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L-DOPA functions as a plant pheromone for belowground anti-herbivory communication

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Abstract

While mechanisms of plant-plant communication for alerting neighbouring plants of an imminent insect herbivore attack have been described aboveground via the production of volatile organic compounds (VOCs), we are yet to decipher the specific components of plant-plant signalling belowground. Using bioassay-guided fractionation, we isolated and identified the non-protein amino acid L-DOPA, released from roots of *Acyrtosiphon pisum* aphid-infested *Vicia faba* plants, as an active compound in triggering the production of VOCs released aboveground in uninfested plants. In behavioural assays, we show that after contact with L-DOPA, healthy plants become highly attractive to the aphid parasitoid *(Aphidius ervi)*, as if they were infested by aphids. We conclude that L-DOPA, originally described as a brain neurotransmitter precursor, can also enhance immunity in plants.

Keywords: Plant-plant signalling, Root exudates, Aphids, Parasitoids, VOC, plant immunity

Introduction

Plant communication with other organisms mainly relies on the release of constitutive or stress-induced chemical signals that travel both through the air headspace or the soil matrix (Bruin & Dicke 2001; Karban 2008; Erb *et al.* 2015). In the rhizosphere, comprising the complex soil environment in close contact with plant roots, plants contribute a steady production of root exudates, including ions, free oxygen and water, enzymes, mucilage, and a variety of other secondary metabolites (Rovira 1969). Once released, root exudates can function as signals regulating plant-microbe (Badri & Vivanco 2009), plant-animal (Johnson & Rasmann 2015) and plant-plant interactions (Bais *et al.* 2006). Belowground plant-plant communication has been proven to mediate key ecological interactions, such as competition and facilitation, in both natural and applied systems, and several molecules have been identified as key agents of chemical communication (van Dam & Bouwmeester 2016).

Emerging evidence indicates that belowground plant-plant communication can also serve to signal neighbouring plants of a recent aboveground insect herbivore attack. For instance, it was shown that a warning signal can run through the common mycelial network of the arbuscular mycorrhizal fungi to alert neighbouring healthy plants of current aphid attack (Babikova *et al.* 2013). It was also previously demonstrated that uninfested *Vicia faba* (Fabaceae) plants maintained in the same pot together with plants infested by the pea aphids *Acyrthosiphon pisum* (Homoptera: Aphididae) became more attractive towards the aphid parasitoids *Aphidius ervi* (Hymenoptera: Braconidae) than when placed in the same pot with healthy plants (Guerrieri *et al.* 2002). This change in attractiveness was not observed when root contact was prevented among plants that had their aerial parts in close proximity, and thus freely exchanging aboveground volatile organic compounds (VOCs) (Guerrieri *et al.* 2002). These results were further confirmed using hydroponic growing conditions. Uninfested *V. faba* plants placed in hydroponic solution that was previously used to grow aphid-infested plants became attractive to *A. ervi* parasitoids, whereas placing them in the hydroponic solution of uninfested plants did not change their attractiveness (Guerrieri *et al.* 2002).

Accordingly, as shown in the *Vicia*-aphid-parasitoid system, plant-plant signalling can also occur within the rhizosphere. Since it only works when roots are in contact, we hypothesized that such belowground plant-plant signalling is mediated by a systemically translocated root-borne elicitors. We therefore predicted that insect herbivore-damaged plants would be induced to produce a unique blend of molecules that elicits a response in neighbouring plants if in contact through the soil matrix. Because herbivore-damaged plants can modify their internal chemistry (Karban & Baldwin 1997) to either directly become more toxic to herbivores (Farmer & Ryan 1992) or indirectly by attracting herbivore natural enemies via the emission of VOCs above and belowground (Kost & Heil 2006; Heil 2008; Dicke & Baldwin 2010), we also predicted that response elicitation in neighbouring plants could be observed in the form of changes in leaf chemistry aboveground (Bezemer & van Dam 2005). Here, we report on

a series of plant-plant communication bioassays and bioassay-guided fractionation analyses that ultimately characterized the amino acid L-DOPA, a known neurotransmitter precursor, as one of the elicitors released by the roots of aphid-damaged *V. faba* plants. We show that root contact with L-DOPA altered the aboveground headspace chemical profile of healthy plants, which then attracted more aphid parasitoids than plants not treated with L-DOPA.

Materials and Methods

Insects - The parasitoid *Aphidius ervi* was reared on its natural host, the pea aphid *Acyrthosiphon pisum* maintained on potted broad bean (*Vicia faba*) plants, cv. Aquadulce (Guerrieri *et al.* 1993). Aphid and parasitoid cultures were kept in separate environmental chambers at 20±1°C, 75±5% relative humidity, and 18L: 6D photoperiod. Insect parasitoids used in the bioassays were reared as synchronized cohorts by exposing heavily infested plants for 24 h to 1-day-old mated females; after a week, the resultant mummies were clipped from the plant and isolated in glass test tubes (60 x 8 mm) plugged with cotton wool. Experimental females were used within the first day after emergence, mated, and fed with a 50% honey solution. All experiments were conducted 3 hr from the onset of the photophase.

