Chrysolina herbacea modulates jasmonic acid, cis-(+)-12-oxophytodienoic acid, (3R,7S)-jasmonoyl-l-isoleucine, and salicylic acid of local and systemic leaves in the host plant Mentha aquatica

A. Occhipinti; S. Atsbaha Zebelo; A. Capuzzo; M. Maffei; G. Gnavi. Chrysolina herbacea modulates jasmonic acid, cis-(+)-12-oxophytodienoic acid, (3R,7S)-jasmonoyl-l-isoleucine, and salicylic acid of local and systemic leaves in the host plant Mentha aquatica. JOURNAL OF PLANT INTERACTIONS. 6(2-3) pp: 99-101.
DOI: 10.1080/17429145.2010.545282

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Abstract

Little information is available on the interaction between herbivorous insects and plants storing terpenoids. In this work we describe the response of the essential oil plant *Mentha aquatica* to the specialist herbivore *Chrysolina herbacea*. Feeding from *C. herbacea* induced a significant increase of jasmonic acid (JA) in both local and systemic *M. aquatica* leaves, whereas the content of the JA precursor, *cis*-(+)-12-oxophytodienoic acid (OPDA), was increased in local leaves and decreased in systemic leaves. The JA conjugate, (3R,7S)-jasmonoyl-l-isoleucine (JA-Ile), was slightly increased in herbivore wounded (HW) local leaves, whereas its content significantly increased in systemic leaves. Herbivory by *C. herbacea* did not increase the content of salicylic acid (SA); however, SA showed a two-fold increase in HW systemic leaves. Our results indicate that also in plants producing direct defences, such as the essential oil plant *M. aquatica*, JA, and SA signalling is triggered by herbivory just like in plants that respond with indirect defence.

Keywords: *Mentha aquatica* , *Chrysolina herbacea* , jasmonic acid, *cis*-(+)-12-oxophytodienoic acid (OPDA), (3R,7S)-jasmonoyl-l-isoleucine (JA-Ile), salicylic acid (SA),

Introduction

The strategy for defence in aromatic plants as *Mentha aquatica* is direct defence, through the constitutive production of terpenoids in specialized tissues, the glandular trichomes (Maffei 2010 Maffei, M. 2010. Sites of synthesis, biochemistry and functional role of plant volatiles. *South Afr J Bot.*, 76: 612–631.). These plants pose apparent chemical barriers to potential herbivore colonists, and seem accessible to relatively few insect lineages, possibly pre-adapted by use of chemically similar or related host plants (Farrell and Mitter 1994 Farrell, BD and Mitter, C. 1994. Adaptive radiation in insects and plants – time and opportunity. *Amer Zool.*, 34: 57–69.). As some insects become adapted to these metabolites, interactions between the two organism groups occasionally lead to highly specific relationships, like in the case between *M. aquatica* and the herbivore *Chrysolina herbacea*. *M. aquatica*, or watermint, is a perennial plant belonging to the Lamiaceae which produces leaf glandular trichomes secreting volatile organic compounds (VOCs) of various chemical composition (Malingré and Maarse 1974 Malingré, TM and Maarse, H. 1974. Composition of essential oil of Mentha aquatica. *Phytochemistry.*, 13: 1531–1535.; Jerkovic and Mastelic 2001

