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Apparent inhibition of induced plant volatiles by a fungal pathogen prevents airborne communication between potato plants

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Abstract

Plant communication in response to insect herbivory has been increasingly studied, whereas that involving pathogen attack has received much less attention. We tested for communication between potato (*Solanum tuberosum*) plants in response to leaf infection by the fungal pathogen *Sclerotinia sclerotiorum*. To this end, we measured the total amount and composition of volatile organic compounds (VOCs) produced by control and infected emitter plants, as well as tested for induced resistance of receiver plants exposed to VOCs from emitters. We further tested for changes in the expression of defensive genes due to pathogen infection. Fungal infection did not significantly affect the total amount or composition of VOCs produced by emitter plants. Correspondingly, we found no evidence of higher resistance to the pathogen in receiver plants exposed to VOCs from infected emitters relative to control emitters. Molecular analyses indicated that pathogen infection drove a down-regulation of genes coding for VOC precursors, potentially explaining the absence of pathogen effects on VOC emissions and thus of communication. Overall, these results indicate no evidence of airborne communication between potato plants in response to fungal infection and point at pathogen inhibition of VOC emissions as a likely explanation for this result.

KEYWORDS

defensive genes, emitter plants, plant communication, receiver plants, *Sclerotinia sclerotiorum*, *Solanum tuberosum*, volatile organic compounds

1 | INTRODUCTION

Plants perceive and respond to complex blends of volatile organic compounds (VOCs) emitted by conspecific and heterospecific neighbouring plants (Heil & Karban, 2010; Karban, Yang, & Edwards, 2014). Such responses involve either priming (a physiological process by

which plants prepare to respond more rapidly or intensely to impending biotic stress; Mauch-Mani, Baccelli, Luna, & Flors, 2017) or full induction of defences by 'receiver' plants when exposed to incoming VOCs released by attacked neighbouring plants ('emitters'), ultimately boosting plant resistance against herbivores (Karban, 2015; Moreira & Abdala-Roberts, 2019). This phenomenon of plant–plant airborne interaction through VOCs, hereafter referred to as communication, has been mainly reported with respect to attack by

phytophagous insects in over 30 plant species, including agricultural crops and tree species (Heil & Karban, 2010; Karban et al., 2014), and is now recognized as an important (and potentially widespread) modulator of plant defensive responses and induced resistance.

Plant pathogens are common in many types of plant communities, and are also important in cultivated systems (Burdon & Thrall, 2008; Morris & Moury, 2019). In some instances, particularly in tropical communities, they are arguably equally or more important drivers of plant abundance and diversity than herbivores (Bagchi et al., 2014; Bell, Freckleton, & Lewis, 2006; Freckleton & Lewis, 2006) and have a pervasive influence on plant defence expression (Biere & Goverse, 2016; Cipollini & Heil, 2010). Accordingly, considerable effort has been invested in understanding the physiological and biochemical basis of induced plant resistance to pathogens (Glazebrook, 2005; Hatcher, Moore, Taylor, Tinney, & Paulb, 2004). Research has shown that pathogens often trigger the upregulation of potent defensive responses in their host plants, although in some cases they have also been shown to manipulate or inhibit plant defences by dampening or fully suppressing induced responses (Abramovitch & Martin, 2004; Cui et al., 2005; Moreira, Abdala-Roberts, & Castagneyrol, 2018). In particular, several studies have reported on the effects of pathogens on plant VOCs (reviewed by Hammerbacher, Coutinho, & Gershenzon, 2019), with likely implications for plant communication. In an early study, Shulaev, Silverman, and Raskin (1997) found that increased emissions of methyl salicylate by wild tobacco plants infected with the tobacco mosaic virus boosted resistance against this pathogen in neighbouring healthy plants. Similarly, Riedlmeier et al. (2017) reported that α-pinene and β-pinene emitted by _Arabidopsis thaliana_ plants infected with _Pseudomonas syringae_ conferred resistance against this pathogen in uninfected neighbouring plants. In addition, another recent study found changes in VOCs emissions in response to insect and pathogen attack in tomato plants, which were in turn associated with increased resistance in healthy plants that were exposed to VOCs from these infected plants (Zhang et al., 2019). These findings suggest that pathogen-driven plant communication is potentially widespread (Heil & Adame-Álvarez, 2010; Quintana-Rodriguez et al., 2015; Yi, Heil, Adame-Álvarez, Ballhorn, & Ryu, 2009), and call for further work uncovering its mechanisms by capitalizing on already-existing knowledge of the physiological and molecular basis of plant–pathogen interactions (Hammerbacher et al., 2019).