Plants - Plant material in hydroponic solution: Broad bean seeds (*Vicia faba* L., cultivar Aquadulce) were soaked in water for 24 h, then potted in vermiculite and kept in a controlled environment room at 20°C. After 5 days, the seedlings were gently removed from the vermiculite, the seed coat discarded and the roots rinsed with water, carefully removing any vermiculite residue. Two seedlings were then placed in a glass beaker containing a hydroponic solution made with Murashige and Skoog basal salt mixture (2 g L^{-1}, Duchefa Biochemies, The Netherlands) and placed in a glasshouse (20°C, L:D 16:8 h). Each beaker was wrapped in aluminium foil to hold the plants in position and to prevent the light from reaching the roots. Every 2-3 days, the hydroponic solution was renewed. For further experiments, specifically after identification 0.1 ppm or 0.01 ppm of the active compounds in the attractive root exudate blends (see methods below), each pure compound (L-DOPA or D-DOPA) was added to the beakers with clean hydroponic solution and two seedlings were transferred into it and kept as described above for 24 h before testing them in the wind-tunnel.

Plant material in soil: Broad bean seeds (*Vicia faba* L., cultivar Aquadulce) were soaked in water for 24 h, then potted (2 plants/pot) in sterile soil and kept in a glasshouse at 20±2°C. The distal end of a Teflon tube (20 cm, 1 cm diameter) covered with parafilm and pinched with a nail to make holes along 5 cm was inserted in each pot and as close as possible to plant roots. After 14 days, 0.1 ppm of each pure compound (L-DOPA or D-DOPA) were syringed through the apical end of the Teflon pinched tube emerging from the soil and left for 24 h before testing them in the wind-tunnel.

Collection and bioassay-guided fractionation of root exudates and identification of L-DOPA in the finally active fraction - After a renewal of hydroponic solution, half of the beakers, containing two-week-old plants, were infested with 100 mixed-age *A. pisum* (P+A). In our experiments, we considered an infestation well above the calculated thresholds of 50 aphids feeding for 72 hours needed to record a change in the behaviour of the aphid parasitoid *A. ervi* (Guerrieri et al, 1999). Nonetheless, the aphid population tested corresponds to an initial state of infestation considering that a single female aphid colonizing a plant reproduce by telytokous parthenogenesis and viviparity resulting in the production of tenth of nymphs each starting reproducing in a few days. After 3 days, the hydroponic solution from uninfested (P) and infested (P+A) plants was collected and filtered using filter paper to remove any debris. Organic compounds present in the solutions were extracted by solid-phase extraction (SPE) from P and P+A solutions (~10 beakers equalling ~2 L per replicate). The SPE columns were 6 ml cartridges containing Evolute C18 sorbent (500 mg, Biotage, UK). The cartridges were conditioned prior to extraction using HPLC grade methanol (2 ml), followed by displacement by distilled water (2 ml). The extractions were performed using a VacMaster-10 SPE manifold (IST, UK). The cartridges were then extracted with methanol (2 ml). This was repeated 40 times. Ten replicates (~100 beakers) were combined and the resulting solution was rotary evaporated to dryness. The compounds were re-dissolved into HPLC water or ethanol (5 ml, 50 µl per beaker) for bioassay or further fractionation and chemical analysis. For the identification of the DOPA enantiomer, chiral separation was achieved on an ACE 5 C18 column (250 mm × 4.6 mm; 5 µm particle size; Thermo Scientific, USA). The mobile phase was 1 mM CuSO4, 3 mM phenylalanine, 0.01% trifluoracetic acid, 1% acetonitrile in HPLC H2O. The flow rate was maintained at 1 mL min-1 or 0.5 mL min-1 and isocratic conditions for 20 min (Wu *et al.* 2006; Husain *et al.* 1994). Detection was at 280 nm, injected volume was 10 µL. 1 mg/mL DOPA standard concentrations were used. C18 root exudate extracts were analysed and fractionated on an ACE 5 C18 column (250 mm × 10 mm; 5 µm particle size; Thermo Scientific, USA) by HPLC (Shimadzu prominence, Shimadzu Corporation, Kyoto, Japan). The mobile phase A was 5% formic acid in HPLC H2O, and mobile phase B was acetonitrile. The flow rate was maintained at 1 mL min-1, starting with isocratic conditions at 5% B for 10 min, then linear gradient program to 60:40 (A:B) at 25 min, to 30:70 at 40 min, to 5:95 at 41 min and isocratic for 5 min, then to 95:5 at 45 min and isocratic for 5 min. Three fractions were collected at 0-15min (Fraction 1), 15-40min (Fraction 2) and from 40-55 min (Fraction 3). Fraction 1 was then fractionated into four sub-fractions 0-6min (Fraction 1a), 6-12 min (Fraction 1b), 12-24 min (Fraction 1c) and 24-55 min (Fraction 1d). Detection was at 280 nm, injected volume was 10 µL.

