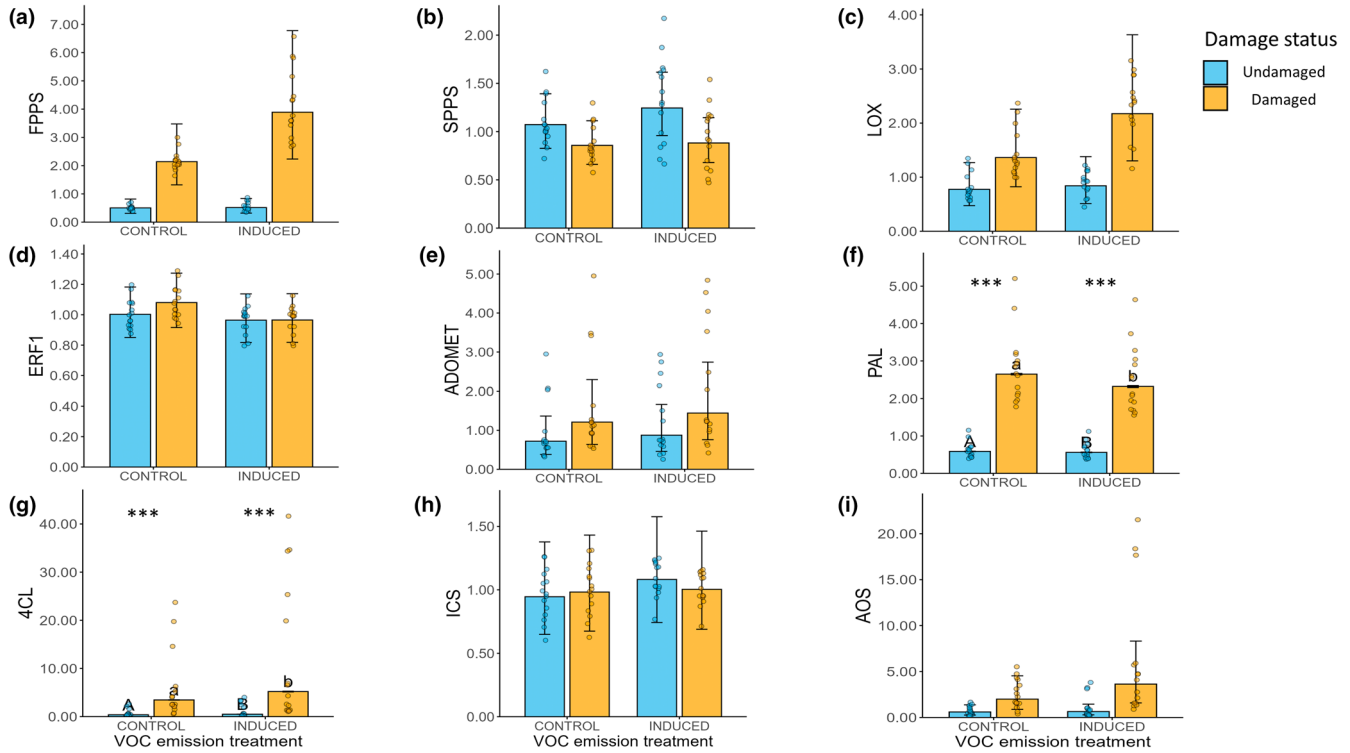

FIGURE 4 Effect of volatile organic compound (VOC) emission treatment (control: Constitutive emissions; induced: Emissions from *Spodoptera exigua*-damaged plants) on the expression of defence-related genes in undamaged and *S. exigua*-damaged potato (*Solanum tuberosum*) plants. Genes analysed: (a) farnesyl pyrophosphate synthase 1-like (*FPPS*), (b) solanesyl diphosphate synthase 3 (*SPPS*), (c) lipoxygenase (*LOX*), (d) ethylene response factor 1 (*ERF1*), (e) S-adenosylmethionine synthase 2 (*ADOMET*), (f) phenylalanine ammonia-lyase-like (*PAL*), (g) 4-coumarate-Co ligase 2 (*4CL*), (h) isochorismate synthase chloroplastic-like (*ICS*) and (i) allene oxide synthase (*AOS*). Bars represent estimated marginal means (EMMs) and 95% CIs based on GLMMs ($N = 15$). Data points (faded) are also shown. Asterisks above bars indicate significant differences between damage treatments (***$p < 0.001$), and different capital or lowercase letters indicate significant differences ($p < 0.05$) based on Tukey's post hoc tests between VOC emission treatments for undamaged and damaged plants, respectively. Statistical test results are provided in Table 2.