In this study, we investigated whether airborne communication occurred between potato (_Solanum tuberosum_) plants in response to leaf infection by the generalist fungus _Sclerotinia sclerotiorum_, a common pathogen in potato plantations in both the Old and New World. To this end, we used a commercial variety of potato commonly cultivated worldwide and measured the severity of pathogen infection (i.e. a proxy of induced resistance) for 'receiver' plants exposed to VOCs released by 'emitter' plants infected by the pathogen versus intact (control) emitters. To assess chemical and molecular changes potentially mediating communication, we tested pathogen effects on emitter total emission and composition of VOCs, as well as the expression of genes associated with induced defences. We

additionally evaluated whether pathogen infection resulted in induced resistance against subsequent pathogen infection in receiver plants and compared any such response to induced resistance via VOCs. By addressing these goals, the present study provides a novel assessment of plant communication in response to pathogen infection in potato plants and further assesses its underlying plant chemical- and molecular-level responses. Results contribute to a better understanding of the mechanisms by which pathogens affect plant defences and its consequences for plant volatile emissions and communication.

2 | MATERIAL AND METHODS

2.1 | Study system

Solanum tuberosum L. (Solanaceae) is a perennial herb that grows up to 60 cm high and propagates via seeds and tubers. It was first domesticated in modern-day southern Peru and north-western Bolivia between 8,000 and 5,000 BC (Hijmans & Spooner, 2001), and later introduced to Europe from the Americas during the second half of the 16th century. Currently, _S. tuberosum_ is the world's fourth-largest food crop after maize, wheat, and rice (FAOSTAT, 2017).

Due to the intensification of agriculture, potato plantations in both the Old and New World have faced many challenges in the last decades, including elevated threat of pests and diseases (Alyokhin, Vincent, & Giordanengo, 2013). Notably, the generalist fungus Sclerotinia stem rot [_S. sclerotiorum_ (Lib.) de Bary, Helotiales: Sclerotiniaceae] is a highly damaging disease in potato (Bolton, Thomma, & Nelson, 2006), causing the so-called 'white mold' disease in stems and leaves (Johnson & Atallah, 2014). It is classified as a necrotrophic pathogen and infection can rapidly spread to the whole plant, reaching the main stem in 4–5 days (Bolton et al., 2006). Infection eventually causes the decay of damaged tissues, eventually leading to plant death (Bolton et al., 2006).

2.2 | Effect of pathogen infection on emitter VOCs and plant communication

In July 2019, we individually planted potato tubers (_S. tuberosum_ L. cv. Kennebec) in 4-L pots containing potting soil with peat (Gramoflor GmbH & Co. KG Produktion, Vechta, Germany). Plants were grown in a glasshouse under controlled light (minimum 12 hr per day, photosynthetically active radiation = 725 ± 19 µmol m^{-2} s^{-1}) and temperature (10°C night, 25°C day), and were watered twice a week. Three weeks after tuber planting, we placed pairs of plants of similar size in $37.5 \times 37.5 \times 96.5$ cm plastic cages to prevent cross-communication between replicate pairs. One plant of each pair acted as the emitter and the other was the receiver (emitter height: 26.38 ± 1.26 cm, receiver height: 28.44 ± 1.40 cm). Within each cage, emitter and receiver plants were placed 20 cm apart to avoid direct physical contact. We randomly assigned emitter plants to one of two treatments: (a) _S. sclerotiorum_ infection (i.e. pathogen-induced plants) or (b) control

(intact plants). In total, there were 44 cages (22 per treatment) for a total of 44 receivers and 44 emitter plants. For the induction treatment, we followed methods for pathogen culturing and inoculation from previous work with *Brassica* plants (Madloo, Lema, Francisco, & Soengas, 2019). Briefly, inoculation consisted of applying three punctures on the upper side of two newly developed leaves using an awl of 1 mm diameter, and then adding agar plugs (0.4 cm in diameter) containing *S. sclerotiorum* mycelia on the punctured site. For the control treatment, we punctured the leaves as above to control for slight mechanical damage caused by puncturing but did not add the pathogen-containing agar. Following from previous work testing for induced changes VOCs emissions that used a similar methodology (e.g. Allmann et al., 2013), puncturing applied was assumed to have a negligible effect on the induction of VOCs and therefore not affect the test of the pathogen induction treatment. Two days after pathogen inoculation, we removed emitter plants to measure their VOC emissions whereas receivers remained inside the cages for a bioassay of induced resistance (see ahead).

We collected aboveground VOCs produced by emitter plants following Rasmann, Erwin, Halitschke, and Agrawal (2011). Briefly, we bagged plants with a 2 L Nalophan bag, and we trapped VOCs on a charcoal filter (SKC sorbent tube filled with Anasorb CSC coconut-shell charcoal) for 90 min at a rate of 0.25 L min^{-1}. We eluted traps with 150 μL dichloromethane (CAS#75–09-2, Merck, Dietikon, Switzerland) to which we had previously added two internal standards [*n*-octane (CAS#111–65-9) and nonyl acetate (CAS#143–13-5), 200 ng of each in 10 μL dichloromethane]. We subsequently injected 1.5 μL of each sample onto an Agilent 7890B Gas Chromatograph (GC) coupled with a 5977B Mass Selective Detector fitted with a 30 m × 0.25 mm × 0.25 μm film thickness HP-5MS fused silica column (Agilent, Santa Clara, CA). We operated the GC in split-less mode with helium as the carrier gas (flow rate 1 mL min^{-1}). The GC oven temperature program was: 3.5 min hold at 40°C, 5°C min^{-1} ramp to 250°C, and 1 min hold at 250°C. We identified volatile terpenes using the library NIST Standard Reference Database 1A v17 and by comparing with commercial standards when available. We measured the total emission of individual VOCs as a proportion of the internal standards (Moreira, Nell, Katsanis, Rasmann, & Mooney, 2018).

