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Aboveground herbivory induced jasmonates disproportionately reduce plant reproductive potential by facilitating root nematode infestation

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Abstract

Different plant feeders, including insects and parasitic nematodes, can influence each other by triggering systemic changes in their shared host plants. In most cases, however, the underlying mechanisms are unclear, and the consequences for plant fitness are not well understood. We studied the interaction between leaf feeding *Manduca sexta* caterpillars and root parasitic nematodes in *Nicotiana attenuata*. Simulated *M. sexta* attack increased the abundance of root parasitic nematodes in the field and facilitated *Meloidogyne incognita* reproduction in the glasshouse. Intact jasmonate biosynthesis was found to be required for both effects. Flower counts revealed that the jasmonate-dependent facilitation of nematode infestation following simulated leaf attack reduces the plant's reproductive potential to a greater degree than would be expected from the additive effects of the individual stresses. This work reveals that jasmonates mediate the interaction between a leaf herbivore and root parasitic nematodes and illustrates how plant-mediated interactions can alter plant's reproductive potential. The selection pressure resulting from the demonstrated fitness effects is likely to influence the evolution of plant defense traits in nature.

KEYWORDS

Ditylenchus sp., *Manduca sexta*, *Nicotiana attenuata*, *Pratylenchus hexincisus*, jasmonates, nicotine, plant fitness, plant resistance, root parasitic nematodes

1 | INTRODUCTION

Plant-mediated interactions between spatially and temporally separated herbivores influence community composition and ecosystem processes (Bardgett, Wardle, & Yeates, 1998; Loreau & Mazancourt, 2013; Wardle et al., 2004) and have therefore been studied in great detail (Bezemer & van Dam, 2005; Bezemer, Wagenaar, van Dam, & Wäckers, 2003; Blossey & Hunt-Joshi, 2003; Harrison & Karban, 1986; Masters, Brown, & Gange, 1993; Ohgushi, 2005; Soler, Erb, & Kaplan, 2013; Wurst, van Dam, Monroy, Biere, & van der Putten, 2008). Interactions between leaf and root feeders in particular have received much attention (Arce et al., 2017; Coppola, Soler, Rao, & Corrado, 2017; Erb et al., 2015; Erb, Robert, Hibbard, & Turlings,

2011; Huang et al., 2017; Kaplan, Sardanelli, & Denno, 2009; Robert et al., 2012; Soler et al., 2007). Several studies demonstrate, for instance, that leaf herbivore attack increases the abundance and performance of root parasitic nematodes (Alston, Schmitt, Bradley Jr, & Coble, 1993; Kafle, Hänel, Lortzing, Steppuhn, & Wurst, 2017; Kaplan et al., 2008; Kaplan et al., 2009; Russin et al., 1989; Russin, McGawley, & Boethel, 1993). Despite the increasing number of studies showing effects of leaf feeders on root herbivores and parasites, the systemic shoot–root signals mediating these effects are unclear.

Jasmonates have been suggested to regulate plant-mediated interactions between above and below ground herbivores (Erb, Lenk, Degenhardt, & Turlings, 2009; Erb, Ton, Degenhardt, & Turlings, 2008; Papadopoulou & van Dam, 2017; van Dam & Heil, 2011; van

Dam & Oomen, 2008). Foliar jasmonate treatments reduce gall formation by root parasitic nematodes in tomato (Bhattarai et al., 2008; Cooper, Jia, & Goggin, 2005; Fan et al., 2015; Fujimoto et al., 2011; Zinovieva, Vasyukova, Udalova, & Gerasimova, 2013), rice (Kyndt et al., 2017; Nahar, Kyndt, de Vleesschauwer, Höfte, & Gheysen, 2011), soybean (Hu, You, Li, Hua, & Wang, 2017), oat (Soriano, Asenstorfer, Schmidt, & Riley, 2004), and thale cress (Gleason, Leelarasamee, Meldau, & Feussner, 2016; Ozalvo et al., 2014). Genetic approaches also show that jasmonate signalling can decrease plant susceptibility to root nematodes (Kammerhofer et al., 2015; Nahar et al., 2011; Ozalvo et al., 2014), albeit with some exceptions (Gao et al., 2008). Strikingly, however, leaf herbivory increases jasmonate levels in damaged and systemic tissues and at the same time increases susceptibility to nematodes (Alston et al., 1993; Kafle et al., 2017; Kaplan et al., 2008; Kaplan et al., 2009; Machado et al., 2013; Russin et al., 1989; Russin et al., 1993). The exact role of herbivory induced, endogenous jasmonates in the interaction between leaf and root attackers remains to be investigated.

Changes in plant primary and secondary metabolism downstream of systemic shoot–root signals have been implicated in above–below-ground interactions (Bezemer & van Dam, 2005; van Dam et al., 2003). Induced phenols, glucosinolates, terpenoids, and root volatiles, for instance, were accompanied by a decreased performance of root feeders on leaf-infested plants (Bezemer et al., 2003; Bezemer, Wagenaar, van Dam, van der Putten, & Wäckers, 2004; Erb et al., 2008; Erb et al., 2011; Erb et al., 2015; Gill, Sandoya, Williams, & Luthe, 2011; Huang et al., 2017; S. N. Johnson et al., 2012; Robert et al., 2012; Soler et al., 2007; Tindall & Stout, 2001). Using a genetic approach, Erb et al. (2015) found that leaf-herbivory induced changes in conjugated root phenolics were required for the deterrence of a root feeding herbivore. Increased nematode performance in response to leaf attack was suggested to result from the increased allocation of carbohydrate towards roots in response to leaf infestation (Kaplan et al., 2008; Masters et al., 1993). However, despite increased invertase activity and short-term changes in carbon allocation following elicitation (Gómez et al., 2012; Schwachtje et al., 2006), roots often suffer strong carbon deprivation in response to leaf attack (Castrillón-Arbeláez, Martínez-Gallardo, Arnaut, Tiessen, & Délano-Frier, 2012; Ferrieri, Agtuca, Appel, Ferrieri, & Schultz, 2013; Gómez et al., 2012; Machado et al., 2013; Machado, Zhou, et al., 2017).