Jerkovic, I and Mastelic, J. 2001. Composition of free and glycosidically bound volatiles of Mentha aquatica L. *Croat Chem Acta.*, 74: 431–439.). The plant is toxic owing to the presence of the oxygenated monoterpenes (+)-pulegone and (+)-menthofuran, while viridiflorol from the essential oil and (S)-naringenin from an ethanolic extract have been isolated by bioassay-guided fractionation using binding to the gamma aminobutyric acid (GABA)-benzodiazepine site. At least 24 species of insect herbivores have been specifically recorded as feeding on *M. aquatica* (Horwood 1919 Horwood, AR. 1919. *British wild flowers – in their natural haunts*, London: The Gresham Publishing Company. ; Warren 1993 Warren, PH. 1993. Insect herbivory on water mint – you can't get there from here. *Ecography.*, 16: 11–15.), among these *C. herbacea*, also known as the mint beetle, is quite diffuse in mint fields. The feeding behavior of this beetle has been recently described (Bienkowski 2009 Bienkowski, AO. 2009. Feeding behavior of leaf-beetles (Coleoptera, Chrysomelidae). *Zool Z.*, 88: 1471–1480.). Since herbivore feeding induces alteration in the aromatic profile of essential oil-producing plants like *M. aquatica* the issue is both ecologically and economically relevant (Valladares et al. 2002 Valladares, GR, Zapata, A, Zygadlo, J and Banchio, E. 2002. Phytochemical induction by herbivores could affect quality of essential oils from aromatic plants. *J Agric Food Chem.*, 50: 4059–4061. ; Banchio et al. 2005 Banchio, E, Zygadlo, Y and Valladares, GR. 2005. Effects of mechanical wounding on essential oil composition and emission of volatiles from Minthostachys mollis. *J Chem Ecol.*, 31: 719–727., 2007 Banchio, E, Valladares, G, Zygadlo, J, Bogino, PC, Rinaudi, LV and Giordano, W. 2007. Changes in composition of essential oils and volatile emissions of Minthostachys mollis, induced by leaf punctures of Liriomyza huidobrensis. *Biochem Syst Ecol.*, 35: 68–74.). The plant hormone jasmonic acid (JA) is an ubiquitous signal for tissue injury and for the subsequent activation of defence responses to many, if not most, insect herbivores (Howe and Jander 2008 Howe, GA and Jander, G. 2008. Plant immunity to insect herbivores. *Annu Rev Plant Biol.*, 59: 41–66.). JA exerts its protective effects by regulating a wide range of defence-related processes, including the synthesis of toxic secondary metabolites (Pauwels et al. 2009 Pauwels, L, Inzé, D and Goossens, A. 2009. Jasmonate-inducible gene: what does it mean?. *Plant J.*, 14: 87–91.). *Cis*-(+)-12-oxophytodienoic acid (OPDA) is the end product of the plastid-localized part of the JA biosynthesis pathway (Wasternack and Kombrink 2010 Wasternack, C and Kombrink, E. 2010. Jasmonates: structural requirements for lipid-derived signals active in plant stress responses and development. *Acs Chem Biol.*, 5: 63–77.). Among JA–amino acid-conjugates, (3R,7S)-jasmonoyl-l-isoleucine (JA-Ile) is the major biologically active molecule perceived by the COI1–JAZ receptor complex (Fonseca et al. 2009 Fonseca, S, Chini, A, Hamberg, M, Adie, B, Porzel, A, Kramell, R, Miersch, O, Wasternack, C and Solano, R. 2009. 7-iso-Jasmonoyl-l-isoleucine is the endogenous bioactive jasmonate. *Nat Chem Biol.*, 5: 344–350.). Salicylic acid (SA)-signalling pathways were originally defined as being involved in antipathogenic responses and in systemic-acquired resistance (SAR) (Dong 2001 Dong, XN. 2001. Genetic dissection of systemic acquired resistance. *Curr Opin Plant Biol.*, 4: 309–314.). The relationship between the JA- and SA-signalling has often been shown to be antagonistic. This antagonism may reflect SA-mediated suppression of JA biosynthesis by reducing the expression of genes encoding JA-biosynthetic enzymes, with the reciprocal SA and JA antagonism of gene expression being dose dependent (Clarke et al. 2009 Clarke, SM, Cristescu, SM, Miersch, O, Harren, FJM, Wasternack, C and Mur, LAJ. 2009. Jasmonates act with salicylic acid to confer basal thermotolerance in Arabidopsis thaliana. *New Phytol.*, 182: 175–187. and references therein).

In a previous work we described the chemical interaction between *M. aquatica* and *C. herbacea* by evaluating the ability of the herbivore to locate and recognize plant chemical cues and the capacity of the host plant to respond to herbivory by emitting deterring molecules (Atsbaha Zebelo et al., submitted). In this work, we analyze the effect of *C. herbacea* herbivory on OPDA, JA, JA-Ile, and SA production in herbivore wounded (HW) and mechanically wounded (MD) local and systemic *M. aquatica* leaves.