Wind tunnel bioassays - For each experimental condition, a total of ten plants grown hydroponically or in soil as described above were used and tested in a wind-tunnel (see Guerrieri *et al.* (1999) for details) daily in a random order to reduce any bias related to the time of the experiments. One hundred parasitoid females were tested singly for each target in no-choice experiments, and observed for a maximum of 5 min. The percentage of response (oriented flights, landings on the target) to each target plant was calculated. The parameters of the bioassay were set as follows: temperature, 20 ± 1 °C; 65 ± 5% RH; wind speed, 25 ± 5 cm s-1; distance between releasing vial and target, 50 cm; PPFD at releasing point, 700 µmol m^2 s^{-1}.

Air entrainment of plants treated with synthetic L-DOPA and D-DOPA - After bean plants were grown in hydroponic solution for 10 days, the hydroponic solution was replaced (200 mL) and treated with L-DOPA (10 µg), D-DOPA (10 µg) or HPLC water (control, 10 µL) (n=15 replicates/treatment). After 24 h, the bean plants were enclosed in Multi-Purpose Cooking Bags [poly(ethyleneterephthalate)] or PET, volume 3.2 L, ~12.5 µm thickness, max. 200°C, Sainsbury's Supermarkets Ltd., London, UK]. The bottom of the bag was enclosed around the top of the beaker containing the hydroponic solution. The inlet was fitted to the open end of the bag, and the outlet was fitted to a corner of the bag after cutting off with scissors. Air that had been purified by passage through an activated charcoal filter (BDH, 10-14 mesh, 50 g) was pushed into (750 mL/min) and pulled (700 mL/min) out of the bags. Volatiles were trapped onto Tenax (50 mg; Supelco, Bellefonte, USA) held in glass tubing (5 mm outer diameter) by two plugs of silanised glass wool. The Tenax was conditioned by washing with dichloromethane (2 mL), followed by redistilled diethyl ether (2 mL) and heating at 132°C for 2 h under a stream of purified nitrogen. After 24 h, the Tenax tubes were sealed in glass ampoules in an atmosphere of nitrogen and stored at -20°C until analysis. Volatile sample analysis Tenax tubes were inserted into the OPTIC PTV unit of a GC (30->250°C ballistically at a rate of 16°C/s) connected to a Micromass Autospec Ultima magnetic sector mass spectrometer (Waters, Milford, MA). The GC (Agilent 6890 N) was fitted with a 50 m × 0.32 mm i.d. × 0.52 µm film thickness HP-1 column (Agilent, Santa Clara, CA, USA). Ionization was by electron impact (70 eV, 220°C). The GC oven temperature was maintained at 30°C for 5 min and then programmed to increase at 5°C/min to 250°C, with a 70-min run time. The identity of peaks was confirmed by comparison of their GC and GC–MS properties with those of authentic standards (see Sasso *et al.* (2007) for details), and by GC peak enhancement using authentic samples. The enantiomeric composition of linalool was already determined as (*R*)-linalool for this plant by (Webster *et al.* 2008). Quantification of compounds was achieved by the single-point external standard method with a series of C7-C22 alkanes, where the amount of an analyte was estimated using the peak area of the nearest alkane peak, the amount of which was known.

Statistical analysis - The number of parasitoids responding to each target was compared with a G-test for independence with William's correction using the RVAideMemoire package (Hervé 2018) in R (R Development Core Team 2020). The resulting values of G were compared with the critical values of Chi-square. To assess differences in VOCs across DOPA treatments, we first performed a Distance-based redundancy analysis (*dbRDA*) after pareto-transformation of the data and based on Gower distance (*capscale* function in *vegan*, (Oksanen *et al.* 2013). The amount of DOPA and other peaks in the P and P+A extracts was compared using ANOVA (p=0.05) investigating the effect of `treatment`, `peak number` and `treatment × peak number`. Peak area/weight values were square root-transformed for the analysis. We visualized the clusters of species across the three treatments (control, D-DOPA, and L-DOPA) using linear discriminant analysis on the VOCs data matrix (*lda* function in the *mass* package (Ripley *et al.* 2013)). Next, to measure the interactive effect of treatment and VOCs identity on VOCs production, we run a two-way generalized linear model (function *glm* in R stats) on $\log_{10}$-transformed data using a Poisson family distribution. Model fit results were followed by Fisher's Least Significant Difference (LSD) test for detecting treatment effects across individual VOCs ($p < 0.05$)

Results

Bioassay-guided fractionation - To measure the activity of the root exudates released by damaged plants, we sampled *V. faba* root exudate extracts using reverse-phase (C_{18}) solid-phase extraction (SPE) from uninfested plants (plants without aphids: Plant only: P), and pea aphid (*A. pisum*)-infested plants (Plant+Aphid: P+A). Using wind-tunnel bioassays, we show that about four times more *A. ervi* oriented to (G test, $\chi = 44.800$, $p < 0.001$) and landed on (G test, $\chi = 10.303$, $p = 0.001$) *V. faba* plants grown in hydroponic solution treated with P+A extract compared to those treated with P alone (Fig. 1A,B). The chemical signal present in P+A root exudate was then identified by bioassay-guided fractionation giving three fractions of different polarity. Seven times more *A. ervi* oriented to and landed on *V. faba* plants treated with *fraction 1* (the most polar fraction) from P+A, compared with the similar HPLC fraction of P (Fig. 1C; G test, $\chi = 45.297$, $p < 0.001$; G test, $\chi = 11.514$, $p < 0.001$). No significant synergistic effects of combining fractions were observed for oriented flights and landings (Fig. 1C; G test, $\chi = 3.306$, $p = 0.069$; G test, $\chi = 0.471$, $p = 0.492$). *Fraction 1* was then further fractionated into four subfractions (Fig. 1A-D) of different polarities, of which the *a* and *d* subfractions showed the most significant effect in eliciting the indirect defence in terms of oriented flights (Fig. 1D; G test, $\chi = 38.339$, $p < 0.001$, G test, $\chi = 43.625$, $p < 0.001$, respectively) and in terms of landings (Fig. 1D; G test, $\chi = 20.723$, $p < 0.001$, G test, $\chi = 14.748$, $p < 0.001$, respectively). Thus by further analysing *fraction 1a* using peak enhancement by co-injection with enantiomerically pure authentic standards, we identified L-DOPA (RT=4.276 min under our HPLC conditions) (Fig. 1E) as one key active compound mediating plant-plant communication. The estimated amount of exuded L-DOPA by infested plants was 5.67