Shortly after collecting emitter VOCs (i.e. later the same day), we performed a bioassay to test whether exposure to VOCs from infected emitters boosted pathogen resistance in receiver plants relative to those exposed to VOCs from control emitters. For this, we inoculated two newly developed leaves per receiver plant with *S. sclerotiorum* following the same procedure described above for the inoculation and induction of emitter plants. Two days after receiver inoculation, we sampled infected leaves and photographed them with a Nikon COOLPIX P100 digital camera (10.3 effective megapixels, ×26 zoom NIKKOR). We estimated the percentage of necrotic area due to pathogen infection using ImageJ software (version 1.52a; LOCI, University of Wisconsin) and averaged values across leaves to obtain the mean value per plant.

In September 2019, we repeated the previously described communication experiment but using larger plants (emitters:

53.82 ± 2.09 cm; receivers: 50.00 ± 2.30 cm) and a slightly different methodology. In this case, we increased tofour days the amount of time receiver plants were exposed to emitters prior to conducting the resistance bioassay for receivers. After this exposure period, emitters were removed from cages to collect their VOCs, receivers were inoculated shortly after the same day, and measurements of necrotic area of receivers were conducted three (rather than two as above) days later. Based on this, relative to the first experiment, this second one involved two additional days of infection since inoculation before emitter VOCs were collected, whereas for receivers it involved two additional days of exposure to emitters and one additional day since inoculation for the assessment of induced resistance. In addition, compared to the first experiment, this experiment involved a slightly larger sample size with 50 cages (25 per treatment).

We jointly analysed the above experiments by running a general linear model testing for the effects of emitter induction treatment (control vs. *S. sclerotiorum* infection), experiment, and their interaction (all fixed factors) on total VOC emission by emitter plants using PROC GLM in SAS (SAS 9.4 System, SAS, Cary, NC) (Littell, Milliken, Stroup, Wolfinger, & Schabenberger, 2006). We also included height of emitter plants as a covariate to account for differences in size which may influence induced responses, as well as log-transformed total VOC emission to achieve normality of residuals. In addition, we ran a permutational multivariate analysis of variance (PERMANOVA) model also testing for an effect of induction treatment, experiment, and their interaction on VOC composition (using abundances of each compound). This analysis was based on 10,000 permutations and was performed with the 'vegan' package in R ver. 4.0.2 software (Oksanen et al., 2016). To visualize these results, we conducted a principal coordinates analysis based on Bray–Curtis pairwise dissimilarities, and graphed the centroids of each induction treatment effect (Moreira et al., 2019). We also identified influential VOCs as those having strong associations ($R^2 > 0.50$) for the first two ordination axes (using 'envfit' in vegan; (Oksanen et al., 2016), and displayed these relationships using biplot arrows with length scaled to R^2 values.

We then ran a generalized linear model also testing for the effects of emitter induction treatment (control vs. *S. sclerotiorum* infection), experiment, and their interaction (all fixed factors) on the percentage of necrotic leaf area of receiver plants. For this, we used PROC GLIMMIX in SAS 9.4 (Littell et al., 2006) with a binomial distribution error and logit-link function. We again included height of receiver plants as a covariate.

2.3 | Effect of induction treatment on emitter gene expression

To assess molecular changes associated with the expression of emitter plant defences due to pathogen infection, in October 2019 we individually planted tubers of *S. tuberosum* L. cv. Kennebec in 4-L pots containing potting soil with peat. Plants were grown as described in the previous experiments. Three weeks after tuber planting, we placed potato plants in a 37.5 × 37.5 × 96.5 cm plastic cage (plant

height: 29.65 ± 1.56 cm). As above, we assigned plants to one of two treatments: (a) subjected to *S. sclerotiorum* infection (i.e. pathogen-induced plants) or (b) control (intact plants). In total, there were 26 cages (13 per treatment) for a total of 26 plants. Methodological procedures used for pathogen-induced and control groups were the same as those described in the previous experiments. Three days after pathogen exposure, we collected three leaves per plant, immersed them in liquid nitrogen and kept them at −80°C until subsequent DNA extraction.

