

Plant genetic relatedness and volatile-mediated signalling between *Solanum tuberosum* plants in response to herbivory by *Spodoptera exigua*

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ABSTRACT

It has been proposed that plant-plant signalling via herbivore-induced volatile organic compounds (VOCs) should be stronger between closely related than unrelated plants. However, empirical tests remain limited and few studies have provided detailed assessments of induced changes in VOCs emissions across plant genotypes to explain genetic relatedness effects. In this study, we tested whether airborne signalling in response to herbivory between *Solanum tuberosum* (potato) plants was contingent on plant genetic relatedness, and further investigated genotypic variation in VOCs potentially underlying signalling and its contingency on relatedness. We carried out a greenhouse experiment using 15 *S. tuberosum* varieties placing pairs of plants in plastic cages, i.e. an emitter and a receiver, where both plants were of the same genotype or different genotype thereby testing for self-recognition, an elemental form genetic relatedness effects. Then, for half of the cages within each level of relatedness the emitter plant was damaged by *Spodoptera exigua* larvae whereas for the other half the emitter was not damaged. Three days later, we placed *S. exigua* larvae on receivers to test for emitter VOC effects on leaf consumption and larval weight gain (i.e. induced resistance). In addition, we used a second group of plants subjected to the same induction treatment with the same *S. tuberosum* varieties to test for herbivore-induced changes in VOC emissions and variation in VOC emissions among these plant genotypes. We found that herbivory drove changes in VOC composition but not total emissions, and also observed quantitative and qualitative variation in constitutive and induced VOC emissions among varieties. Results from the bioassay showed that the amount of leaf area consumed and larval weight gain on receiver plants exposed to damaged emitters were significantly lower compared to mean values on receivers exposed to control emitters. However, and despite genotypic variation in induced VOCs, this signalling effect was not contingent on plant genetic relatedness. These findings provide evidence of VOCs-mediated signalling between *S. tuberosum* plants in response to *S. exigua* damage, but no evidence of self-recognition effects in signalling contingent on variation in VOC emissions among *S. tuberosum* varieties.

Emitter induction treatment boosted herbivore resistance in receiver potato plants, but this signalling effect on receivers was not contingent on emitter-receiver relatedness.

1. Introduction

Plants perceive and respond to complex blends of volatile organic compounds (VOCs) emitted by conspecific and heterospecific neighbouring plants (Heil and Karban, 2010; Karban et al., 2014a). These responses frequently involve either priming or induction of defences by

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undamaged “receiver” plants when exposed to incoming VOCs released by herbivore-damaged neighbours (“emitters”), ultimately leading to heightened resistance against herbivory in receiving plants (Karban, 2015). This form of plant-plant signalling mediated by VOCs, also termed “plant-plant communication”, has been documented in over 40 plant species, including several agricultural crops and forestry species (Heil and Karban, 2010; Karban et al., 2014a), and is now a well-accepted phenomenon that has significantly enriched research and theory on plant defensive mechanisms. Further, and based on this growing evidence, methods that exploit VOCs-mediated plant communication have been proposed as a complementary strategy for promoting sustainable plant protection against pests and diseases (Stenberg et al., 2015; Pickett and Khan, 2016; Turlings and Erb, 2018), including the exogenous application of VOCs to mediate plant defence priming (Turlings and Erb, 2018; Ninkovic et al., 2021). Accordingly, a new stage in our understanding of plant-plant signalling and its application in crop management promises to unfold in the following decades, with particular emphasis on research aimed at uncovering ecologically key volatiles and the contingency of VOC induction on environmental conditions.

Recent advances highlight that VOC blends emitted by attacked plants can vary due to multiple biotic and abiotic factors with likely consequences on plant signalling (Moreira and Abdala-Roberts, 2019). One potentially important factor driving variation in plant VOC-mediated is plant genetic relatedness (Karban, 2021). A number of studies have shown that signalling is stronger among closely related plants than among unrelated ones, presumably dictated by genetically-based differences in VOC emissions dictating signalling specificity (Karban and Shiojiri, 2009; Karban et al., 2014b, 2016; Moreira et al., 2016, 2018a). Accordingly, some authors have invoked kin selection as a mechanism for the origin and maintenance of stronger plant communication among conspecifics (Karban and Shiojiri, 2009; Karban et al., 2013), arguing that communication between non-related individuals should be costly for the emitter plants as they would be increasing competitors’ fitness at the expense of their own (Heil and Ton, 2008; Heil and Karban, 2010). Alternatively, others have argued that plant-plant signalling has evolved as a by-product of plants using VOCs for rapid and efficient intra-plant signalling (between organs of the same plant, e.g. different branches in tree crown) when signalling via vascular connectivity is limited or less efficient (Frost et al., 2007; Heil and Silva Bueno, 2007). This debate points to the need for assessing the mechanisms that underlie the occurrence and strength of signalling, including the volatile compounds (and receptors) mediating genotype specificity in signalling (Turlings and Erb, 2018; Bouwmeester et al., 2019). A better understanding of plant genetically-based specificity in signalling will shed light on relatedness effects and the origins of plant-plant signalling, as well as inform VOC-based crop protection methods.