µg/g/day and by uninfested plants was 4.95 µg/g/day (ANOVA, df=1, p=0.001). Subsequent bioassays using pure compounds showed that about 5 times more *A. ervi* oriented to (G test, $\chi = 48.643$, $p < 0.001$) and about 3 times more landed on (G test, $\chi = 16.794$, $p < 0.001$), *V. faba* plants grown in hydroponic solution treated with L-DOPA relative to when treated with D-DOPA (at both concentrations of 0.1 ppm and 0.01 ppm) and relative to untreated *V. faba* plants (Fig. 1F), indicating enantiomers -dependent activity. No dose-dependent effect was noted for L-DOPA in terms of oriented flights (Fig. 1F; 0.01ppm: 35.4% *vs* 0.1ppm: 48.4%; G test, $\chi = 3.378$, $p = 0.066$) and landings (Fig. 1F; 0.01ppm: 18.7% *vs* 0.1ppm: 24.7%; G test, $\chi = 0.656$, $p = 0.418$). These response patterns were subsequently confirmed by performing experiments with plants grown in soil and treated with synthetic L-DOPA at a dose of 0.1 ppm (Fig. 1F; G test, $\chi = 27.496$, $p < 0.001$; G test, χ 11.121, $p < 0.001$). While we found that fraction 1d was also attractive, we were not able to fully elucidate the molecular structure of each molecule in that faction. We therefore opted to only focus on the activity of L-DOPA in this study, but we acknowledge that other compounds in the root exudate extract might also activate neighbouring plant's defences.

Induction of VOCs in neighbouring plants - By means of gas chromatography coupled to mass-spectrometry (GC-MS) analysis of the leaf headspace of *V. faba* plants grown in hydroponic solution with L-DOPA, or D-DOPA isomers, we found a total of nine compounds which varied significantly across treatments (Fig. 2; ANOVA based on 999 permutations, $F_{2,24} = 2.08$, $p = 0.034$). Across all VOCs, we also found that some compounds were more induced than others by L-DOPA (treatment effect; LR $\chi = 10.306$, $p = 0.006$; and VOCs by treatment interaction; LR $\chi = 11.601$, $p = 0.771$). Specifically, we show that L-DOPA-treated plants released 10 times and 5 times more methyl salicylate, 3 times and 4 times more of the sesquiterpene (*E*)-ocimene, 3 times and 7 times more (*E*)-caryophyllene than control (untreated) and D-DOPA treated plants, respectively (Fig. 3).

Discussion

The emerging paradigm is that plants may detect chemicals, released from conspecific or heterospecific neighbouring plants, and in response change their physiology or chemistry (Arimura *et al.* 2000; Karban 2008). Aboveground, the main players of plant-plant signalling are the volatile organic compounds (VOCs), particularly those released in response to biotic stresses. In this context, an ever-growing body of literature is showing that VOCs emitted by herbivore-damaged plants increase resistance of neighbouring undamaged plants (Karban *et al.* 2014). Responses in the receiving plants include priming, which leads to enhanced defence induction upon subsequent insect attack (Erb *et al.* 2015), or full induction of direct (Moreira *et al.* 2016) or indirect (i.e., the attraction of natural enemies of the herbivores) defences (Turlings & Erb 2018).