We ground leaf material in a mortar until a fine powder and then performed total RNA isolation on a Maxwell RSC Instrument (Promega, Madison, WI) using Maxwell 16 LEV plant RNA Kit (Promega, Madison, WI), following the manufacturer's instructions, except for the addition of Fruit Mate (TaKaRa, Shiga, Japan) in the homogenization solution of the Promega RNA kit, in a ratio of 1:1. We used 1 μg of total RNA for first-strand cDNA synthesis, using GoScript(TM) Reverse Transcription System (Promega, Madison, WI) following the manufacturer's instructions and finally we diluted (1:2 v:v) this extract with nucleotide-free water. We amplified specific sequences of 12 genes (three housekeeping and nine target genes) in a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) using SYBR Green (GoTaq qPCR Master Mix, Promega). Three of the genes analysed are terpene synthase genes (Dudareva, Klempien, Muhlemann, & Kaplan, 2013; Zhou & Pichersky, 2020), namely: farnesyl pyrophosphate synthase 1-like (FPPS) which is the precursor of sesquiterpenes, solanesyl diphosphate synthase 3 (SPPS) which is the precursor of monoterpenes and lipoxygenase (LOX) which is the precursor of green leaf volatiles (Table S1). The other six genes analysed are involved in the three main pathways of plant defence induction and are involved in both local and systemic acquired resistance to herbivores and pathogens (Chen, Zheng, Huang, Lai, & Fan, 2009; Turner, Ellis, & Devoto, 2002; Wang, Li, & Ecker, 2002), namely: ethylene response factor 1 (ERF1) and *S*-adenosylmethionine synthase 2 (ADOMET) which are involved in the ethylene pathway, phenylalanine ammonia-lyase-like (PAL), 4-coumarate-CoA ligase 2 (4CL) and isochorismate synthase chloroplastic-like (ICS) which are involved in the salicylic acid pathway, and allene oxide synthase (AOS) which is involved in the jasmonic acid pathway (Table S1). The primers used were previously published in the literature or were designed based on gene sequences published in NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using PRIMER 3 (Rozen & Skaletsky, 2000). More information about genes, primer sequences and annealing temperatures for PCR amplification are shown in Table S1. We calculated real-time amplification efficiencies for each gene from the slopes of the standard curves using LinRegPCR V12.17 (Ruijter et al., 2009; Tuomi, Voorbraak, Jones, & Ruijter, 2010). We used BestKeeper software to rank the housekeeping genes and we selected RPN7 gene to normalize the data (Pfaffl, Tichopad, Prgomet, & Neuvians, 2004). We followed the mathematical model proposed by Pfaffl (2001) for relative quantification in RT-PCR to calculate relative expressions for each gene. We tested the effect of induction treatment on the expression of each gene using general linear

models with PROC GLM in SAS ver. 9.4. We also included total height of plants as a covariate and log-transformed LOX, ICS and AOS data to achieve normality of residuals.

2.4 | Effect of pathogen infection on induced resistance by infected plants

We additionally evaluated whether pathogen infection resulted in induced resistance against subsequent pathogen infection and compared these results to any such induced resistance via VOCs. To this end, in September 2020 we individually planted tubers of *S. tuberosum* L. cv. Kennebec in 4-L pots containing potting soil with peat. Plants were grown as described in the previous experiments. Three weeks after tuber planting, we placed one potato plant in a 37.5 × 37.5 × 96.5 cm plastic cage (plant height: 21.34 ± 0.72 cm). As above, we assigned plants to one of two treatments: (a) subjected to *S. sclerotiorum* infection (i.e. pathogen-induced plants) and (b) control (intact plants). In total, there were 38 cages (19 per treatment) for a total of 38 plants. Methodological procedures used for pathogen-induced and control treatments were the same as those described in the previous experiments. To test for induced resistance, three days after initial *S. sclerotiorum* inoculation we inoculated two newly developed leaves on each control and pathogen-infected plant following the same inoculation procedures described above. Three days after, we collected these two latter infected leaves and photographed them with a Nikon COOLPIX P100 digital camera. We estimated the percentage of necrotic area due to pathogen infection using ImageJ software and averaged values across leaves to obtain a mean value per plant.

We ran a generalized linear model testing for the effects of initial induction treatments (control vs. *S. sclerotiorum* infection) on the percentage of necrotic area of new leaves after subsequent pathogen inoculation. For this, we used PROC GLIMMIX in SAS 9.4 (Littell et al., 2006) with a binomial distribution error and logit-link function. We again included plant height as a covariate.

3 | RESULTS

3.1 | Pathogen effects on emitter VOCs and receiver induced resistance

We detected a total of 39 VOCs in the headspace of emitter potato plants, of which 32 were positively identified (Table S2). The three most abundant compounds which accounted, on average, for 67.42 ± 7.90% of total VOC emission across samples were: β-caryophyllene, (Z)-β-farnesene and α-cubenene (Table S2). We found that the induction treatment (control vs. *S. sclerotiorum* infection) did not significantly affect the total production (back-transformed log data: control = 255.85 ± 52.77 ng hr^{-1}; induced = 322.29 ± 66.51 ng hr^{-1}) (Table 1 and Figure 1a) or composition of VOCs released by emitter plants (PERMANOVA: Table 1 and

TABLE 1 Results from general linear models testing the effect of induction treatment (control vs. *S. sclerotiorum* infection) on total VOCs released by emitter potato (*S. tuberosum*) plants and the percentage of leaf necrotic area due to pathogen infection for receiver potato plants

Response	DF$_{num,den}$	Treatment (T)		Experiment (E)		T × E		Plant size	
		F	p	F	p	F	p	F	p
VOC emission	1, 87	0.73	.395	12.18	**<.001**	0.01	.905	0.00	.971
VOC composition	1, 87	1.36	.209	4.01	**.020**	1.98	.075	9.29	**<.001**
Leaf necrotic area	1, 88	0.21	.652	37.47	**<.001**	0.03	.868	25.94	**<.001**

