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This is the author's manuscript

Original Citation:

Availability:
This version is available http://hdl.handle.net/2318/83581 since 2016-10-11T16:09:38Z

Published version:
DOI:10.1080/17429145.2010.545282

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(Article begins on next page)
This is the author's final version of the contribution published as:


The publisher's version is available at: http://www.tandfonline.com/doi/abs/10.1080/17429145.2010.545282

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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

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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...

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 (Atsbahe 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. H₂-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, H₂-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 \textit{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. \textit{Curr Opin Plant Biol.}, 11: 428–435.; Koo and Howe 2009 Koo, AJK and Howe, GA. 2009. The wound hormone jasmonate. \textit{Phytochemistry.}, 70: 1571–1580.). \textit{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. \textit{Cis}-(+)-12-oxophytodienoic acid (OPDA), jasmonic acid (JA), and (3R,7S)-jasmonoyl-l-isoleucine (JA-Ile) levels in local and systemic leaves of \textit{Mentha aquatica} in response to \textit{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.


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.
References