Another open question in the field of plant-mediated interactions between herbivores is how plant-mediated effects translate into changes in plant fitness (Arce et al., 2017; Kroes et al., 2016; X. Li, Li, & Meng, 2017; Luo et al., 2016; Pineda, Soler, Pastor, Li, & Dicke, 2017; Rasmann et al., 2012; Rodriguez-Saona, Chalmers, Raj, & Thaler, 2005; Schöning & Wurst, 2016). In coyote tobacco, for instance, mirids decrease caterpillar growth, and the fitness penalties of caterpillar attack are reduced when plants are co-colonized by mirids (Kessler & Baldwin, 2004). However, whether the observed changes in plant performance are the result of the plant-mediated interactions among herbivores, or whether they reflect changes in induced responses of plants under dual attack (Stam et al., 2014) remains difficult to evaluate. The elucidation and manipulation of the regulatory signals which govern plant-mediated interactions might help to fill this knowledge gap.

In this study, we evaluated the impact of *Manduca sexta*-induced jasmonates on the abundance and reproduction of root parasitic nematodes in the laboratory and the field. We furthermore measured the consequences of *M. sexta*-induced susceptibility to *Meloidogyne incognita* using jasmonate-deficient plants and quantified the impact of *M. sexta* leaf attack on root primary and secondary metabolites. Manipulating the key hormone governing the interaction between *M. sexta* and *M. incognita* allowed us to assess the influence of plant-mediated interactions on plant reproductive potential.

2 | MATERIAL AND METHODS

2.1 | Plant material and planting conditions

Two *Nicotiana attenuata* Torr. Ex. Watson plant lines were used for experiments: i) a transgenic control line transformed with an empty vector construct (EV) and ii) a jasmonate deficient inverted repeat allene oxide cyclase transformed line (irAOC; Bubner, Gase, Berger, Link, & Baldwin, 2006; Kallenbach, Bonaventure, Gilardoni, Wissgott, & Baldwin, 2012). Before planting, all seeds were surface sterilized and germinated on Gamborg's B5 media (Krügel, Lim, Gase, Halitschke, & Baldwin, 2002). For glasshouse experiments, the seedlings were transferred to Teku pots (Pöppelmann GmbH & Co. KG, Lohne, Germany) 10 days after germination, and 10 to 12 days later, the seedlings were planted into 1-L pots filled with washed sand. Plants were grown as described (Krügel et al., 2002). For the field experiment, seeds of the transformed *N. attenuata* lines were imported under APHIS notification number 07-341-101n, and experiments were conducted under notification number 06-242-02r. Plants were grown as described (Machado, McClure, Herve, Baldwin, & Erb, 2016; Schuman, Barthel, & Baldwin, 2012).

2.2 | Phytohormone measurements

To profile *M. sexta* herbivory-induced systemic changes in jasmonate levels, we measured jasmonic acid (JA) and jasmonoyl-L-isoleucine (JA-Ile) in local, treated leaves and in non-treated petioles, stems, systemic leaves, and roots following standard procedures (*n=5*) (Ferrieri et al., 2015; Machado et al., 2013). To simulate *M. sexta* attack, we rolled a pattern wheel three times along each side of the midvein. The resulting wounds were immediately treated with 10 μl of 1:5 diluted *M. sexta* oral secretions (W + OS) (Machado et al., 2013; Machado, Zhou, et al., 2017). Treated leaves and untreated systemic plant tissues were harvested at different time points, flash frozen in liquid nitrogen, and stored at −80°C for chemical analysis. Intact plants were harvested in a similar manner and used as controls. JA and JA-Ile were quantified as previously described (Ferrieri et al., 2015; Machado, Robert, et al., 2016).

2.3 | Effect of aboveground herbivory on root primary and secondary metabolites

To investigate the impact of systemically induced jasmonates on root primary and secondary metabolism, we quantified soluble sugars, starch, free amino acids, and nicotine upon simulated *M. sexta* attack

in jasmonate competent EV and jasmonate-biosynthesis deficient irAOC plants ($n = 5$). For this, we treated the plants with simulated herbivory attack as described above. Simulated herbivory attack treatments were carried out on three leaves every other day for three times to obtain nine treated leaves in total (Machado et al., 2013). Six days after the first treatment (i.e., 2 days after the last treatment), roots were harvested, washed, dried, and frozen in liquid nitrogen. Soluble sugars and starch were quantified as described elsewhere (Machado, Arce, Ferrieri, Baldwin, & Erb, 2015; Machado, Baldwin, & Erb, 2017; Robert et al., 2014). Nicotine was quantified as described elsewhere (Jimenez-Aleman, Machado, Baldwin, & Boland, 2017; Jimenez-Aleman, Machado, Görls, Baldwin, & Boland, 2015). For amino acid quantification, 100 mg of ground plant material were extracted in acidified methanol (50% MeOH, 49.5% H_2O, 0.05% acetic acid). After centrifugation, supernatants were 10-times diluted in water containing 10 µg/ml algal amino acids uniformly labelled with ^{13}C and ^{15}N (Isotec, Miamisburg, US). The liquid chromatography–mass spectrometry conditions were as described elsewhere (Docimo et al. 2012).