In this study, we tested whether VOC-mediated airborne communication between *Solanum tuberosum* L. (Solanaceae) plants in response to leaf herbivory by larvae of the generalist insect *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) was contingent on plant genetic relatedness, as well as further investigated VOC blends and compounds potentially underlying any such specificity. For this, we carried out a greenhouse experiment with 15 *S. tuberosum* varieties where we placed pairs of plants in plastic cages, i.e. an emitter and a receiver, where both plants were of the same genotype or of a different genotype. Then, for half of the cages within each level of relatedness the emitter plant was damaged by *S. exigua* larvae whereas for the other half the emitter was not damaged (controls). After exposure to emitter VOCs, we placed caterpillars on receivers and measured the amount of leaf damage and larval weight gain to test for induced resistance due to plant-plant signalling and whether any such effects were contingent on plant genetic relatedness, namely self-recognition. In addition, we performed a second greenhouse experiment under the same conditions using the same varieties to measure total emission and composition of VOCs in control and

damaged plants to test for differences in emissions among *S. tuberosum* varieties presumably underlying possible effects of relatedness on signalling. By addressing plant genetic relatedness effects and quantitative and qualitative changes in VOC emissions, the present study provides an insight into plant-based specificity in communication with potential applications in agricultural management of *S. tuberosum* pests.

2. Results

2.1. Effects of herbivory on emitter VOC emission

We detected a total of 29 VOCs in the headspace of *S. tuberosum* plants (Table 1). There was a significant effect of *S. tuberosum* variety (range: 38.09 ± 10.63 tetralin-equivalent ng h^{-1} to 1361.57 ± 381.21 tetralin-equivalent ng h^{-1}), but not of emitter induction (control = 230.24 ± 23.10 tetralin-equivalent ng h^{-1} ; induced = 292.16 ± 29.22 tetralin-equivalent ng h^{-1}) or its interaction with *S. tuberosum* variety, on total VOC emissions (Table 2). Follow-up analyses by individual compound, however, indicated that plants damaged by *S. exigua* significantly increased the emission of 12 compounds: nonatriene, butanoic acid, 3-hexenyl ester, (E)-, β -elemene, (E)- β -farnesene, germacrene D, β -cadinene, β -selinene, zingiberene, β -bisabolene, β -sesquiphellandrene, an unidentified sesquiterpenoid and viridiflorol (Table 1). In addition, analyses of total VOCs conducted separately for control and induced plants to assess quantitative genotypic variation in emissions indicated a significant effect of *S. tuberosum* variety on both constitutive ($F_{14,43} = 7.74$, $P < 0.001$) and induced ($F_{14,43} = 5.43$, $P < 0.001$) total VOC emissions, with up to ca. 35- and ca. 63-fold variation among varieties, respectively (Fig. 1).

On the other hand, PERMANOVA indicated significant effects of emitter induction, *S. tuberosum* variety, and their interaction on VOCs composition (Table 2). Emitter induction explained ca. 2% of the variation in VOCs composition, with the first two axes of the ordination together accounting for 49.52% of the variation due to this treatment (32.24% and 17.28%, respectively; Fig. 2). Variation in VOCs composition due to emitter induction was mainly associated with the amount of β -caryophyllene ($R^2 = 0.15$, $P < 0.001$) and β -bisabolene ($R^2 = 0.13$, $P < 0.001$). In addition, PERMANOVAs performed separately for control and induced plants to assess qualitative genotypic variation in emissions indicated a significant effect of *S. tuberosum* variety on the composition of both constitutive (DF = 14, 43, Pseudo-F = 4.50, $P < 0.001$) and induced (DF = 14, 43, Pseudo-F = 5.91, $P < 0.001$) VOCs (Fig. 3A and B). *Solanum tuberosum* variety explained 54.67% of the compositional variation in constitutive VOCs, with the first two axes together accounting for 63.42% of the variation in VOCs due to this effect (45.40% and 18.02% respectively) (Fig. 3A). Differences due to *S. tuberosum* variety were mainly associated with the amount of β -caryophyllene ($R^2 = 0.67$, $P < 0.001$) and δ -elemene ($R^2 = 0.53$, $P < 0.001$) (Fig. 3A). In addition, variety explained 61.58% of the compositional variation in induced VOCs, with the first two axes together accounting for 62.05% of the variation in VOCs across varieties (42.03% and 20.02% respectively) (Fig. 3B). Compositional variation in induced VOCs across varieties was mainly associated with the amount of β -caryophyllene ($R^2 = 0.64$, $P < 0.001$) and β -bisabolene ($R^2 = 0.61$, $P < 0.001$) (Fig. 3B).

2.2. Signalling effects on receiver plants

The emitter induction treatment significantly affected caterpillar consumption and weight gain on receiver plants (Table 3). Specifically, we found that the percentage of leaf area consumed by *S. exigua* on receiver plants exposed to damaged emitters was, on average, 39% lower compared to that of receivers exposed to control emitters (control = $22.32 \pm 3.23\%$; herbivory = $13.72 \pm 2.35\%$) (Fig. 4A). Likewise, caterpillar weight gain was, on average, 43% lower on receiver plants exposed to damaged emitters compared to that of receivers exposed to control emitters (control = 18.75 ± 2.66 mg; herbivory = 10.65 ± 1.85

Table 1

Means (\pm SE) for emission of individual volatile organic compounds (tetralin-equivalent ng h⁻¹) identified by GC-MS under two treatments (control vs. herbivory by *Spodoptera exigua*) in *Solanum tuberosum* (potato) emitter plants from 15 different varieties. RT = Retention times. KRI = Kovats retention index used for identification of compounds without commercial standards (KRI_c for calculated values and KRI_e for expected values from the NIST database).