Note: For VOC composition, we used a permutational multivariate analysis of variance (PERMANOVA) model (using log-transformed data). Because the experiment was carried out in two replicate iterations, we also included the effect of the experimental iteration and its interaction with induction treatment in the statistical model. Plant size (of emitters for VOCs and of receivers for pathogen infection) measured as total height was used as a covariate. *F*-values, degrees of freedom and associated significance levels (*p*) are shown. Significant *p*-values (*p* < .05) are in boldface.
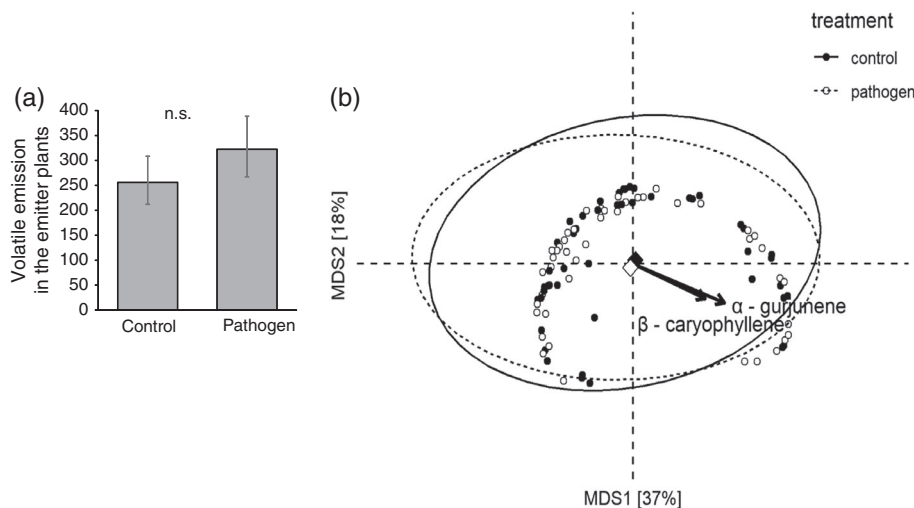Abbreviation: VOC, volatile organic compound.

FIGURE 1 (a) Effects of induction treatment (control vs. leaf infection by *S. sclerotiorum*) on the total emission of volatile organic compounds (in nanograms per hour, back-transformed from log data) by emitter potato (*S. tuberosum*) plants. Bars are least-square means ± *SEM* (N = 47). (b) Unconstrained ordinations showing the effects of emitter induction treatment on the log-transformed composition of volatile organic compounds produced by emitter plants. Biplot arrows show associated linear trends with volatiles, scaled to reflect relative magnitude of effects based on R^2 values (R^2 > 0.50, *p* < .001). The emitter induction treatment ordination displays control and pathogen-induced centroids (represented as diamonds) and 95% ellipses. The first two axes of this ordination accounted for 55% of the treatment effect in volatile composition (37 and 18%, respectively). Statistics are shown in Table 1. n.s., non-significant

Figure 1b). In addition, the induction treatment did not significantly affect the percentage of necrotic leaf area of receiver plants (control = 25.79 ± 2.20%, induced = 25.17 ± 2.22%) (Table 1 and Figure 2). These results remained consistent across experiments as shown by the non-significant treatment by experiment interaction (Table 1).

3.2 | Pathogen effects on the expression of plant defence-related genes

For terpene synthase genes, we found that the induction treatment significantly affected the expression of LOX and FPPS, but not SPPS (Table 2 and Figure 3a–i). Specifically, expression of LOX and FPPS was 54 and 33% lower, respectively for infected plants relative to controls (Figure 3a,b). For genes associated with general induced

resistance responses, we found that the induction treatment significantly affected expression of ERF1, PAL and ICS, but not ADOMET, 4CL or AOS (Table 2). Specifically, the expression of PAL was 175% greater for infected plants compared to controls (Figure 3f), whereas the expression of ERF1 and ICS was 41 and 85% lower, respectively for infected plants (Figure 3d,h).

3.3 | Inducted resistance in response to pathogen infection

Prior pathogen infection significantly affected subsequent pathogen infection (Figure 4). Namely, plants previously infected with the pathogen exhibited a 23% lower mean percentage of necrotic leaf area after subsequent inoculation with the pathogen (9.56 ± 0.93%) compared to plants not previously infected (12.37 ± 0.93%) (Figure 4).
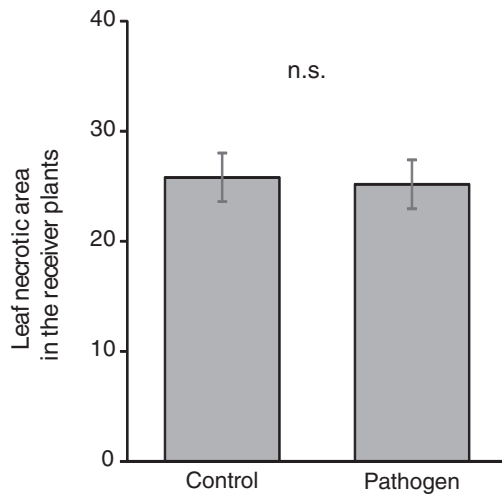
FIGURE 2 Effects of emitter induction treatment (control vs. leaf infection by *S. sclerotiorum*) on the percentage of leaf necrotic area for receiver potato (*S. tuberosum*) plants. Bars are least-square means ± *SEM* (*N* = 47). Statistics are shown in Table 1. n.s., non-significant