4 | DISCUSSION

Our results demonstrate that exposure to artificial VOC blends mimicking those released by plants damaged by *S. exigua* feeding boosts resistance in potato plants. Namely, we found that the feeding bioassay showed that exposure to the induced VOCs blend drove a reduction in herbivory relative to plants exposed to the control VOCs blend. In consonance with this finding, genetic analyses revealed higher expression of *4CL* under induced VOC exposure and, importantly, a greater expression of this gene in response to damage under the VOCs induced emissions than under the control emissions treatment, consistent with the idea of an effect of VOC priming on defence induction in potato plants. Together, these concurrent findings contribute in a novel way to a mechanistic understanding of volatile blend-specific signalling effects and molecular-level responses behind defence induction and resistance in potato.

The damage bioassay showed that potato plants exposed to the induced VOCs blend exhibited a lower proportion of leaf area loss to *S. exigua* feeding compared to plants exposed to the control VOCs blend. This finding is indicative of greater resistance in plants exposed to induced VOC blends, with similar outcomes reported in other crop studies making use of artificial emitters. For example, in maize plants (*Zea mays*), exposure to a blend of GLVs, including (Z)-3-hexenal, (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate, enhanced resistance to *S. exigua* in wheat plants, as evidenced by elevated JA production and VOC emissions. In such study, the response was triggered by simulated *S. exigua* damage, including mechanical wounding and regurgitant application (Engelberth et al., 2004). Likewise, artificial exposure to nonatriene, a compound also included in our VOC blend, increased resistance of sweet potato (*Ipomoea batatas*) plants to *Spodoptera lituria*: larvae feeding on nonatriene-exposed plants gaining significantly less mass compared to larvae feeding on unexposed plants (Meents et al., 2019). Admittedly, the observed reduction in damage on potato plants was relatively modest. It is possible that the strength and persistence of VOC perception in receiver plants is limited under controlled exposure, leading to partial or transient priming of defensive pathways rather than a full activation of resistance (Witkowski et al., 2025). Also, *S. exigua* is a highly polyphagous herbivore possibly capable of compensating for induced plant defences through adjusted feeding rates, detoxification mechanisms or altered host use as shown in previous work with this pest (Breeschoten et al., 2019; Paudel et al., 2013). On the

other hand, we cannot reject the possibility that the observed reduction in leaf damage reflects an early stage of a stronger response that could become more pronounced under repeated exposures to damage (Girón-Calva et al., 2012; Shiojiri et al., 2012). Even so, effects equal to or even stronger than that observed may be of lesser importance for crop yield under field conditions (Karban, 2015; Lynch et al., 2006). In this sense, trade-offs between defence induction and plant growth (Brosset & Blande, 2022; Yip et al., 2019; Züst & Agrawal, 2017) may constrain benefits to crop production via boosted resistance and should be accounted for. As a first step, our findings point to the need for field assessments to test for the strength of signalling effects on crop resistance and resulting implications for yield.