Compound	RT	KRI _c	KRI _e	Control	Herbivory
β -myrcene ^a	11.90			1.734 \pm 0.585	0.586 \pm 0.234
Nonatriene	15.88	1114.23	1116	0.000 \pm 0.000	5.285 \pm 1.023
Butanoic acid, 3-hex-enyl ester, (E)-	17.79	1184.25	1185	0.053 \pm 0.037	7.840 \pm 0.940
Dodecane ^a	18.10			7.112 \pm 0.695	7.378 \pm 0.568
Tridecane ^a	20.96			3.615 \pm 0.407	3.627 \pm 0.358
δ -elemene	22.02	1339.19	1338	1.333 \pm 0.313	2.176 \pm 0.554
α -copaene	23.08	1378.02	1376	2.752 \pm 0.637	3.770 \pm 0.849
β -elemene	23.47	1392.31	1391	6.779 \pm 1.634	13.184 \pm 3.088
α -gurjunene ^a	23.91			8.076 \pm 1.585	8.110 \pm 1.819
β -caryophyllene	24.16			213.542 \pm 27.030	243.286 \pm 36.355
β -copaene	24.50	1432.36	1432	1.550 \pm 0.363	2.587 \pm 0.643
trans- α -bergamotene	24.66	1438.67	1440	3.642 \pm 0.745	5.752 \pm 1.242
cis- β -farnesene	24.81	1444.59	1445	7.354 \pm 1.512	9.458 \pm 2.306
(+/-)-gymnomitrene	24.86	1449.33	1452	1.076 \pm 0.275	1.344 \pm 0.272
trans-geranylacetone ^a	25.02			3.213 \pm 0.484	3.413 \pm 0.424
(E)- β -farnesene ^a	25.14			28.262 \pm 4.903	62.363 \pm 16.363
Acoradien	25.46	1469.85	1471	6.608 \pm 1.381	6.870 \pm 1.292
Germacrene D	25.75	1481.69	1481	25.226 \pm 5.617	47.174 \pm 11.342
β -cadinene	25.84	1485.24	1485	9.081 \pm 1.625	15.275 \pm 3.072
β -selinene	25.90	1487.61	1486	0.327 \pm 0.140	1.847 \pm 0.440
Zingiberene	26.08	1494.71	1495	4.199 \pm 0.580	8.249 \pm 1.245
Bicyclogermacrene	26.15	1497.47	1496	11.989 \pm 2.021	13.951 \pm 2.584
β -bisabolene ^a	26.45			4.672 \pm 1.091	10.003 \pm 1.560
γ -cadinene	26.57	1514.75	1513	0.310 \pm 0.119	0.673 \pm 0.244
β -sesquiphellandrene	26.81	1524.69	1524	11.305 \pm 2.687	20.829 \pm 4.783
Palustrol	27.87	1568.63	1568	0.159 \pm 0.079	0.471 \pm 0.200
Unidentified sesquiterpenoid	28.11	1578.57		14.808 \pm 2.621	22.694 \pm 4.953
Caryophyllene oxide ^a	28.23			4.569 \pm 0.775	2.691 \pm 0.457
Viridiflorol	28.83	1608.95	1611	2.231 \pm 0.457	4.008 \pm 0.986

We performed *P*-value adjustments using the False Discovery Rate for *P* < 0.05 to avoid inflating Type I error due to multiple testing. Individual VOCs that significantly differed within treatments (*P* < 0.05) are in bold.

^a Compounds identified with commercial pure standards.

mg) (Fig. 4B). However, there was no significant interaction between emitter induction and genetic relatedness on either the amount of leaf herbivory or caterpillar weight gain (Table 3, Fig. 4A and B).

Table 2

Effects of emitter induction treatment (control vs. herbivory by *Spodoptera exigua*), plant variety, and their interaction on total emission of VOCs (linear model, LM) and VOC composition (PERMANOVA) in *Solanum tuberosum* (potato) plants from 15 different varieties. We included height of plants as a covariate. Shown are *F*-values/Pseudo-*F* for each factor, degrees of freedom and associated significance levels (*P*-value), as obtained from LM and PERMANOVA, respectively. Significant *P*-values are highlighted in bold.

	Total VOC emissions			VOC composition	
	DF _{num,den}	<i>F</i>	<i>P</i>	Pseudo- <i>F</i>	<i>P</i>
Treatment (T)	1, 87	2.32	0.131	5.06	0.002
Variety (V)	14, 87	11.52	< 0.001	8.34	< 0.001
T \times V	14, 87	1.09	0.378	1.69	0.003
Plant height	1, 87	6.13	0.015	16.72	< 0.001

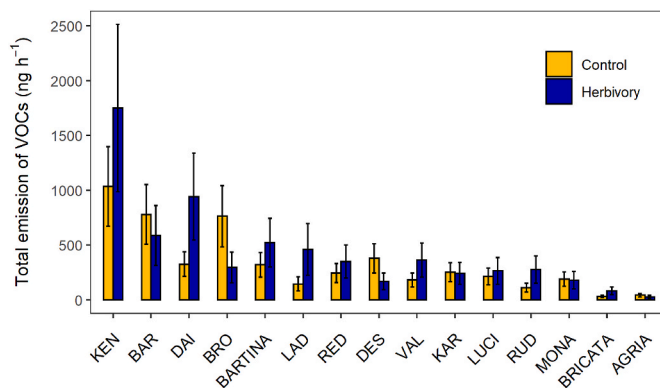


Fig. 1. Total emission of volatile organic compounds (VOCs, in nanograms per hour) emitted by *Solanum tuberosum* (potato) plants from 15 different varieties in constitutive conditions (control) and herbivore-induced conditions (after *Spodoptera exigua* feeding). Bars are back-transformed least-square means \pm SE from a linear model (*N* = 8).