2.4 | Effect of *M. sexta*-induced jasmonates on root parasitic nematode abundance in the field

To investigate the effect of *M. sexta*-induced jasmonates on root parasitic nematode abundance in the field, we planted wild type (EV) and jasmonate-biosynthesis deficient irAOC *N. attenuata* seedlings at the field station of the Lytle Ranch Preserve (St. George, UT, USA), induced them with simulated *M. sexta* herbivory and isolated, identified, and quantified root-associated nematode communities. IrAOC plants show a 95% reduction in *M. sexta*-induced jasmonates (Kallenbach et al., 2012; Machado et al., 2013). To simulate *M. sexta* herbivory, half of the plants were treated by wounding and application of 10 µl of 1:5 diluted *M. sexta* oral secretions (W + OS) 3 weeks after transplantation ($n=6$) (Machado et al., 2013; Machado, Zhou, et al., 2017). The treatments were carried out on three leaves, every other day for three times to obtain nine treated leaves per plant. Five weeks after transplantation into the field, roots were excavated and transferred together with their surrounding soil into ziplock bags and stored in camping coolers. The next day, the samples were placed on a custom-made mist extractor (McClure, Nischwitz, Skantar, Schmitt, & Subbotin, 2012). After 48 hr of extraction, nematodes were collected on a 500 mesh (24-µm-pore-size), sieved and resuspended in 10 ml of distilled water. Plant parasitic nematodes were then observed under a stereomicroscope, identified by morphological characteristics, and counted. Identification of *Pratylenchus hexincisus* was confirmed by Alex Y. Ryss, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia.

2.5 | Effect of *M. sexta*-induced jasmonates on root nematode reproductive fitness in the glasshouse

To determine whether *M. sexta*-induced jasmonates affect the reproductive potential of root parasitic nematodes, we induced wild type (EV) and jasmonate deficient (irAOC) plants by simulated *M. sexta* herbivory, infested them with *M. incognita* infective juveniles, and

quantified the number of nematode eggs ($n = 20$). Plants were induced as described above. Intact plants and wounded plants treated with water (W + W) instead of *M. sexta* oral secretions were used as controls ($n = 20$). Six days after the first *M. sexta* herbivory treatment, that is, 2 days after the last treatment, ten thousand *M. incognita* infective juveniles were released into the pots. Five weeks after the last *M. sexta* induction treatment, the roots of an additional subset of plants were harvested and washed, and nematode egg masses were collected by washing the roots with 1% sodium hypochlorite (Arce et al., 2017). The resulting egg suspension was passed through a 20-µm-mesh sieve and thoroughly washed with tap water. Eggs were suspended in water and counted under a light stereomicroscope. After egg collection, the roots were weighed. According to our preliminary experiments, this nematode density results in 38.8 ± 7.08 galls/plant within 5 weeks, which corresponds to an intermediate level of infestation and does not affect plant growth drastically (Bridge & Page, 1980). Similar nematode densities are frequently used in other studies (Arce et al., 2017; Barker & Weeks, 1991; Hanounik & Osborne, 1975; Kafle et al., 2017).

2.6 | Effect of *M. sexta*-induced jasmonates on root gall numbers

To estimate the influence of herbivory induced jasmonates on nematode establishment, jasmonate producing and jasmonate-deficient plants were subjected to simulated *M. sexta* attack and inoculated with *M. incognita* nematodes as indicated above. Two weeks after the first treatment, the plants were harvested, and the numbers of galls were counted.

2.7 | Quantification of the impact of the plant-mediated interaction between *M. sexta* and *M. incognita* on plant reproductive potential

To assess whether the *M. sexta*-induced, jasmonate-dependent facilitation of *M. incognita* performance influences *N. attenuata* reproduccion, we measured flower production as a proxy of the plant's reproductive potential in control plants, plants attacked by *M. incognita*, plants subjected to simulated *M. sexta* attack, and plants subjected to both stresses ($n = 8$). From the measured effect sizes of plants that were subjected to a single stress, we calculated the potential additive effect of both stresses on flower production by summing up the single stress effect sizes. *Manduca sexta* attack was simulated as described above. Seven days after the first simulated *M. sexta* herbivory treatment, ten thousand *M. incognita* infective juveniles were released in the roots as described above. Intact plants served as controls. Five weeks later, flowers were counted on each plant. Earlier experiments have shown that the number of flowers after 5 weeks is a good proxy for total flower and seed capsule production (Hettenhausen, Baldwin, & Wu, 2012; Machado, McClure, et al., 2016).

2.8 | Statistical analysis

All dataset were analysed by ANOVA using Sigma Plot 12.0 (Systat Software Inc., San Jose, CA, USA) unless otherwise stated. Normality and equality of variance were verified using Shapiro–Wilk and

Levene's tests, respectively. Holm–Sidak post hoc tests were used for multiple comparisons. Datasets from experiments that did not fulfill the assumptions for ANOVA were natural log-, root square-, or rank-transformed before analysis. Principal component analysis was carried out in MetaboAnalyst 3.0 using default settings (Xia & Wishart, 2016).

3 | RESULTS

3.1 | Simulated *M. sexta* attack induces local and systemic jasmonate accumulation

Upon simulated *M. sexta* attack, JA and JA-Ile levels increased in treated leaves within 10 min (Figure 1a,b). Within 30 min, systemic accumulation was visible in the petioles, stems, and systemic leaves (Figure 1c–h). Two hours after induction, an increase in JA and JA-Ile was observed in the roots (Figure 1i,j).