TABLE 2 Results from general linear models testing for the effects of induction treatment (control vs. *S. sclerotiorum* infection) on relative expression levels of genes associated to defences in potato (*S. tuberosum*) plants, namely: (a) lipoxygenase (LOX), (b) farnesyl pyrophosphate synthase 1-like (FPPS), (c) solanesyl diphosphate synthase 3 (SPPS), (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)

Gene	$DF_{num,den}$	Treatment		Plant size	
		F	*p*	*F*	*p*
LOX	1, 23	6.36	**.019**	4.84	**.038**
FPPS	1, 23	10.44	**.004**	0.01	.943
SPPS	1, 23	0.02	.880	1.18	.288
ERF1	1, 23	6.84	**.016**	0.07	.798
ADOMET	1, 23	2.12	.159	0.01	.958
PAL	1, 23	12.40	**.002**	6.63	**.017**
4CL	1, 23	0.24	.625	3.86	.062
ICS	1, 23	25.95	**<.001**	0.01	.988
AOS	1, 23	1.71	.203	2.95	.099

Note: Emitter plant size measured as total height was used as a covariate. *F*-values, degrees of freedom and associated significance levels (*p*) are shown. Significant *p*-values (*p* < .05) are in boldface.

4 | DISCUSSION

Our findings indicated that infection by *S. sclerotiorum* did not cause significant changes in total VOC emissions or composition of emitter potato plants, and that fungal infection in emitter plants did not affect the level of pathogen resistance of neigbouring receiver plants. These results counter previous studies which have found effects of pathogen infection on plant VOC emissions which in turn boost resistance in neighbouring uninfected plants, including both wild (Riedlmeier et al., 2017; Shulaev et al., 1997) and cultivated (Castelyn, Appelgryn, Mafa, Pretorius, & Visser, 2015; Quintana-Rodriguez et al., 2015; Zhang et al., 2019) species. One possible explanation for these findings is that the induction treatment was not strong enough to produce detectable changes in VOCs emissions. However, our methodological approach closely resembled previous work measuring plant responses to pathogen infection (reviewed by Hammerbacher et al., 2019; Sharifi, Lee, & Ryu, 2018), and, importantly, results remained unchanged across experiments despite differences in plant size, emitter exposure time to pathogen infection, and receiver exposure time to emitter VOCs. Moreover, expression levels of two of the three terpene synthase genes studied (LOX and FPPS), as well as two genes associated with general induced resistance responses (ERF1 and ICS), were significantly down-regulated after *S. sclerotorium* infection, suggesting that the fungus impaired the induction of plant VOCs emissions, therefore preventing plant-to-plant communication. At the same time, we found evidence of induced resistance for infected plants which shows that, contrary to among-plant VOC-mediated induced responses, within-plant induced responses are not hampered by prior pathogen infection.