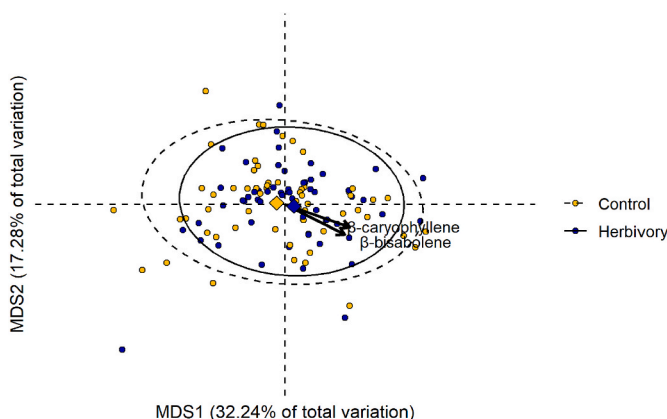


Fig. 2. Unconstrained ordination (PCoA) showing the effects of emitter induction treatment (control vs. herbivory by *Spodoptera exigua* feeding) on the composition of VOCs released by *Solanum tuberosum* (potato) plants from 15 different varieties. Biplot arrows represent linear associations with the two most influential volatiles based on their *R*² values scaled to reflect relative magnitude of effects. Diamonds represent the centroids for each emitter induction treatment and associated 95% ellipses. The first two axes together accounted for 49.52% of total variation in volatile composition due to differences among emitter induction treatment.

3. Discussion

Our findings indicated that herbivory by *S. exigua* drove qualitative (compositional) but not quantitative (total emission) changes in VOC

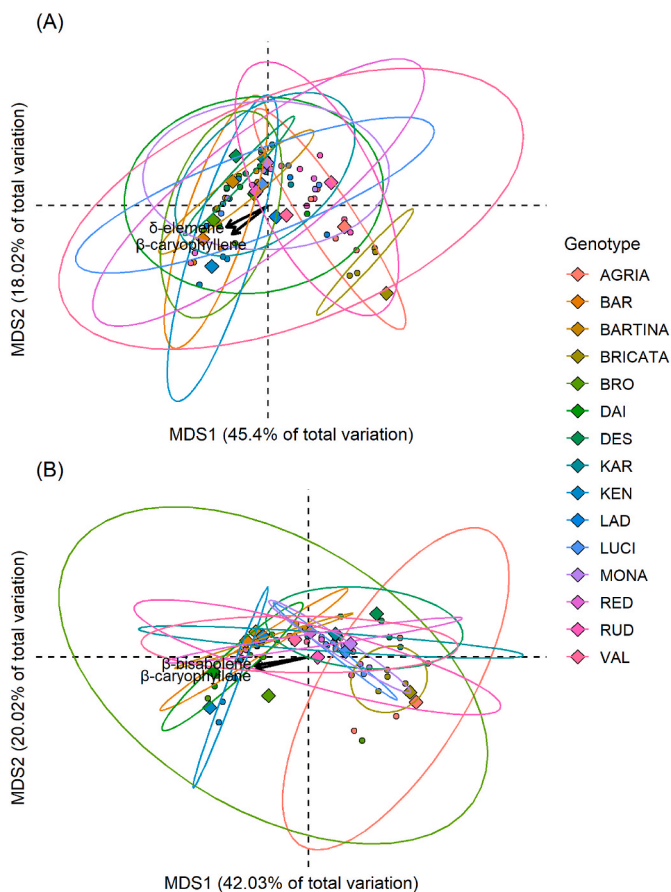


Fig. 3. Unconstrained ordination (PCoA) showing the effect of *Solanum tuberosum* (potato) variety ($N = 15$) on the composition of volatile organic compounds (VOCs) in (A) constitutive conditions (control) and (B) herbivore-induced conditions (after *Spodoptera exigua* feeding). Biplot arrows represent linear associations with the two most influential volatiles based on their R^2 values scaled to reflect relative magnitude of effects. Diamonds represent the centroids for each *S. tuberosum* variety and associated 95% ellipses. The first two axes together accounted for 63.42% and 62.05% of total variation due to *S. tuberosum* varieties in constitutive and induced VOC composition respectively.

Table 3

Results from general linear mixed models showing the effects of emitter induction treatment (control vs. herbivory by *Spodoptera exigua*), plant genetic relatedness (emitter and receiver of the same or different variety, i.e., genotype self- and non-self), and their interaction on the percentage of leaf area consumed and weight gain of *S. exigua* larvae feeding on receiver *Solanum tuberosum* (potato) plants from 15 different varieties after exposure to emitter VOCs. We included individual plant as a random effect (statistics not reported) and height of receiver plants as a covariate. F-values, degrees of freedom and associated significance levels (P -value) are shown. Significant P -values ($P < 0.05$) are in bold.