3.2 | Simulated *M. sexta* attack reduces root nutritional quality in a jasmonate-dependent manner

To investigate whether the increase in JA and JA-Ile in the roots of leaf-induced plants is associated with changes in root metabolism, we measured free amino acids, soluble sugars, starch, and nicotine in the roots of EV and jasmonate-deficient irAOC plants following simulated *M. sexta* attack. Principal component analysis revealed strong jasmonate-dependent metabolic changes in the roots of plants that were subjected to simulated *M. sexta* attack
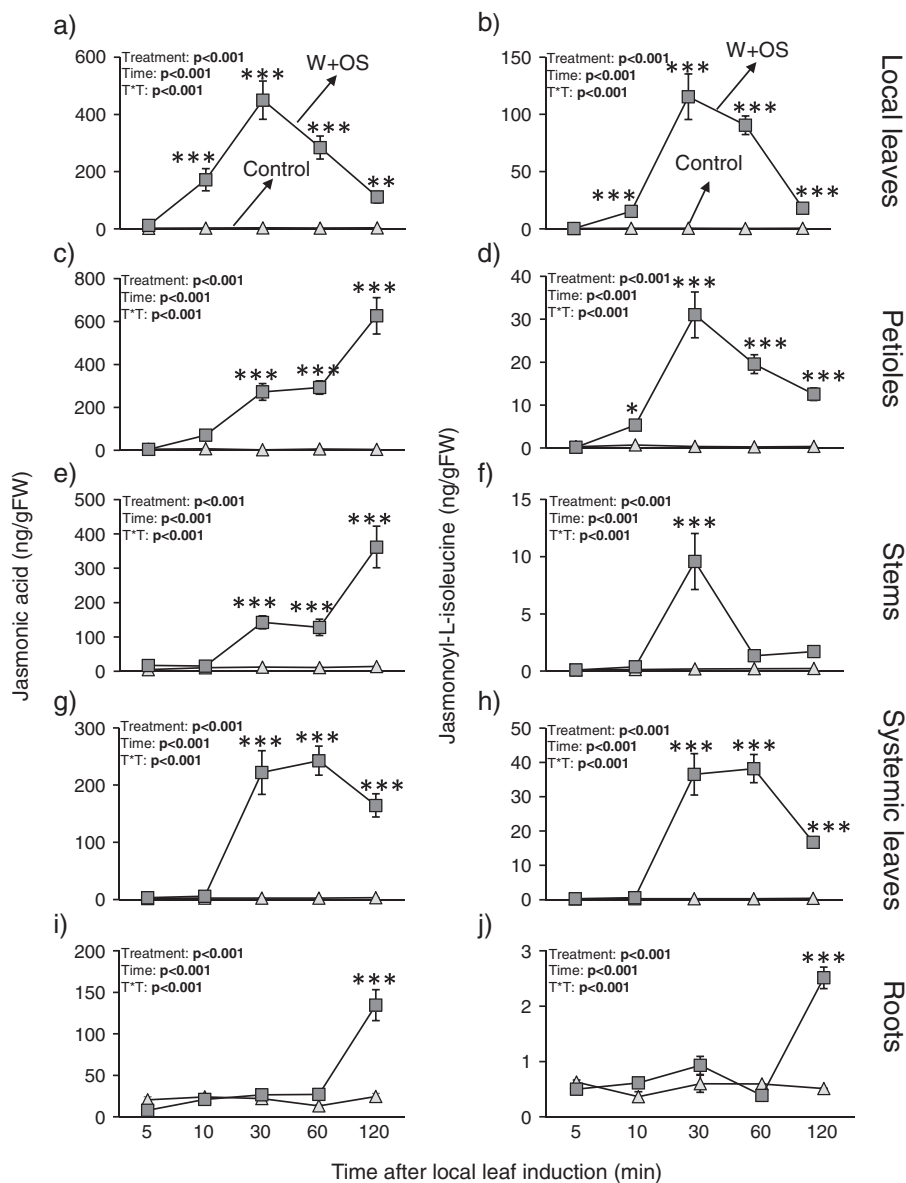

FIGURE 1 Jasmonates are induced locally and systemically in response to simulated *Manduca sexta* attack. Average (±SE) jasmonic acid levels (a, c, e, g, i) in local treated leaves (a) or in untreated petioles (c), stems (e), systemic leaves (g), or roots (i). Average (±SE) jasmonoyl-L-isoleucine levels (b, d, f, h) in local treated leaves (b) or in untreated petioles (d), stems (f), systemic leaves (h), or roots (j). Asterisks indicate statistically significant differences between treatments within time point and tissue (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Control = intact plants; W + OS = wounded and *M. sexta* oral secretion-treated plants

(Figure 2a). In particular, lower levels of glucose, fructose, sucrose, and proline and higher nicotine contents were observed in the roots of EV plants treated with *M. sexta* oral secretions compared to control and wounded plants (Figure 2b–d and Table S1). In contrast, no changes in carbohydrate pools or nicotine were observed in