Induced VOC emissions also affected gene expression, namely *PAL* and *4CL*, both associated with the SA pathway, albeit contrastingly. While *PAL* was significantly downregulated, *4CL* was strongly upregulated in response to exposure to the induced VOC blend. Importantly, the VOC emissions treatment influenced gene expression levels in response to herbivory: We found a markedly higher expression of *4CL* in response to damage under the induced VOCs blend relative to that achieved under the control VOCs blend, consistent with the idea of VOC-related priming of the induction of defensive metabolites associated with this gene (see discussion ahead). The fact that *PAL* instead showed a weaker increase in expression under the induced than control VOCs blend might suggest a process of 'fine-tuning' within the phenylpropanoid pathway in response to prior exposure to induced VOCs. Under this view, in response to damage, plants exposed to induced VOCs appear to reduce to a greater extent overall input capacity via *PAL* while enhancing the flux through the specific metabolic pathway associated with *4CL* induction. This mechanism has been suggested to optimize the use of available hydroxycinnamic acids for the synthesis of defence compounds such as lignins and flavonoids associated with *4CL*, rather than for SA or other derivatives associated with *PAL* (Dixon et al., 2002; Fraser & Chapple, 2011; Gallego-Giraldo et al., 2011). Such compounds act as both physical (structural) and chemical defences, including barriers against attackers and the deterrence of feeding, development and oviposition of herbivores. For instance, lignin deposition in the cells correlates with enhanced insect resistance, and flavonoids act as effective chemical defences against chewing insects (Grover et al., 2022; Yang et al., 2025; Yao et al., 2023; Zhang et al., 2022). Relatedly, other studies have reported the upregulation of genes associated with flavonoid biosynthesis (e.g. chalcona sintasa, *CHS*, and chalcone isomerase, *CHI*, homologues) implicating a priming effect in response to exposure to artificial VOCs or volatile blends (Li et al., 2024; Witkowski et al., 2025).

Surprisingly, we detected no effect of the VOC emission treatment on the expression of *AOS* in response to damage (i.e. no significant interaction), a key enzyme in JA biosynthesis (Farmer & Goossens, 2019; León & Sánchez-Serrano, 1999). Defence against chewing insects is typically dependent on the JA pathway (Baldwin et al., 1997; Thaler et al., 2012; Zhang et al., 2017), and previous studies have reported priming effects on JA-related genes following artificial VOC exposure (Li et al., 2024; Pérez-Hedo et al., 2021; Witkowski et al., 2025). This discrepancy may be explained by a shortened priming phase, during which gene expression peaks within 24 h after attack and declines after 48 h; this transient response may have gone undetected in our experiment, as we measured expression 48 h after *S. exigua* feeding. That said, we did find a trend for a greater increase in *AOS* expression in response to damage under induced than control VOC emissions, suggesting a priming effect was present but was not strong enough to be statistically detected given the above limitation. Interestingly, in one of the above JA-related studies, Witkowski et al. (2025) reported that priming associated with the expression of JA-related genes in *Solidago altissima* did not result in higher resistance to the generalist insect *Helicoverpa zea*. Based on our findings, however, we cannot rule out that an upregulation of *AOS* or other JA-related genes contributed to damage reduction in potato plants exposed to induced VOCs. Still, it is noteworthy that despite the marked increase in *4CL* expression, the reduction in herbivore damage was relatively modest. This apparent mismatch between transcriptional activation and phenotypic outcome, together with the absence of a clear induction of JA-related genes, could be indicative of active suppression of plant defence end products by the herbivore. Indeed, previous studies in *Arabidopsis thaliana* have shown that effector compounds present in the saliva of *S. exigua* can activate the SA signalling pathway, which in turn antagonizes JA-mediated defences that are typically effective against chewing herbivores (Paudel et al., 2013). Such hormonal crosstalk could attenuate JA-dependent defence responses, resulting in only partial resistance despite strong upregulation of genes such as *4CL*. In sum, the observed gene expression changes could reflect an activated but functionally constrained defence state, where herbivore-driven suppression limits the translation of transcriptional responses into effective protection.