	Leaf area consumed			Larval weight gain		
	DF ^{num,den}	F	P	DF ^{num,den}	F	P
Treatment (T)	1, 72	4.78	0.032	1, 64	6.47	0.013
Relatedness (R)	1, 72	0.41	0.522	1, 65	0.66	0.421
T × R	1, 72	0.77	0.384	1, 65	0.20	0.658
Plant height	1, 75	3.86	0.053	1, 68	1.14	0.289

emissions. In addition, we found significant differences among *S. tuberosum* varieties in total emissions and composition for both constitutive (controls) and induced (herbivory) plants. In turn, results from the plant-plant signalling experiment indicated, as expected, that

the emitter induction treatment boosted herbivore resistance in receiver plants, but this signalling effect was not contingent on emitter-receiver relatedness (i.e., non-significant genetic relatedness by induction interaction). Therefore, while *S. tuberosum* varieties exhibited substantial qualitative and quantitative differences in VOC emissions, particularly for induced plants which emitted blends presumably responsible for signalling, such variation had no detectable consequence for plant-plant signalling effects on induced resistance. Together, these findings provide strong evidence for VOC-mediated plant-plant signalling in response to herbivory across *S. tuberosum* varieties, and suggest that this signalling is insensitive to genetically-based differences in VOC emissions across the studied varieties.

Unexpectedly, *S. exigua* feeding had no detectable effect on total VOC emissions. However, analyses of individual compounds indicated that, relative to controls, induced plants exhibited significant increases in several compounds such as nonatriene, β -bisabolene and β -sesquiphellandrene, some of which are known or suspected to play a role in resistance against insects in potato and other crops, including direct action as feeding deterrents or toxicity as well as involvement in signalling. For example, nonatriene increased resistance against *S. littoralis* in sweet potato (Meents et al., 2019) and to the spider mite *Tetranychus urticae* in lima bean (Arimura et al., 2000). Similarly, β -bisabolene increased resistance against the whitefly *Bemisia tabaci* in cotton (Tamilselvan et al., 2021), and β -sesquiphellandrene reduced growth of *S. littoralis* in maize (Erb et al., 2011). Likewise, Meents et al. (2019) found that application of synthetic nonatriene caused a significant induction of trypsin protease inhibitory activity in sweet potato cultivars, which resulted in reduced larval performance. Results also indicated compositional changes in VOCs due to *S. exigua* damage, consistent with observed changes in the abundances of several compounds due to the induction. Thus, while no effect of induction on total VOCs was detected, the induction of some compounds over others presumably drove compositional changes in emission blends. Further work testing for the ecological function of these compounds in *S. tuberosum* is needed, including both their direct effects on plant pests as well as their role in mediating plant-plant signalling effects on direct and indirect defence.

Concomitant with herbivore-induced VOC changes, we found that plant-plant signalling boosted receiver resistance to *S. exigua* in *S. tuberosum* plants. Namely, receiver plants exposed to VOCs from herbivore-induced emitters exhibited greater resistance to *S. exigua* as evidenced by reduced leaf damage and larval growth relative to receivers exposed to VOCs from control emitters. The fact that induction of specific individual compounds, rather than total emissions, and resulting blend compositional changes were presumably responsible for induced resistance in receiver plants suggests that signalling was mediated by specific compounds or blends of induced VOCs. These results support previous findings in a number of other crop and non-crop species (Karban et al., 2014b; Ninkovic et al., 2021) including previous research by our group on *S. tuberosum* (Vázquez-González et al., 2022). Namely, we found no evidence of signalling between *S. tuberosum* plants in response to herbivory by the specialist leaf-feeder *Leptinotarsa decemlineata* (Abdala-Roberts et al., 2022), possibly due to the suppression of induced VOCs by this insect. Similarly, infection by the generalist leaf pathogen *Sclerotinia sclerotiorum* (Moreira et al., 2021) appeared to also inhibit signalling between *S. tuberosum* plants. Combined, these and our present study suggest enemy-induced specificity in VOCs-mediated signalling in *S. tuberosum* plants and points to the necessity of attacker-specific strategies for effective pest management using VOCs (Moreira et al., 2018a). It is also plausible that damage by *S. exigua* induces VOC blends that boost resistance against other attackers, calling for further work on VOC-mediated signalling in response to attacks by multiple enemies. Finally, while signalling did not induce phenolic compounds in this study (see Table S1 and Fig. S1 in the Supplementary Material), further work is needed to identify other compound types mediating VOC-mediated induced resistance.