jasmonate deficient irAOC plants upon simulated *M. sexta* attack (Figure 2b–d). Although root proline levels were reduced upon simulated herbivory attack in a jasmonate dependent manner, no changes in total amino acid levels were observed in induced plants (Figure 2d, Table S1).
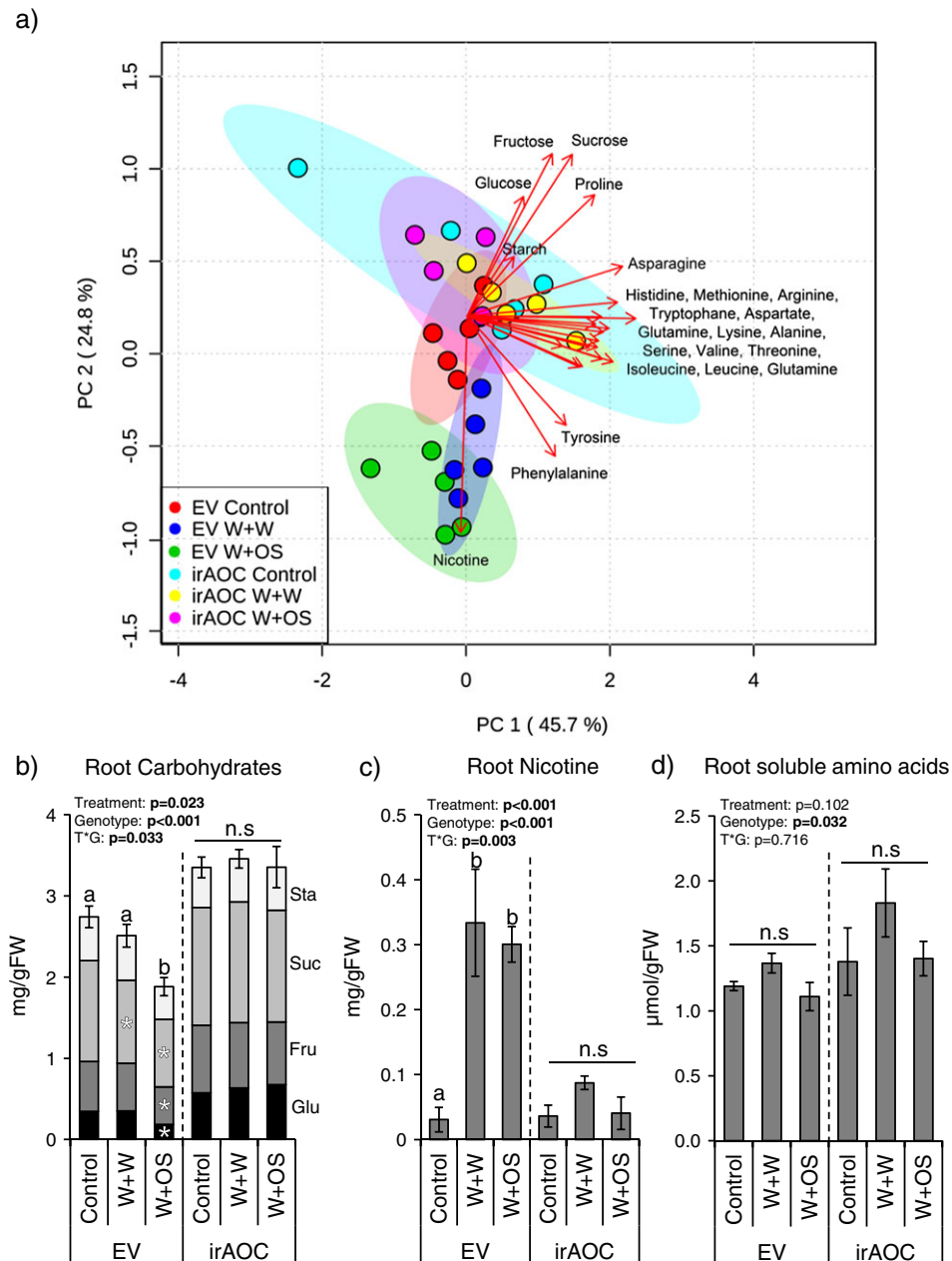
FIGURE 2 Leaf herbivory strongly impacts root metabolism. (a) Principal component analysis of the root metabolic profiles in response to simulated leaf herbivory attack. Highlighted areas denoted 95% confidence intervals. Vectors display the relationship between metabolites and treatments. Vector lengths denote the magnitude of the relationship and the direction whether it is positive or negative. (b) Average (±SE) root carbohydrate content. Black bars: Glucose (Glu), dark grey bars: Fructose (Fru), light grey bars: Sucrose (Suc), and white bars: Starch (Sta). Different letters indicate statistically significant differences between treatments within genotypes ($p < 0.05$). Asterisks indicate statistically significant differences for individual metabolites between W + W- or W + OS-treated plants and control plants within genotypes. (c) Average (±SE) root nicotine content. Different letters indicate statistically significant differences between treatments within genotypes ($p < 0.05$). (d) Average (±SE) root amino acid content. Different letters indicate statistically significant differences between treatments within genotypes ($p < 0.05$). Thirty plants were analysed ($n = 5$). EV = jasmonate-competent, empty vector-transformed *Nicotiana attenuata* plants; irAOC = jasmonate-biosynthesis deficient allene oxide cyclase *N. attenuata* plants; Control = intact plants; W + W = wounding and water-treated plants; W + OS = wounded and *Manduca sexta* oral secretion-treated plants; n.s = not significant

3.3 | Simulated herbivore attack increases the abundance of parasitic nematodes in the field in a jasmonate-dependent manner

To understand the ecological consequences of leaf-herbivory induced root jasmonates, we quantified the abundance of root parasitic nematodes in jasmonate-competent EV and jasmonate-deficient irAOC plants in the field. We observed that _N. attenuata_ roots were attacked by _P. hexincisus_ and _Ditylenchus sp._ nematodes (Figure 3). In jasmonate competent EV plants, simulated _M. sexta_ attack increased the number of _P. hexincisus_ individuals more than fourfold (Figure 3a). In contrast, leaf induction had no effect on _P. hexincisus_ abundance in jasmonate-deficient irAOC plants. Overall, _P. hexincisus_ was more abundant in the root systems of irAOC plants than in EV plants (Figure 3). No effects of treatment or genotype were observed for _Ditylenchus sp._ (Figure 3b).

3.4 | Simulated herbivory attack increases _M. incognita_ performance in a jasmonate-dependent manner

To understand the effect of _M. sexta_ attack on root parasitic nematodes in more detail, we conducted experiments under controlled conditions in the glasshouse. As our efforts to rear _P. hexincisus_ or _Ditylenchus sp._ were unsuccessful, we used the model parasitic nematode _M. incognita_ instead. _Meloidogyne incognita_ infests tobacco roots in natural and agricultural ecosystems (Kaplan et al., 2008; Kaplan et al., 2009; Tedford & Fortnum, 1988). We did not observe any significant changes in the number of galls induced by nematodes on EV or jasmonate-deficient irAOC plants 2 weeks after nematode infestation (Figure S1). However, on jasmonate-competent EV plants, leaf wounding significantly increased the number of _M. incognita_ eggs 5 weeks after nematode infestation (Figure 4a). This effect was further increased by treating the wounds with _M. sexta_ oral secretions, and the effect was independent of root biomass (Figure 4a,b). In untreated plants, nematode performance did not differ between EV and jasmonate-deficient irAOC plants (Figure 4). In contrast to EV plants, wounding or simulated _M. sexta_ attack did not change nematode performance in irAOC plants. From these results, we conclude that

jasmonate signalling is required for the wound- and _M. sexta_-induced facilitation of _M. incognita_ performance.