The lack of effect of *S. sclerotiorum* on emitter VOCs, combined with the negative effects of infection on expression levels of terpene synthase genes, strongly suggests that this pathogen inhibits the induction of volatile compounds and that this in turn prevents effective communication with intact neighbouring plants. Accordingly, considerable work has shown that pathogens produce molecules, so-called effectors, aimed at manipulating plant defences for the pathogen's benefit, including the suppression of induced plant defences through biochemical mechanisms which modulate the plant's defence-signalling network (reviewed by Abramovitch & Martin, 2004; Pieterse & Dicke, 2007). For example, the necrotrophic fungus *Botrytis cinerea*, closely related to *S. sclerotiorum*, has been shown to suppress the jasmonic acid (JA) pathway in tomato (*Solanum lycopersicum*) plants (El Oirdi et al., 2011). Likewise, studies with tobacco and rice plants have shown that *P. syringae* and *Xanthomonas oryzae* suppress induced plant defences (Espinosa, Guo, Tam, Fu, & Alfano, 2003; Lee, Chung, Kang, Chung, & Lee, 2016), including reduced or altered VOC emissions (Lee et al., 2016; for soil symbiont effects on plant VOCs see Rasmann, Bennett, Biere, Karley, & Guerrieri, 2017). In addition, pathogen manipulation of plant defences has also been shown to result in increased vector attraction and facilitate the spread of vector-borne plant diseases (reviewed by Biere & Bennett, 2013). Further work is needed to determine the importance of pathogen-caused changes in VOCs involved in plant communication, plant-herbivore, and plant-centred tri-trophic interactions (e.g. Martini, Pelz-Stelinski, & Stelinski, 2014). Current challenges are determining whether there are overall differences in the prevalence or strength of plant communication involving herbivore versus pathogen attack, and whether there are relevant guild-specific effects in each case. Likewise, a better understanding of the implications for plant communication of
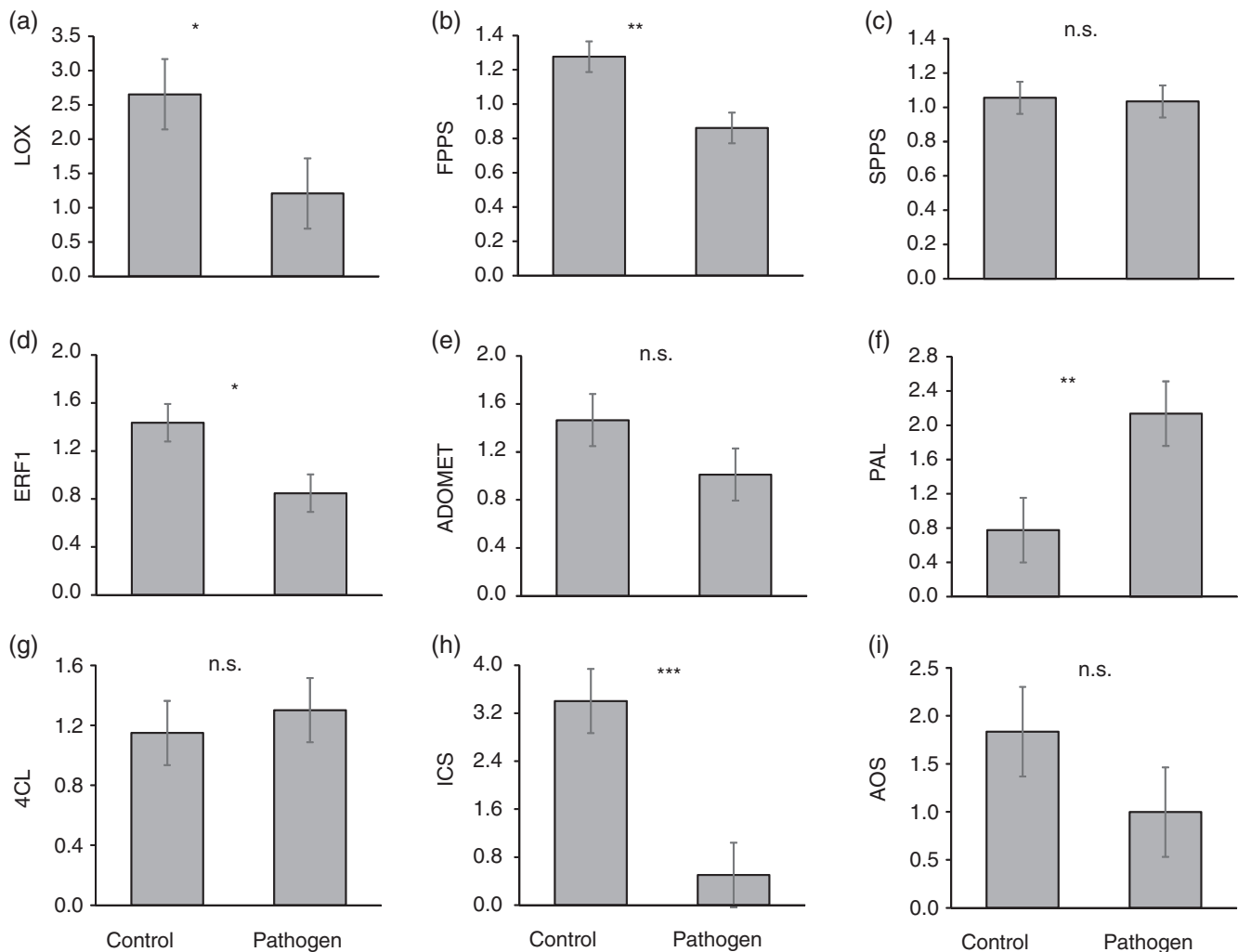
FIGURE 3 Effects of induction treatment (control vs. leaf infection by *S. sclerotiorum*) on the expression defence-related genes in potato (*S. tuberosum*) plants, namely: (a) lipoxygenase (LOX), (b) farnesyl pyrophosphate synthase 1-like (FPPS), (c) solanesyl diphosphate synthase 3 (SPPS), (d) ethylene 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). Bars are least-square means ± *SEM* (*N* = 23). For LOX, ICS and AOS, we show back-transformed log data. Asterisks above the bars indicate significant differences between induction treatments at *$p < .05$, **$p < .01$ and ***$p < .001$. Statistics are shown in Table 2. n.s., non-significant

interactions involving joint attack by insect herbivores and pathogens is necessary (Cardoza, Alborn, & Tumlinson, 2002). A recent meta-analysis by Moreira, Abdala-Roberts, and Castagneyrol (2018) showed that plant-mediated interactions between attackers (insect herbivores and pathogens) were generally weaker and less consistent when involving pathogens, but it remains unknown whether such patterns hold with respect to VOCs emissions and plant communication.