Despite qualitative and quantitative differences in induced VOCs

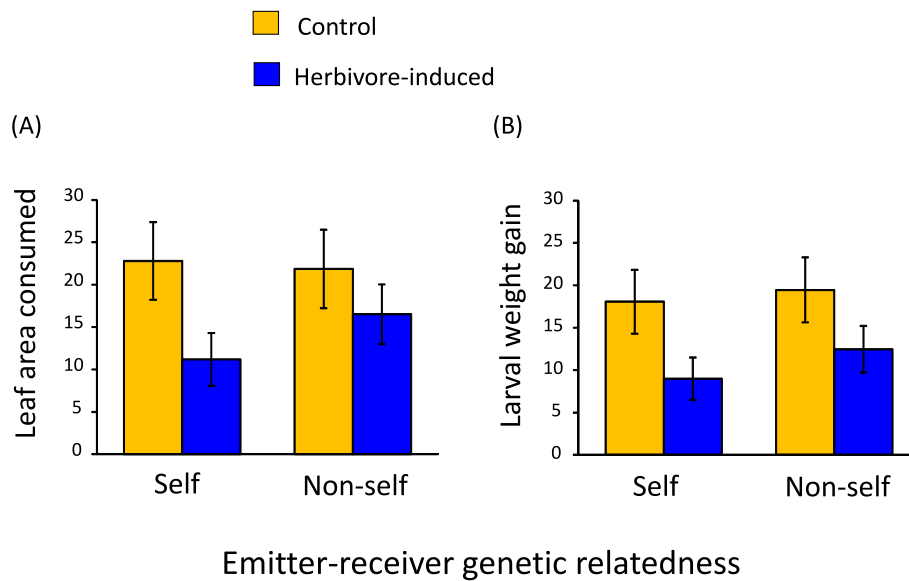


Fig. 4. Effect of emitter induction treatment (control vs. herbivory by *Spodoptera exigua*) on *S. exigua* larvae (A) percentage of leaf area consumed and (B) weight gain (in mg) on receiver *Solanum tuberosum* (potato) plants from 15 different varieties in cases where emitter-receiver pairs were of the same (self) or different (non-self) variety. Bars are back-transformed least-square means \pm SE extracted from a general linear mixed model (N = 20–23).

among *S. tuberosum* varieties (linear model and PERMANOVA for damaged plants), we found no effect of emitter-receiver genetic relatedness on signalling (i.e. no evidence of self-recognition). The few studies conducted so far testing for effects of genetic relatedness argue that plants can discriminate between volatile cues released by genetically close vs. distant relatives and respond more strongly to VOCs from related individuals (e.g. Karban and Shiojiri, 2009; Karban et al., 2013, 2014b, 2016; Moreira et al., 2016). Instead, our work agrees with a previous investigation reporting that VOC-mediated communication in the shrub *Baccharis salicifolia* due to aphid feeding was not contingent on whether emitter and receiver plants were of the same or different genotype (Moreira et al., 2018b). In such study, and consistent with our present study, authors also found significant genotypic variation in VOCs emissions (Moreira et al., 2018b). In addition, in a multi-species study Pearse et al. (2012) found that signalling effects due to herbivory in neighbouring plants depended on genetic relatedness in only one of their three studied annual species. Thus, although *S. exigua*-induced changes in individual VOCs and in VOCs composition presumably played a role in signalling between *S. tuberosum* plants, VOC variation among varieties (in particular induced VOCs) was presumably not biologically relevant (or strong enough). This suggests that a core group of induced compounds (e.g. concentration thresholds of specific compounds and/or compound abundance ratios; Rosenkranz et al., 2021) mediates signalling between *S. tuberosum* plants and that such compounds or blends remain relatively unchanged (i.e. within certain limits) despite quantitative and qualitative variation in induced VOCs across varieties.

Further work evaluating in more detail the influence of genetic relatedness among genotypes or varieties is needed. This would include sequencing of varieties to correlate genetic distance with the strength of signalling effects in non-self emitter-receiver pairs. This genetic data could inform experimental designs that explicitly test for variety emitter-receiver compositions with varying degrees of relatedness. In addition, a related aspect would be to assess the influence of domestication history on signalling. Research has shown that cultivated varieties are more susceptible to pests compared to their wild relatives, mainly as a result of selection for traits that increase productivity at the expense of defensive traits, or even selection directly aimed at reducing physical or chemical defences to increase palatability for human consumption (Turcotte et al., 2014; Whitehead et al., 2017). These

domestication effects have also been shown to affect VOC emissions, frequently decreasing total production or altering blends and the abundances of specific compounds (Rodríguez-Saona et al., 2011; Chen et al., 2015; Rowen and Kaplan, 2016). As a result, domestication has likely altered or disrupted plant-plant signalling, but our understanding of such effects is still limited. Studies could compare signalling effects within wild vs. domesticated varieties, as well as test for effects of domestication history by replicating non-self emitter-receiver variety combinations with contrasting breeding histories (e.g., breeding for pest vs. abiotic resistance, or different types of vegetative traits). Finally, additional work assessing the role of specific compounds or blends (e.g. using artificial emitters) informed by prior detailed work on genetically based variation in VOC emissions can provide important mechanistic insight on signalling and its contingency on plant genetically-based phenotypic variation associated with relatedness and domestication history.

4. Conclusions

Most methods currently used to control *S. exigua* on *S. tuberosum* rely on neonicotinoid insecticide application (Kuhar et al., 2013). While alternative methods such as breeding for increased insect and pathogen resistance have been developed (Garrido et al., 2017), strategies for sustainable pest control remain limited (e.g. crop rotation, intercropping, insect pheromone traps, insect-food supplements). Our results indicate that VOC emissions in *S. tuberosum* plants could be used to enhance plant resistance in response to damage by *S. exigua*, a finding that warrants further tests with other insect pests. Future greenhouse experiments followed by field trials testing for signalling effects in response to different attackers (including crossed induced resistance by one attacker on another) and the role of specific compounds or blends are needed to integrate information on different interaction outcomes and candidate VOCs responsible for them. Once this basic knowledge is obtained, target VOCs can be synthesized and applied in field trials as a management tool for pest control in *S. tuberosum* (e.g. with integrated pest management schemes).