3.5 | _M. sexta_-induced, jasmonate-dependent facilitation of root nematodes imposes additional fitness costs to _N. attenuata_

To quantify how the _M. sexta_-induced, jasmonate-dependent facilitation of _M. incognita_ performance influences the reproductive potential of _N. attenuata_, we measured flower production in EV and irAOC plants in response to the different treatment combinations. Both simulated _M. sexta_ herbivory and _M. incognita_ infestation decreased flower production of EV plants (Figure 5a). Both stresses together strongly reduced flower production in EV plants. Interestingly, this effect was larger than the expected additive effect of the individual stresses (Figure 5a,b). Simulated _M. sexta_ herbivory and _M. incognita_ infestation also decreased flower production of irAOC plants (Figure 5a). However, in irAOC plants, the effect of both stresses together was not different from the effect of _M. incognita_ alone (Figure 5a) and was not significantly different from the expected additive effect of the individual stresses (Figure 5b).

4 | DISCUSSION

In this study, we show that intact jasmonate signalling is required for leaf herbivory induced facilitation of root parasitic nematode infestation. This facilitation effect reduces the plant's reproductive potential to a greater degree than would be expected from the additive effects of the individual stresses.

Plant-mediated interactions are well known to influence the distribution and abundance of plant-associated organisms (Arce et al., 2017; Bezemer & van Dam, 2005; Bezemer et al., 2003; van Dam et al., 2003; Kaplan et al., 2009; Erb et al., 2013; Erb et al., 2011; Erb et al., 2015). It has also been argued that the resulting impact on plant fitness may drive the evolution of plant defense traits (Erb et al., 2008; M. T. J. Johnson, Vellend, & Stinchcombe, 2009; Stam et al., 2014; van der Putten, Vet, Harvey, & Wäckers, 2001). However, isolating the contribution of plant-mediated effects to fitness differentials in
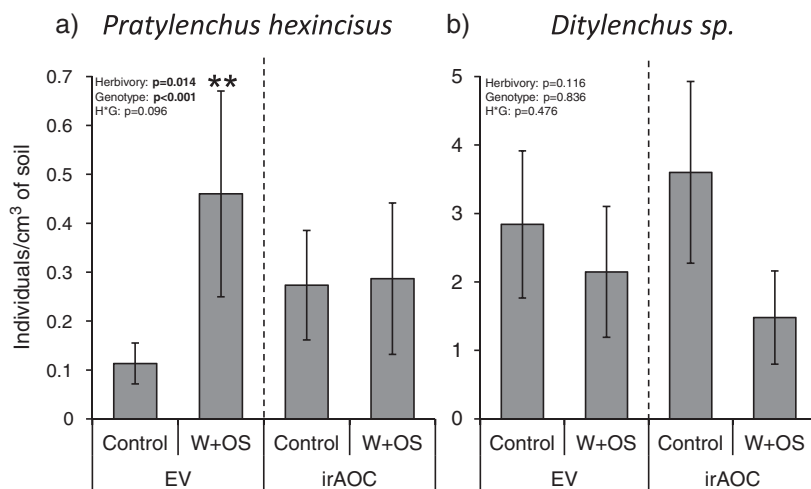

FIGURE 3 Herbivory induced jasmonates increase root parasitic nematode abundance in the field. Average (±SE) number of (a) _Pratylenchus hexincisus_ or (b) _Ditylenchus_ sp. per cm³ of soil. Twenty-four plants were excavated and analysed (n = 6). Asterisks indicate statistically significant differences between treatments within genotypes (**: 0.01 < _p_ < 0.05). EV = jasmonate-competent, empty vector-transformed _Nicotiana attenuata_ plants; irAOC = jasmonate-biosynthesis deficient allene oxide cyclase _N. attenuata_ plants; Control = intact plants; W + OS = wounded and _Manduca sexta_ oral secretion-treated plants

a)

Herbivory: **p=0.014**
Genotype: **p<0.001**
H*G: **p=0.096**

b)

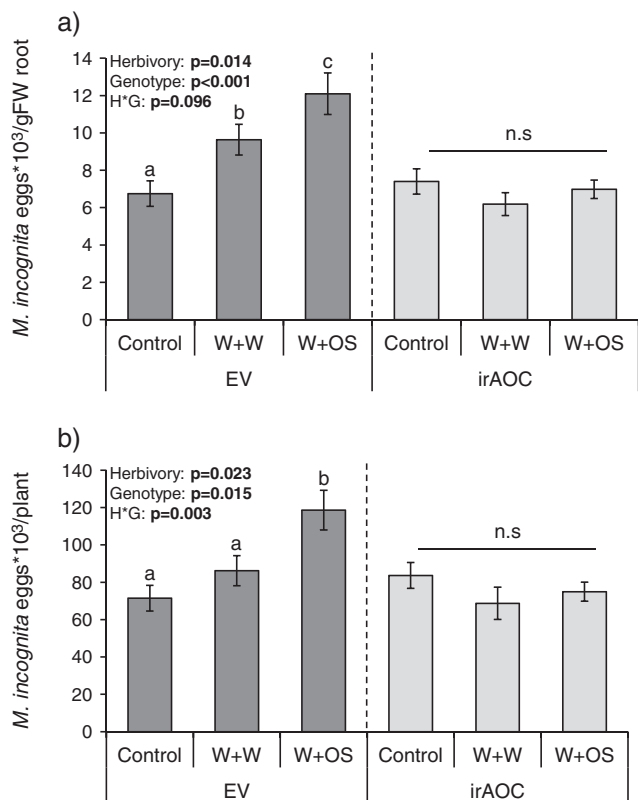Herbivory: **p=0.023**
Genotype: **p=0.015**
H*G: **p=0.003**