Belowground, plant-plant interaction can also rely on the release and perception of chemicals in the form of volatile or non-volatile root exudates (Bais *et al.* 2006), or those that can travel through the mycelial network connecting neighbouring plants (Song *et al.* 2010; Barto *et al.* 2012; Babikova *et al.* 2013). Among the main functions of plant-plant signalling belowground is the kin/non kin recognition, so to alter the development of roots and regulate nutrient and water acquisition. For example, allelopathic rice cultivars generated avoidance patterns in the roots of other rice cultivars and several paddy weed species (Yang & Kong 2017). By far less studied is the role of root exudates in mediating plant-plant communication in response to herbivore attack (Moreira & Abdala-Roberts 2019). For example, it was shown that aphid-free plants became repellent to aphids but attractive to aphid parasitoids when they were connected to aphid-infested plants via a common mycorrhizal mycelial network (Babikova *et al.* 2013). In this example, the mycelia network likely served as conduit for information exchange between the healthy and attacked plants, eliciting in the latter a change in the production and release of aboveground VOCs, particularly methyl salicylate. We here demonstrated that belowground plant-plant communication, involving changes in aboveground VOC production of healthy plants during ongoing aphid attack on neighbouring plants, occurs even in the absence of a fungal connection. Specifically, we found that within the complex root exudates blend, a non-volatile compound, the non-protein amino acid L-DOPA, is exuded by the roots of damaged plants and is perceived as an alarm signal by neighbouring plants. In the soil, amino acids have been shown to occur as "free" (i.e., not covalently bound to any other chemical entity), dissolved in the soil aqueous solution, or bound to soil colloids or to soil organic matter (Vranova et al 2011; Moe, 2013). There is also ample evidence that amino acids can move from the rhizosphere into plant roots (reviewed by Nasholm et al. 2009), and thus move within the soil matrix. Accordingly, we show that by placing L-DOPA in the rhizosphere, the plants sense it somehow, and active VOCs production. However, how long L-DOPA remains in the soil, and how far and how fast this compound can travel in the soil matrix remains an open question that merits future investigations, also by comparing different substates.

Independently of the mechanism of movement in the soil, we show that neighbouring *V. faba* plants responded to the presence of L-DOPA by inducing methyl salicylate, (*E*)-ocimene and (*E*)-caryophyllene production, all compounds known to attract aphid parasitoids (Du *et al.* 1998; Sasso *et al.* 2007, 2009; Babikova *et al.* 2013) and predators (Zhu and Park 2005). For instance, tomato plants attacked by the potato aphid *Macrosiphum euphorbiae* also increased significantly the production of methyl salicylate and (*E*)-caryophyllene, which was linked to the increased attraction of the parasitoid *A. ervi* (Sasso *et al.* 2007; Sasso *et al.* 2009). Similarly, plants treated with *cis*-jasmone, a plant-derived insect feeding-related signal, were more attractive for *A. ervi*, and this attraction was associated with the induction of (*E*)-ocimene (Birkett *et al.* 2000), later confirmed in experiments using transgenic tobacco plants (Cascone *et al.* 2015). The emission of (*Z*)-3-hexenyl acetate, 6-methyl-5-hepten-2-one

306 and (*Z*)-3-hexenol, which are known to attract *A. ervi* (Du *et al.* 1998; Sasso *et al.* 2007; Sasso *et al.*
307 2009), was enhanced, although not significantly, in L-DOPA-treated plants (Fig. 3).
308
309 In addition to being exuded from roots, non-protein amino acids such as L-DOPA can be easily
310 translocated within plant tissues and can be reused or diverted to primary metabolism when needed
311 (Huang *et al.* 2011). The leaves and pods of *V. faba* plants contain high quantities of L-DOPA (Burbano
312 *et al.* 1995), whose presence can affect the community of insect herbivores attacking these plants.
313 Accordingly, it has been shown that L-DOPA is detrimental for most generalist herbivores, whilst it is
314 exploited in different ways by specialists. For example, it was shown that *A. pisum* can sequester this
315 compound, which was reported to provide benefits for wound healing and protection against UVA-
316 radiation (Huang *et al.* 2011). For the other legume specialist aphid, *Aphis fabae*, it was shown that L-
317 DOPA can act as a powerful feeding stimulant (Jördens & Klingauf 1977). Therefore, L-DOPA can be
318 directly co-opted by insect herbivores for their own benefits. In the perpetual battle between plants and
319 insect herbivores, evolution acts on fostering adaptations and counter-adaptions for attacking and
320 defensive strategies (Ehrlich & Raven 1964). In this scenario, plants can only escape the attack of an
321 herbivore by developing more potent means of defence, such as the production of novel toxic secondary
322 metabolites. In response, the herbivores can continue feeding on the plant if they develop means of
323 tolerating or overcoming the novel toxic agent. Conversely, the subtle action of indirect defences,
324 associated to the release of specific VOCs that facilitate the foraging behaviour of predators or
325 parasitoids of the herbivore, is, evolutionarily speaking, invisible to the targeted pest on which no
326 immediate selective pressure is posed (Kessler & Heil 2011). Therefore, broad bean plants seem to have
327 counter-balanced the selective pressure of the specialist aphid *A. pisum* to cope with a toxic compound
328 (L-DOPA) by diverting the function of this compound so to deliver an indirect effect of resistance
329 induced in neighbouring plants. Plant-plant communication regulated by specific elicitors such as L-
330 DOPA amplifies the indirect resistance response to a biotic stress from a single individual to community
331 level. We know that in the same system the release of specific VOCs regulating the attraction of natural
332 enemies is associated to a specific infestation threshold, in terms of number of feeding aphids and
333 duration of their feeding activity (Guerrieri *et al.* 2002). We here show that at the same time the broad
334 bean plant responds to aphid infestation aboveground, as well as belowground, by conveying a specific
335 signal to conspecific neighbours eliciting the release of similar VOCs. The efficiency of parasitoid
336 foraging behaviour relies on the reliability and detectability of plant semiochemicals (Vet & Dicke
337 1992). The amplification of plant responses, from individuals to the entire community, seems to better
338 fulfil both requirements. In fact, herbivore-induced VOCs reliably indicate to parasitoids the presence
339 of their target victim. Moreover, it is worth noting that the VOCs released in response to aphid attack
340 can also function as direct defences. For example, methyl salicylate reduced the number of fixed aphids
341 and the reproductive rate of fixed ones by more than two thirds (Digilio *et al.* 2012). Therefore, to
342 summarize, *V. faba* plants have evolved the ability to perceive stress signals in neighbouring plants both