5. Experimental

5.1. Study system

Solanum tuberosum L. (Solanaceae) is a herbaceous perennial plant that grows up to 60 cm high and propagates via seeds and tubers. This plant was domesticated about 8000 years ago in the Central Andes region (Peru-Bolivia) (Hijmans and Spooner, 2001), and introduced to Europe on the second half of the 16th century. At present, there are more than 4000 edible varieties and is the world's fourth-largest food crop after maize, wheat, and rice (FAOSTAT, 2017).

The intensification of *S. tuberosum* production over the last decades has drastically increased the impact of pests and diseases on this crop. Notably, the generalist beet armyworm *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) is one of the most economically important pests on *S. tuberosum*, which feeds on the foliage causing drastic reductions in growth and yield (Brown and Dewhurst, 1975).

5.2. Signalling effects on receiver plants

In October 2020, we individually planted tubers of 15 potato varieties (*S. tuberosum* L. cultivar [cv. Hereafter] Agria, Baraka, Bartina, Bricata, Brooke, Daifla, Desiree, Kartoffel Gala, Kennebec, Lady Amarilla, Lucinda, Monalisa, Red Scarlet, Rudolph, and Valencia; see Table S2 in the Supplementary Material for more details of these varieties) 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 (10 h per day, Photosynthetically Active Radiation = $725 \pm 19 \mu\text{mol m}^{-2} \text{s}^{-1}$) and temperature (10 °C night, 25 °C day), and were watered twice a week. One month after planting, we grouped *S. tuberosum* plants of similar size in pairs and placed them in $37.5 \times 37.5 \times 96.5$ cm plastic cages. These cages had two frontal holes covered with a mesh allowing vital airflow. One plant of each pair acted as emitter and the other as receiver (emitter height: 29.57 ± 10.89 cm, receiver height: 27.62 ± 12.33 cm). Within each cage, plants were placed 20 cm apart to avoid physical contact. Adjacent cages were spaced by 2 m to prevent cross-signalling between adjacent replicates (Freundlich et al., 2021). In order to test for genetic relatedness effects ("relatedness effect" hereafter), for half of the cages emitter-receiver pairs were of the same variety (self-signalling), while for the other half pairs were of a different variety (non-self-signalling). We selected plants such that all *S. tuberosum* varieties were similarly represented across treatment combinations. Then, to test for signalling in response to herbivory ("emitter induction" treatment hereafter), we randomly assigned half of the emitter plants of each replicate to one of the following treatments: (1) subjected to *S. exigua* herbivory (i.e. induced plants) or (2) control (undamaged plants). This resulted in a fully crossed two-by-two factorial experiment involving relatedness and emitter induction. In total, we established 85 cages: 43 for emitter-induced treatment vs. 42 for control, and 20–23 cages per induction treatment \times self-genotype combination vs. 20–22 per induction treatment \times non self-genotype combination (see Table S3 in the Supplementary Material). For the emitter induction treatment, we placed one third-instar larvae of *S. exigua* on each of two fully-expanded leaves per emitter plant using a fine paintbrush and covered these leaves with a nylon bag to prevent the caterpillar from escaping. For control plants, we also covered two fully expanded leaves but did not add larvae to account for any bagging effect. After three days of herbivore feeding, when damage was on average ca. 20% leaf area consumed, we removed emitter plants from all cages and conducted a bioassay on receiver plants to test whether exposure to VOCs from attacked emitters increased receiver resistance. For this, we placed one third-instar *S. exigua* larvae on each of two fully expanded leaves per receiver plant following the same procedure described above for emitter induction. Prior to the bioassay, we weighted all larvae to the nearest 0.0001 g to control for larval initial weight. We kept larvae on receiver plants for three days and

then collected all leaves subjected to herbivory and photographed them with a Samsung Galaxy A30s (25 effective megapixels, $4 \times$ digital zoom). We estimated the percentage of leaf area consumed using the professional mobile application BioLeaf - Foliar Analysis™ (Brandoli Machado et al., 2016). In addition, we weighted all larvae again to estimate larval weight gain as the difference between the initial and final larval weight. Although we did not measure herbivory on emitters, data on leaf damage for receiver plants exposed to control emitters indicated that there was no significant variation among varieties in herbivory (i.e. susceptibility to *S. exigua*) ($F_{10,50} = 1.91$, $P = 0.064$), implicating that there were no biases in emitter VOC induction across varieties potentially influencing the test of genetically-based specificity in signalling.

Immediately after finishing leaf damage estimations we collected two fully expanded undamaged leaves per plant to test whether exposure to VOCs from damaged emitters boosted receiver defence induction (i.e. priming effect), therefore explaining any such effect of signalling on induced resistance. Leaves were oven-dried them for 48 h at 40 °C and used Ultra-High-Performance Liquid-Chromatograph to quantify phenolic compounds following Moreira et al. (2020). Results from these analyses, however, indicated no significant effect of emitter induction on either hydroxycinnamic acids or flavonoids, the main groups of phenolics found. Accordingly, these results are shown in the supplementary material (Table S1; Fig. S1).