Materials and methods

Plant material and growth conditions

Stolons of *M. aquatica* L. were collected from wild populations, surface sterilized with 70% ethanol for 20 s and with sodium hypochlorite (1% v/v available chlorine) for 5 min, and then rinsed three times with sterile distilled water. Plants were grown in plastic pots with sterilized peat and vermiculite (V/V 4:1) at 23°C and 60% humidity using daylight fluorescent tubes at 270 µE m^{-2} s^{-1} with a photophase of 16 h.

Insect collection and rearing

Adults of *C. herbacea* (Duftschmid) (Coleoptera, Chrysomelidae, Chrysomelinae) were collected by hand picking from infested *M. aquatica* fields. After collection, beetles were reared at 22°C in ventilated glass chambers and fed with *M. aquatica* cuttings. The beetles were starved for 24 h prior the experiments.

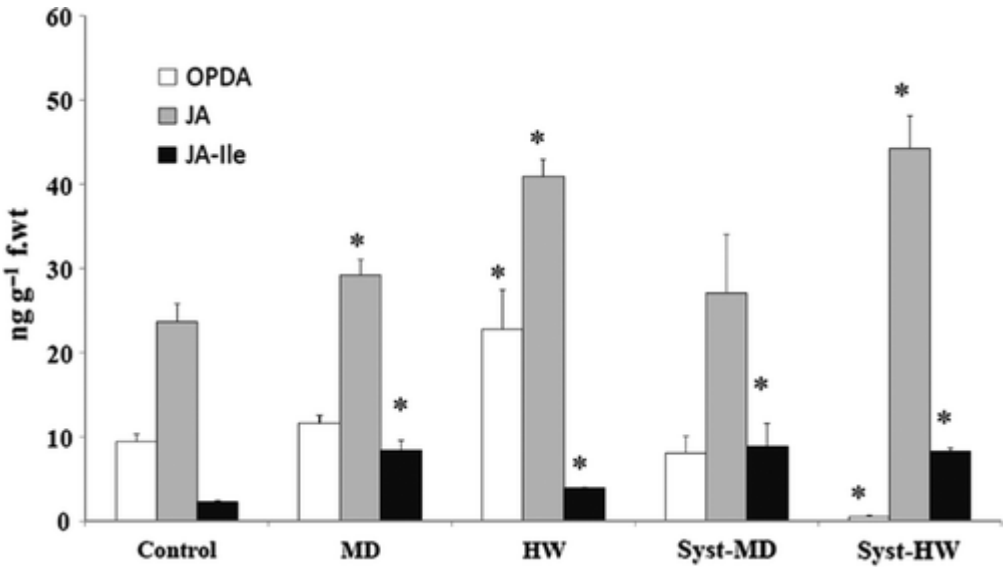
Extraction and determination of cis-(+)-12-oxophytodienoic acid (OPDA), jasmonic acid (JA), (3R,7S)-jasmonoyl-l-isoleucine (JA-Ile), and salicylic acid (SA)