To summarize, our findings demonstrate that artificial exposure of potato plants to VOC blends mimicking emissions under *S. exigua* feeding can reduce insect herbivory on plants. This effect appears to be linked to increased expression of *4CL*, a gene involved in lignin and flavonoid biosynthesis, suggesting a targeted enhancement of chemical defences via these pathways (but without discarding the influence of other genes). These results provide valuable insights into the potential of VOC-mediated induced resistance via, for example, artificial, field-based emitters as a sustainable pest management strategy in this crop (e.g. Tholl et al., 2021), but also point to important challenges in progressing towards more realistic settings and obtaining biologically significant effects. For instance, the metabolic costs associated with de VOC exposure-triggered novo biosynthesis of defence compounds may constrain plant growth and tuber development via trade-offs, potentially reducing yield and therefore leading to economic implications. Additionally, herbivores may adapt to or plastically cope with enhanced plant defences over time, potentially diminishing the medium- to long-term effectiveness of VOC-based strategies. Also, the use of artificial VOC emitters under field conditions faces limitations affecting efficacy related to environmental variability, maintaining chemical stability

and standardized release kinetics, as well as economic and logistical constraints related to large-scale application. The use of artificial emitters, per the approach (methodology and tools) used in our study (e.g. emitters used, greenhouse conditions), should therefore be viewed as an intermediate phase to mechanistically assess the role of compound blends, which then informs efforts that scale-up to develop logistically and economically viable volatile-based pest control strategies under realistic greenhouse or field conditions. Here, VOC-induced defences can be optimized with complementary approaches such as real-time odour-based monitoring systems that detect pest-associated VOCs and lead to timely (e.g. Choi et al., 2025), more effective and less costly defence induction responses. At the same time, VOC-mediated pest control should not be viewed in isolation but rather implemented as part of Integrated Pest Management frameworks that make use of other complementary control methods to maximize efficacy.

Lastly, research gaps worth considering in developing applied research could consider VOC-mediated effects across different time points post-damage, and assess resistance outcomes against different herbivore species or guilds (often involving simultaneous or sequential attacks) and pathogens as well. In the case of potato, greenhouse and field trials could be conducted using other herbivores, including the specialist Colorado potato beetle or the aphid *Myzus persicae*, and test whether ecologically relevant induced blends trigger defence priming in potato and other related crop species. Starting with experiments under controlled conditions and then transitioning to field-based studies using cost-effective technologies will help build the necessary knowledge to design as well as test the applicability and effectiveness of VOC-mediated approaches (e.g. use of artificial emitters) in crop management under realistic agricultural settings.

AUTHOR CONTRIBUTIONS

Xoaquín Moreira and Luis Abdala-Roberts conceived the ideas; Lucía Martín-Cacheda and Xoaquín Moreira designed the methodology; Lucía Martín-Cacheda and Alessio Tei collected the data and performed the experiments; Lucía Martín-Cacheda, Gregory Röder and Alessio Tei performed the chemical and defoliation analyses; Yolanda Arnaiz performed the molecular analyses; Xoaquín Moreira and Gregory Röder contributed reagents, materials and analysis tools; Lucía Martín-Cacheda analysed the data and led the writing of the manuscript. Xoaquín Moreira and Luis Abdala-Roberts contributed critically to the drafts. All authors gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data and code are available from the Zenodo repository: https://doi.org/10.5281/zenodo.20925872 (Martín-Cacheda et al., 2026).

STATEMENT ON INCLUSION

Our study brings together authors from a number of different countries, including scientists based in the country where the study was carried out. All authors were engaged early on with the research and study design to ensure that the diverse sets of perspectives they represent were considered from the onset. Whenever relevant, literature published by scientists from the region was cited.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Diagnostic plots from *DHARMa* for the GLMM of proportion of damage (with all observations: 180).

Figure S2. Diagnostic plots from *DHARMa* for the refitted GLMM of proportion of damage excluding 5 outliers (175 observations).

Figure S3. Diagnostic plots from *DHARMa* for the GLMMs of relative expression of each gene.

Table S1. List of the 10 synthetic VOCs used in the VOC emission treatment (both control and induced).

Table S2. Back-transformed estimated marginal means (EMMs) with their 95% confidence intervals (95% CIs) for gene expression under damage status (undamaged vs. damaged plants).

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