FIGURE 4 Herbivory induced jasmonates increase root parasitic nematode reproductive potential in the glasshouse. Average (±SE) number of *Meloidogyne incognita* eggs (a) per gram of roots or (b) per plant 5 weeks after infestation. Eighty plants were harvested and analysed (*n* = 20). Different letters indicate statistically significant differences between treatments within genotypes (*p* < 0.05). EV = jasmonate-competent, empty vector-transformed *Nicotiana attenuata* plants; irAOC = jasmonate-biosynthesis deficient allene oxide cyclase *N. attenuata* plants; Control = intact plants; W + W = wounding and water-treated plants; W + OS = wounded and *Manduca sexta* oral secretion-treated plants; n.s = not significant

multiattacker situations has been hampered by the lack of molecular tools to manipulate plant-mediated interactions (Schöning & Wurst, 2016; Kessler & Baldwin, 2004; Soler et al., 2013; Soler et al., 2007). In this study, the identification of jasmonates as the signals that mediate the facilitation of parasitic nematodes following leaf attack provides direct evidence that the plant-mediated effect reduces the plant's reproductive potential. This finding is consistent with the hypothesis that plant-mediated effects have the potential to influence the evolution of plant defense traits (Carmona & Fornoni, 2013; Fox, 1981; Lankau & Strauss, 2007; Poelman & Kessler, 2016).

Several studies have demonstrated that leaf attack increases the susceptibility of plant roots towards parasitic nematodes (Alston et al., 1993; Kafle et al., 2017; Kaplan et al., 2008; Kaplan et al., 2009; Russin et al., 1989; Russin et al., 1993). The increased performance of nematodes on leaf-attacked plants has been proposed to result from increased carbohydrate allocation to the roots (Biere & Goverse, 2016; Kaplan et al., 2009; Masters et al., 1993). Our work confirms that leaf induction specifically facilitates root nematode infestation and increases nematode abundance and reproductive output in the field and the glasshouse in a species-specific manner. However, we

also observed that leaf induction was accompanied by a strong depletion of sugars and starch in the roots (Machado et al., 2013; Machado, Zhou, et al., 2017). These observations are therefore not consistent with the hypothesis that carbohydrates are the facilitators of nematode performance (Kaplan et al., 2009; Biere & Goverse, 2016; Masters et al., 1993). We therefore propose that the herbivory induced facilitation of root nematodes might be achieved through other mechanisms. Facilitation may, for instance, be the result of structural changes in the roots (Erb et al., 2015). Leaf herbivory downregulates lignin biosynthetic genes and alters lignin-associated phenolics in the roots of *N. attenuata* (Gaquerel, Kotkar, Onkokesung, Galis, & Baldwin, 2013), which might reduce the energy required for nematode penetration and establishment or facilitate the formation of giant cells that maximize nutrient availability and fuel nematode reproductive potential (Dhakshinamoorthy, Mariama, Elsen, & de Waele, 2014; Fogain & Gowen, 1995; Ji, Kyndt, He, Vanholme, & Gheysen, 2015; Wuyts et al., 2007). An alternative explanation might be that herbivory changes plant attractiveness by influencing, for instance, root exudation or root volatile emission patterns. Nematodes exploit systemically induced root volatiles for host location (Ali, Alborn, & Stelinski, 2011; Kihika, Murungi, & Coyne, 2017; Massalha, Korenblum, Tholl, & Aharoni, 2017). Further studies might aim at investigating the relative contribution of these effects to herbivory induced facilitation of nematode infestation.

Jasmonates have been proposed as regulatory signals in plant-mediated interactions between leaf- and root-feeding herbivores (van Dam & Heil, 2011; Erb et al., 2008; Erb et al., 2009), and the results presented here are consistent with this hypothesis. In laboratory and field experiments, we observed that leaf herbivory induced jasmonates were required to increase the populations of *P. hexincisus* and *M. incognita* nematodes. However, we also observed that herbivory induced jasmonates did not influence *Ditylenchus sp.* nematode populations, which suggest that the role of systemically induced jasmonates is species-specific.

The majority of studies so far found that jasmonates protect plants against root-feeding nematodes. Our field results also show that constitutive jasmonates are associated with lower numbers of *P. hexincisus* on the roots. Given these observations, it may seem surprising that leaf-herbivory induced jasmonates increase rather than decrease nematode abundance. However, many studies document that induced jasmonates trigger very different phenotypic changes than do the constitutive jasmonate levels (Machado et al., 2013; Machado et al., 2015; Meldau, Ullman-Zeunert, Govind, Bartram, & Baldwin, 2012; Santhanam, Groten, Meldau, & Baldwin, 2014). For instance, induced jasmonates influence the composition of soil microbiota, whereas constitute endogenous jasmonates only have a minor influence (Carvalhais et al., 2013; Kniskern, Traw, & Bergelson, 2007; Santhanam et al., 2014). Thus, we postulate that due to the different plant responses they elicit, constitutive jasmonate levels have a neutral to negative effect on root-feeding nematodes, whereas leaf-herbivory induced jasmonates have a positive effect.