Interestingly, we found a significant and marked increase in PAL expression levels in response to pathogen infection. This enzyme is involved in the salicylic acid signalling pathway, it is a precursor of phenolic compounds biosynthesis (Chen et al., 2009), and its upregulation could explain the observed induced resistance of plants previously infected by this pathogen. Given the observed pattern of down-regulation of terpene synthase genes, taken together these findings suggest that *S. sclerotiorum* has seemingly opposite effects on

different components of the defensive metabolism of potato plants. This pathogen appears to concurrently boost the induction of some types of direct defences (e.g. phenolics associated with PAL) while inhibiting the induction of VOCs (associated with terpene synthase genes), with this having important implications for the outcome of plant–pathogen interactions. A plausible prediction is that *S. sclerotiorum* infection will predominantly affect within-plant induced resistance and the outcome of subsequent plant–pathogen interactions, while weakening or preventing VOC-mediated interactions involving plant–pathogen as well as other interactions (e.g. with insect herbivores or natural enemies that use these compounds as cues). Having said this, ICS and ERF1, associated with the salicylic acid and ethylene pathways (respectively), were down-regulated in response to fungal infection, suggesting an even more complex scenario whereby the fungus can also suppress some components of the salicylic acid
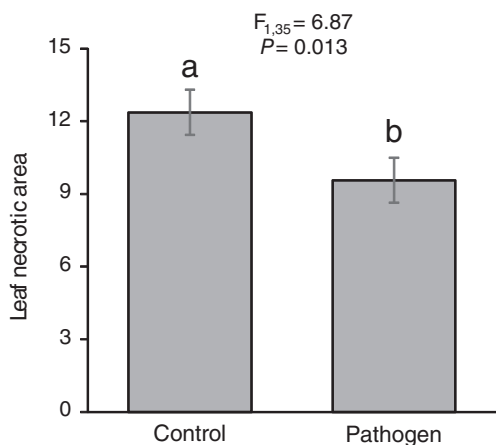
FIGURE 4 Effects of prior inoculation treatment (control vs. leaf infection by *S. sclerotiorum*) on the percentage of necrotic area for new leaves after subsequent pathogen infection on potato (*S. tuberosum*) plants. Bars are least-square means ± *SEM* (*N* = 19). *F*-values, degrees of freedom and associated significance levels (*p*) are shown. Different letter indicates a significant (*p* < .05) difference between control and infected plants

metabolism as well as other metabolic pathways associated to direct or indirect defences.

Before reaching broader generalizations, results should be interpreted in light of several features of our study. One of these is that we only used one potato cultivar (Kennebec) which is known for its fast growth and high yields (Akeley, Stevenson, & Schultz, 1948), and that results could differ for other varieties (Rowen & Kaplan, 2016). Breeding for fast growth has been shown to collaterally reduce defences (Turcotte, Turley, & Johnson, 2014; Whitehead, Turcotte, & Poveda, 2017), including the expression of induced plant defences due to insect herbivory (Moreira, Abdala-Roberts, Gols, & Francisco, 2018) and pathogen infection (Alarcon et al., 2015). Further work with other commercial potato varieties is therefore necessary to assess the generality of our findings. Relatedly, although there are several studies showing plant–plant communication in response to herbivory in domesticated crops (e.g. tomato, barley, maize, cotton; summarized in Heil & Karban, 2010; Karban et al., 2014), comparisons between domesticated varieties and wild accessions are needed to increase inference about the importance of this phenomenon in wild species and to understand domestication effects on VOC-mediated induced defences. In addition, it could also be that a longer exposure time to pathogen infection in emitter plants or of receivers to emitters would have yielded different results. In some species, VOC-mediated induction can take up to a week or more as shown for wheat by Castelyn et al. (2015), who reported induced resistance of receiver plants against leaf rust *Puccinia tritica* after 10 days of exposure to VOCs from emitter plants. Thus, while our results provide good evidence that *S. sclerotiorum* does not boost VOCs induction in emitter plants and that this precludes induced resistance in receiver plants after two or four days of exposure to infected neighbours, further experiments testing longer exposure times (as well as longer

bioassays) should be considered. In addition, detailed assessments of physiological and metabolic changes underlying the induction of defensive end products by receiver plants after response to VOCs exposure are needed.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
Xoaquín Moreira formulated the idea of the manuscript and wrote the first draft of the manuscript. Xoaquín Moreira, Rodrigo R. Granjel, Luis Abdala-Roberts and Pilar Soengas designed the experiment. Xoaquín Moreira, Viviana Pasch, Rodrigo R. Granjel and Pilar Soengas performed the experiment. Xoaquín Moreira, Pilar Fernández-Conradi, Viviana Pasch and Sergio Rasmann performed the chemical analyses. María de la Fuente performed the molecular analyses. Xoaquín Moreira, Sergio Rasmann and Ted C. J. Turlings contributed reagents/materials/analysis tools. Xoaquín Moreira and Carla Vázquez-González analysed the data. Luis Abdala-Roberts, Sergio Rasmann and Ted C. J. Turlings contributed critically to the writing.

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