5.3. Effects of herbivory on emitter VOC emission

In October 2021, we performed a second greenhouse experiment with a different set of plants to test whether quantitative and qualitative variation in the emission of VOCs differ among *S. tuberosum* varieties, a necessary condition for genetic relatedness effects on plant-plant signalling. For this, we planted 120 tubers from the same 15 *S. tuberosum* varieties included in the previous experiment (8 tubers per variety) in 4-L pots containing potting soil with peat. We grew plants exactly as described for the first experiment. One month after germination, we randomly assigned plants to the same induction treatment described above. After three days of *S. exigua* feeding, we removed larvae and collected aboveground VOCs produced by emitter plants following Rasmann et al. (2011). Briefly, we bagged plants with a 2-L Nalophan bag and trapped VOCs on a charcoal filter (SKC sorbent tube filled with anascorb CSC coconut-shell charcoal) for 2 h using a Sidekick 224-52MTX pump (0.25 L min^{-1} airflow of technical air N_2O_2). We eluted traps with 150 μL dichloromethane (Merck, Dietikon, Switzerland) to which we had previously added tetralin as the internal standard (200 ng in 10 μL dichloromethane). We then injected 1.5 μL of the extract for each sample into an Agilent 7890 B gas chromatograph (GC) coupled with a 5977 B mass selective detector (MSD) fitted with a $30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$ film thickness HP-5MS fused silica column (Agilent, Santa Clara, CA, USA). We operated the injection into the GC in pulsed splitless mode (250 °C, injection pressure 15 psi) with helium as the carrier gas. The GC oven temperature programme was: 3.5 min hold at 40 °C, $5 \text{ }^\circ\text{C min}^{-1}$ ramp to 230 °C, then a 3 min hold at 250 °C post run (constant helium flow rate 0.9 mL min^{-1}). The transfer line was set at 280 °C. In the MS detector (EI mode), a 33–350 (m/z) mass scan range was used with MS source and quadrupole set at 230 °C and 150 °C, respectively. We identified volatile terpenes using commercial pure standards and comparing their Kováts indices, calculated relative to the retention times of a series of n-alkanes ($\text{C}_8\text{--C}_{20}$, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) analysed under the same chromatographic conditions, with those reported in the literature. It is important to note that, although our Kováts indices matched well with those previously reported, VOCs should be considered as 'putative' until confirmation with standards. We quantified total emission of individual VOCs using normalized peak areas and expressed it as nanograms per hour (ng h^{-1}). We obtained the normalized peak area of each individual compound by dividing their integrated peak areas by the integrated peak area of the internal standard (Abdala-Roberts et al., 2022), in order to standardize

for variations in the sample volume during the elution process. Reported values for individual VOCs should thus be considered as tetralin-equivalent nanograms of compound released by each plant per hour. The total emission of VOCs of each sample was then obtained by summing the concentrations of individual VOCs.

5.4. Statistical analyses

Effects of herbivory on emitter VOCs. First, to evaluate herbivory effects on quantitative variation in VOC emissions, we used linear models (LMs) testing for effects of emitter induction treatment (two levels: control or herbivory), *S. tuberosum* variety (15 levels), and their interaction (all fixed factors) on the emission of each individual VOC and total VOC emissions (data from the second experiment). In all these models, we also included plant height as a covariate to account for differences in plant size which could affect volatile emissions. In the case of individual VOCs, we performed *P*-value adjustments using the false discovery rate for $P < 0.05$ to avoid inflating Type I error due to multiple testing (Benjamini and Hochberg, 1995). Total VOC emission was log-transformed to achieve normality of residuals. Second, to test for herbivory effects on qualitative variation in VOCs emissions, we ran a permutational multivariate analysis of variance (PERMANOVA) based on 10,000 permutations to test the effects of emitter induction, *S. tuberosum* variety, and their interaction on VOC composition using Bray-Curtis dissimilarity matrices based on abundance of each individual compound. To visualize these results, we conducted a principal coordinate analysis (PCoA) based on pairwise dissimilarities and graphed the centroids of each herbivory treatment effect (Moreira et al., 2021). We also identified the two most influential VOCs as those having the strongest associations with the first two ordination axes based on their R^2 values, and displayed these relationships using biplot arrows with the length scaled to R^2 values.

For both the LMs and PERMANOVA analyses above, we ran follow-up models testing for variation among *S. tuberosum* varieties in VOCs separately for control and damaged plants. Qualitative and/or quantitative differences in emissions among varieties, particularly for induced VOCs, are an important condition for testing an effect of genetic relatedness on signalling, in this case with respect to the emission side of signalling. More genetically distant plants (in our case non-self emitter vs. receiver genotype pairs) are expected to differ more in their emissions and in turn exhibit weaker signalling than more closely related genotypes with similar emissions (in our case the same variety, i.e., self-recognition).

Signalling effects on receiver resistance. To test for signalling effects on receiver induced resistance, we ran a linear mixed model (LMM) testing for effects of emitter induction (control), genetic relatedness (self-, non-self), and their interaction (all fixed factors) on the percentage of leaf area consumed and larval weight gain on receiver plants. The interaction was of particular interest since it tested whether signalling effects in response to herbivory were contingent on emitter-receiver genetic relatedness. In addition, we included plant as a random factor to control for non-independence of each pair of damaged leaves analysed per individual. We also included the height of receiver plants as a covariate to account for differences in size possibly affecting induced responses. Both response variables were square-root-transformed to achieve normality of residuals.

We ran all statistical analyses in R software version 4.2.1 (R Core Team, 2020). We implemented LMs and LMMs using the *lm* and *lmer* functions, respectively from the *lmerTest* package (Kuznetsova et al., 2017). We report back-transformed least-square means and standard errors from these models using the *lsmeans* function from the *lsmeans* package (Lenth, 2016). In addition, we implemented PERMANOVA and ordination methods using the *adonis* and *capscale* functions, respectively from the *vegan* package (Oksanen et al., 2016).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.phytochem.2022.113561>.

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