An additional important aspect revealed by our experiments is that the facilitation of nematode performance reduces the plant's reproductive potential. The costs, expressed as the reduction in number of flowers, associated with this facilitation process are greater than the
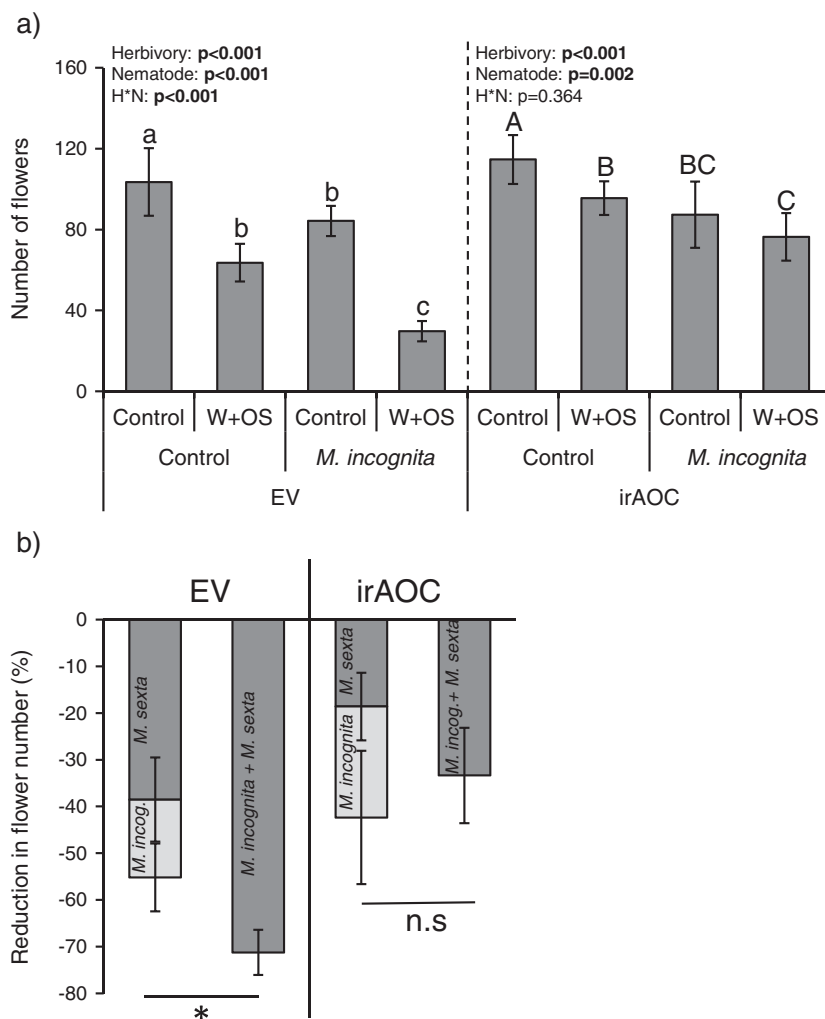
FIGURE 5 Facilitation of nematode performance by leaf herbivory reduces flower production. (a) Average (±SE) number of flowers produced per plant. (b) Average reduction in flower number upon simulated *Manduca sexta* attack, *Meloidogyne incognita* infestation, or both. Sixty-four plants were analysed (*n* = 8). Different letters indicate statistically significant differences between treatments within genotypes (*p* < 0.05). Asterisks indicate significant differences between treatments within genotypes (*: *p* < 0.05). EV = jasmonate-competent, empty vector-transformed *Nicotiana attenuata* plants; irAOC = jasmonate-biosynthesis deficient allene oxide cyclase *N. attenuata* plants; Control = non-induced plants; W + OS = wounded and *M. sexta* oral secretion-treated plants; Control = *M. incognita*-free plants, *M. incognita* = *M. incognita*-infested plants; n. s = not significant

sum of the costs that result from single attacks. Silencing the key hormone that regulates this facilitation process leads to the disappearance of this difference, which directly associates the observed costs with the facilitation process. In other words, the strong reduction in flower numbers of wild type plants that are elicited in their leaves and attacked in their roots by nematodes is not the result of the addition or synergistic interaction of the two stresses, but it is specifically associated with the leaf-herbivory induced facilitation of nematode performance. However, it is important to note that jasmonates also alter the cost of defense induction by *M. sexta*. We can, therefore, not fully exclude that the absence of the synergistic effect in the jasmonate-deficient plants may, at least in part, be driven by a reduction of the physiological interactions which are elicited by dual attack. Further research would be required to understand the facilitation process in sufficient detail to specifically manipulate it without impairing other responses that are elicited by individual attacks.

In conclusion, our work provides evidence for the requirement of intact jasmonate signalling for herbivory induced facilitation of root parasitic nematode infestation. The facilitation process reduces plant reproductive potential beyond the individual or combined effect of both stresses. Jasmonate signalling is a highly polymorphic trait in *N. attenuata* (D. Li, Baldwin, & Gaquerel, 2016, 2015; Machado et al., 2013; Schuman, Heinzel, Gaquerel, Svatos, & Baldwin, 2009), which partially accounts for the asymmetric herbivore damage under natural conditions (Machado,

McClure, et al., 2016; Kallenbach et al., 2012; Steppuhn, Schuman, & Baldwin, 2008). Our experiments suggest that nematode attack might exert selective pressure against jasmonate signalling and could therefore be one of the factors that favours polymorphisms in jasmonate signalling and jasmonate-independent defensive mechanisms in nature (Machado et al., 2015; Machado, Zhou, et al., 2017).

AUTHOR CONTRIBUTIONS

R. A. R. M. conceived the project, designed and performed all the experiments, analysed data, and wrote the manuscript. C. C. M. A. designed and performed experiments, analysed data, and helped to write the manuscript. M. A. M. designed and performed experiments and analysed data. I. T. B. designed and performed experiments, analysed data, and helped to write the manuscript. M. E. conceived the project, designed and performed experiments, analysed data, and wrote the manuscript. All authors read and approved the final version of the manuscript.

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