above- and belowground. Independently of the mode of communication, the healthy perceiving plants induce the production of key volatile compounds that can directly inhibit future aphid infestation, and at the same time, these VOCs can also attract natural enemies of the aphids in their surroundings. However, evolutionarily speaking, why do plants alert their conspecific neighbours of an imminent herbivore attack remains a matter of debate (Kessler and Heil 2011). In this case, we can argue that within an extended and densely packed crop field, the successful detection of an herbivore on a damaged plants by a parasitoid should be very scarce. Therefore, by allowing the signal to be amplified by their neighbours, a set of individual plants should facilitate the foraging success of parasitoids (Vet & Dicke, 1992), whose impact on the aphid population is usually visible with some delay in respect to the action of a predator. In fact, the enhanced release of methyl salicylate induced in our system by L-DOPA, has been shown to be also effective in attracting insect predators such as ladybugs (Zhu and Park, 2005), hence more broadly boosting the biological control of aphid pests.

The discovery of L-DOPA, a neurotransmitter precursor in animals, acting in the rhizosphere as a plant defensive pheromone supports the paradigm of divergent evolutionary outcomes for the activity of the same molecule, spanning the plant and animal kingdoms. Similarly, GABA, another non-protein amino-acidic neurotransmitter found in animal brains, was discovered to function as signalling molecule for plant development and stress response activation against biotic attack (Zimmerli *et al.* 2000). Plants can therefore co-opt broad-spectrum molecules for their own defence response against insect herbivores, whose activity could be exploited to enhance natural crop resistance against insect pests (Conrath *et al.* 2006; Bown & Shelp 2016).