Fifth node undamaged, herbivore fed and mechanically damaged local leaves and sixth to seventh node systemic leaves of *M. aquatica* were collected after 1 h of treatment and placed immediately in liquid nitrogen and kept at-80°C until use for extraction. Mechanical damage was performed with a pattern wheel (Bricchi et al. 2010 Bricchi, I, Leitner, M, Foti, M, Mithöfer, A, Boland, W and Maffei, ME. 2010. Robotic mechanical wounding (MecWorm) vs. herbivore-induced responses: early signaling and volatile emission in Lima bean (Phaseolus lunatus L.). *Planta.*, 232: 719–729.). Compounds were extracted from local and systemic leaves according to Pan et al. (2008 Pan, XQ, Welti, R and Wang, XM. 2008. Simultaneous quantification of major phytohormones and related compounds in crude plant extracts by liquid chromatography-electrospray tandem mass spectrometry. *Phytochemistry.*, 69: 1773–1781.) with minor modifications. The content of OPDA, JA, JA-Ile, and SA in the samples was determined by comparing retention times and mass spectra of standard solutions with a linear gradient in Reverse Phase (RP)-chromatography (ZORBAX Eclipse XDB-C18) and further analysis with a 6330 Agilent Ion Trap LC/MS system. H_2-JA was used as internal standard and precursor ions were detected in negative mode by multiple reaction monitoring (MRM): $[M–H]^–$ 209.0, 211.0, 322.0, 291.0, and 137.0 for JA, H_2-JA, JA-Ile, OPDA, and SA, respectively. The resulting amount of OPDA, JA, JA-Ile, and SA was referred to the total fresh weight of leaves (Pan et al. 2008 Pan, XQ, Welti, R and Wang, XM. 2008. Simultaneous quantification of major phytohormones and related compounds in crude plant extracts by liquid chromatography-electrospray tandem mass spectrometry. *Phytochemistry.*, 69: 1773–1781.). The reported data are the mean values of at least three biological replicates and several technical replicates. Paired *t*-test and Bonferroni adjusted probability were used to assess the difference between treatments and the control and calculated by using Systat 10.

Results and discussion

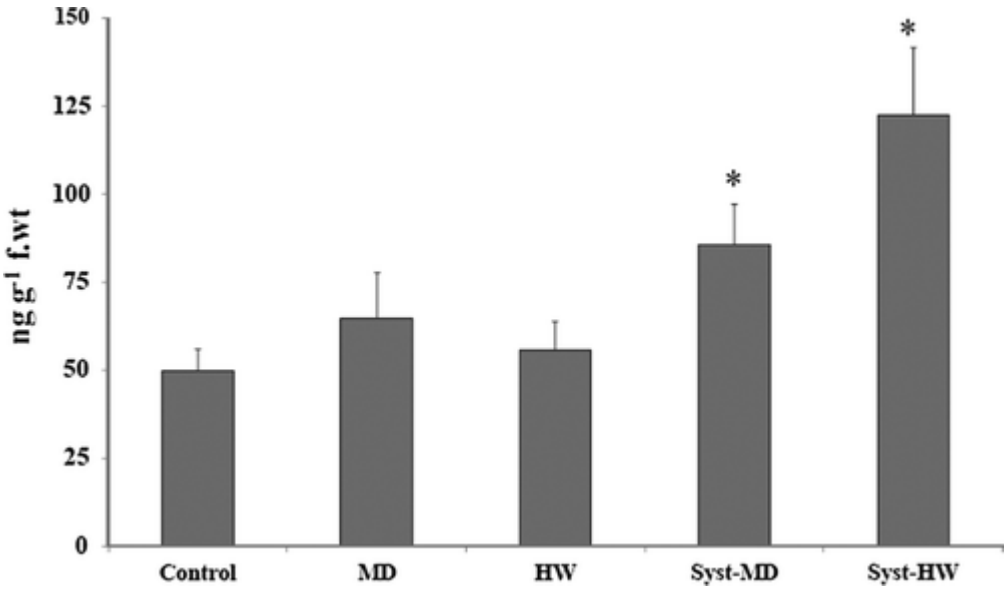
Leaf damage inflicted by mechanical wounding and herbivory are generally considered highly effective triggers for *de novo* JA synthesis (Katsir et al. 2008 Katsir, L, Chung, HS, Koo, AJK and Howe, GA. 2008. Jasmonate signaling: a conserved mechanism of hormone sensing. *Curr Opin Plant Biol.*, 11: 428–435.; Koo and Howe 2009 Koo, AJK and Howe, GA. 2009. The wound hormone jasmonate. *Phytochemistry.*, 70: 1571–1580.). *C. herbacea* feeding caused a significant increase of OPDA in local leaves and a significant decrease of OPDA in systemic leaves, whereas no significant effects on OPDA were found with MD, both in local and systemic leaves (Figure 1). In HW local and in systemic leaves, the significant change in OPDA was accompanied by a significant increase in JA, with respect to control leaves. In MD local leaves, JA showed a small but significant increase with respect to controls, whereas MD systemic leaves showed not significant JA variations, when compared to control leaves. JA-Ile was significantly increased by MD both in local and systemic leaves; whereas, in HW leaves the increase was more evident in systemic than in local leaves (Figure 1).