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Figures

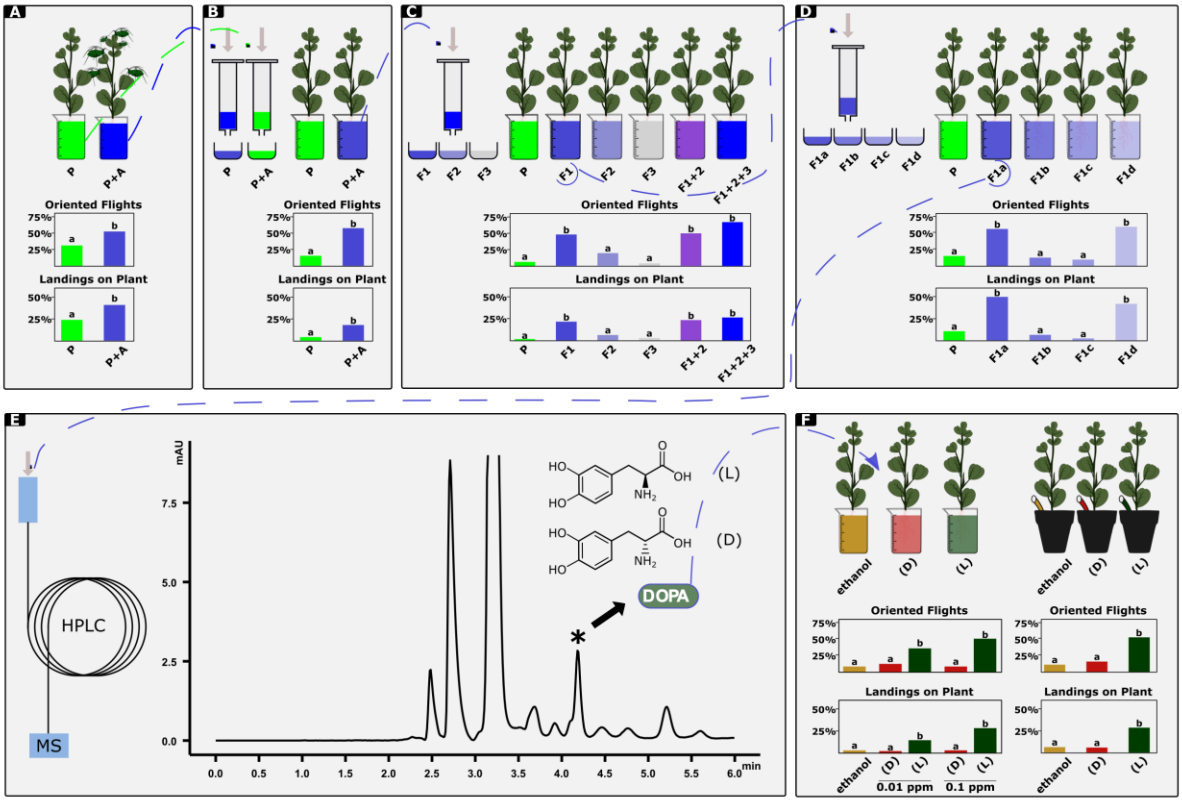

Fig. 1. Workflow for identifying root exudates for mediating plant-plant communication. Barplots show results (in %) of the oriented flights and landings of the aphid parasitoids (*Aphidius ervi*) towards bean plants (*Vicia faba*) grown in hydroponic medium (Murashige and Skooge). Behavioural assays for (**A**) pea aphid (*Acyrthosiphon pisum*)-infested (P+A, blue bar), and uninfested (P, green bar) *V. faba* plants; (**B**) *V. faba* plants treated with C_{18}-SPE collected root exudate extracts from uninfested (P, green bar) and from *A. pisum*-infested (P+A, blue bar) plants; (**C**) (P+A) *V. faba* plants treated with LC Fractions (F1, F2, F3) of the roots exudates of the P+A treatment; (**D**) *V. faba* plants treated with LC F1 subfractions (F1a, F1b, F1c, F1d); (**E**) peak identification of DOPA; (**F**) Behavioural assays for *V. faba* plants treated with synthetic DOPA (L or D) in hydroponic solution (left panels), or in the soil (right panels). Different letters above bars indicate significant differences ($P<0.05$) among treatments.

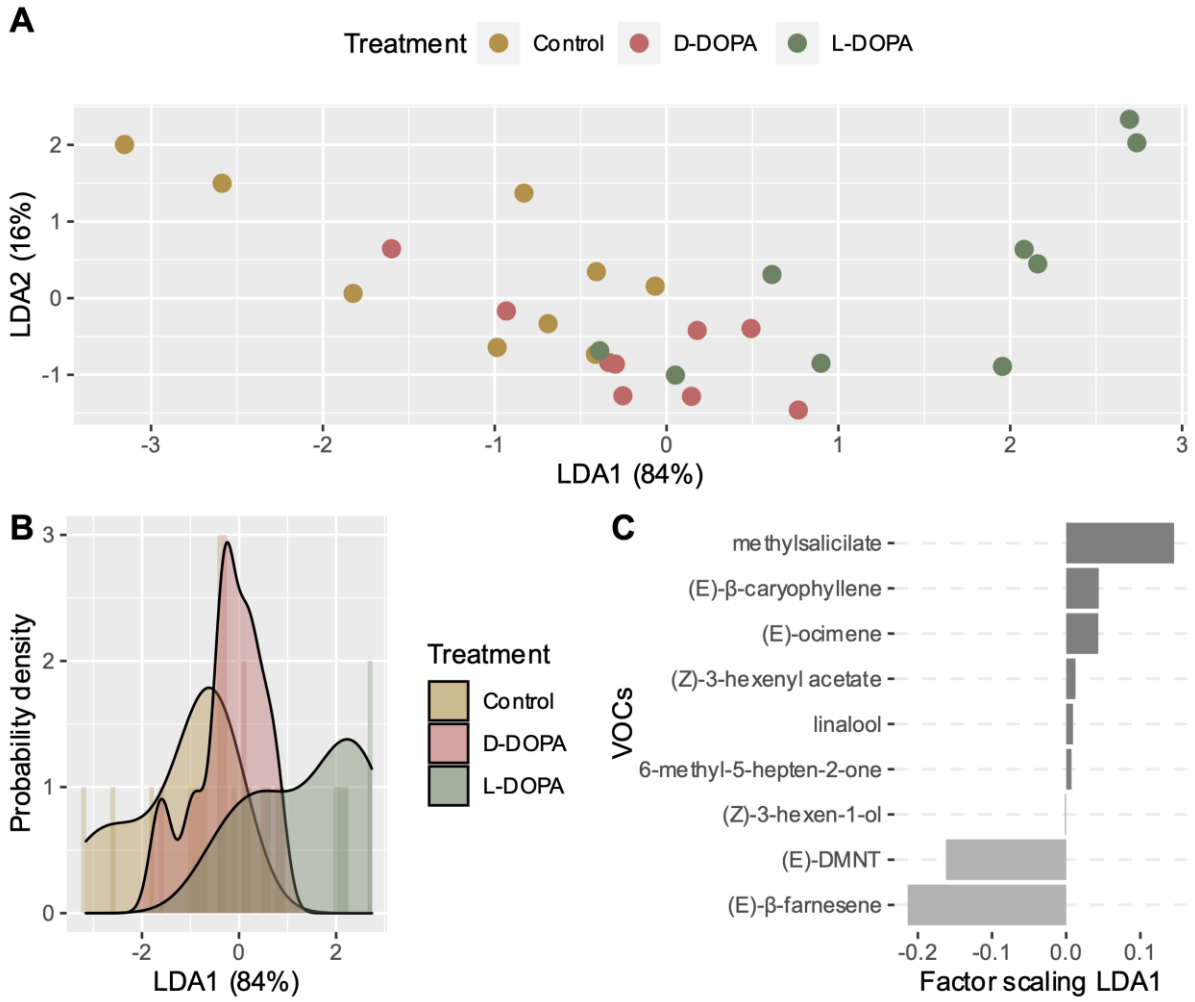

Fig. 2. Linear discriminant analysis (LDA) of aboveground *Vicia faba* volatile organic compounds (VOCs). VOCs were measured on plants grown in hydroponic medium and treated with Ethanol only (brown colors), or treated with either D-DOPA (red colors) or L-DOPA (green colors) at1 ppm. **(A)** LDA biplot distribution of discriminant scores of leaf VOCs profiles across the three treatments. The first linear discriminant (LDA1) explains 83% of the between-group variance, and the second linear discriminant (LDA2) explains 16% of the between-group variance. **(B)** Histograms and density plots showing the distribution of discriminant scores (from LDA1) of leaf VOCs profiles released by plants under the three treatments. **(C)** Discriminant coefficients of LDA1 for each VOCs included in the overall volatile blend. Compounds with negative coefficients (in light grey) reflect negative discriminant scores of leaf VOCs (control and D-DOPA treated plants), while compounds with positive coefficient (in dark grey) reflect positive discriminant scores (L-DOPA treated plants).

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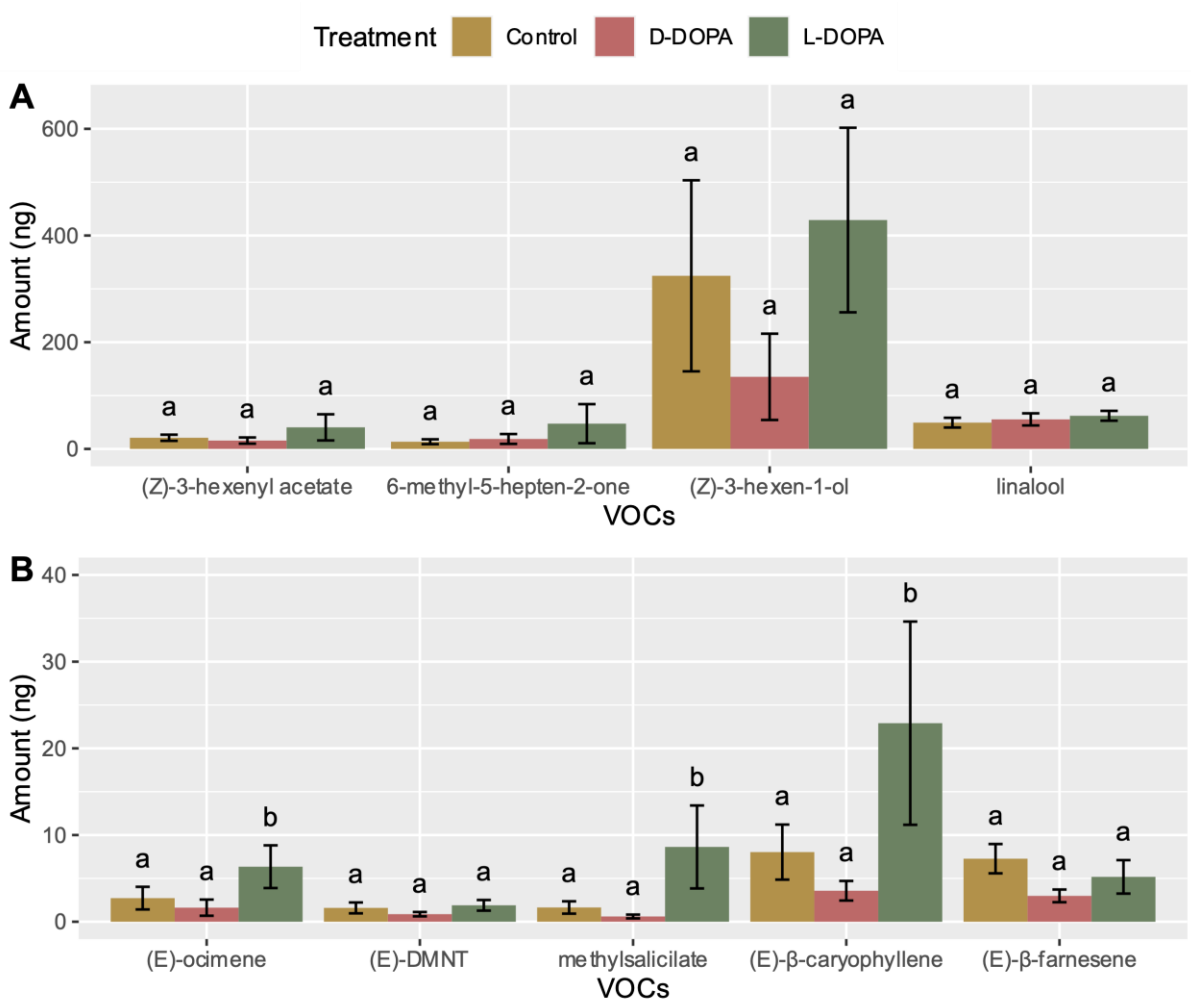

572

573 **Fig.3. Effect of DOPA isomers on aboveground volatile organic compounds (VOCs) production.**
574 Shown are the **(A)** the major and **(B)** the minor VOCs produced by *Vicia faba* leaves, when plants were
575 grown in hydroponic medium and treated with Ethanol only (control, brown bars), or treated with either
576 D-DOPA (red bars) or L-DOPA (green bars) at1 ppm.