Figure 1. *Cis*-(+)-12-oxophytodienoic acid (OPDA), jasmonic acid (JA), and (3R,7S)-jasmonoyl-l-isoleucine (JA-Ile) levels in local and systemic leaves of *Mentha aquatica* in response to *Chrysolina herbacea* herbivory. HW, herbivore wounded leaves; MD, mechanically wounded leaves; Syst-HW, systemic leaves in HW plants; Syst-MD, systemic leaves in MD plants. Asterisks '*' indicate significant differences with respect to control leaves.



SA was not increased neither by HW nor MD in local leaves as compared to control leaves; however, systemic leaves showed a dramatic increase of SA, both in MD and, more consistently, in HW plants (Figure 2).

Figure 2. Salicylic acid levels in local and systemic leaves of *Mentha aquatica* in response to *Chrysolina herbacea* herbivory. HW, herbivore wounded leaves; MD, mechanically wounded leaves; Syst-HW, systemic leaves in HW plants; Syst-MD, systemic leaves in MD plants. Asterisks '*' indicate significant differences with respect to control leaves.



Leaf chewers in general induce only JA signalling (Heil 2008 Heil, M. 2008. Indirect defence via tritrophic interactions. *New Phytol.*, 178: 41–61.); however, in the interaction between *C. herbacea* and *M. aquatica* the increase of JA and JA-Ile was accompanied by a consistent systemic increase in SA. In tomato and in Lima bean, JA is required for the induction of volatile release upon spider mite infestation (Ament et al. 2004 Ament, K, Kant, MR, Sabelis, MW, Haring, MA and Schuurink, RC. 2004. Jasmonic acid is a key regulator of spider mite-induced tolatile terpenoid and methyl salicylate emission in tomato. *Plant Physiol.*, 135: 2025–2037.). However, in lima bean while SA pathway is required for mounting an indirect defence against spider mite, volatile release upon caterpillar feeding seems to be mainly controlled by JA concentrations (Ozawa et al. 2000 Ozawa R , Arimura GI , Takabayashi J , Shimoda T , Nishioka T. 2000 . Involvement of jasmonate- and salicylate-related signaling pathways for the production of specific herbivore-induced volatiles in plants . *Plant Cell Physiol* . 41 : 391 398 .). Our results are in line with those obtained by Leitner and co-workers (2005) in which the chewing herbivore *Spodoptera littoralis* induces a rise of JA in local leaves and a significant increase in systemic leaves of *Medicago truncatula*. As in the interaction between *C. herbacea* and *M. aquatica*, feeding *S. littoralis* on *M. truncatula* was observed to induce no SA variation in local leaves and a significant increase in SA content in systemic leaves (Leitner et al. 2005 Leitner, M, Boland, W and Mithöfer, A. 2005. Direct and indirect defences induced by piercing-sucking and chewing herbivores in *Medicago truncatula*. *New Phytol.*, 167: 597–606.). Interestingly, in systemic HW leaves, the increased content of SA is associated to a significant decrease in OPDA, suggesting a possible SA-mediated suppression of JA biosynthesis (Clarke et al. 2009 Clarke, SM, Cristescu, SM, Miersch, O, Harren, FJM, Wasternack, C and Mur, LAJ. 2009. Jasmonates act with salicylic acid to confer basal thermotolerance in Arabidopsis thaliana. *New Phytol.*, 182: 175–187. and reference therein).

To our knowledge this is the first report on SA, JA, JA-Ile, and OPDA herbivore induction in a plant accumulating terpenoid as a direct defence. Preliminary results from our lab indicate that JA is able to induce also in *M. aquatica* both terpenoid emission and terpenoid gene expression (Atsbaha Zebelo et al., in preparation). The involvement of JA and SA signalling in plants using direct defences, such as aromatic plants, is a new emerging field of research that deserves further studies.

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