0014-4754/96/070739-05$1.50 + 0.20

Airborne methyl jasmonate stimulates the biosynthesis of furanocoumarins in the leaves of celery plants (*Apium graveolens*)

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Received 19 May 1995; accepted 20 December 1995

Abstract. When leaves of *Apium graveolens* are exposed to vapours of methyl jasmonate (MeJa) or fed through the petiole with aqueous solutions of jasmonic acid (Ja), the levels of natural furanocoumarins, in particular xanthotoxin and bergapten, are greatly increased. The induction becomes manifest after application of ≥ 20 nmol of either MeJa or Ja. Levels of xanthotoxin and bergapten begin to increase approximately 24 h after application of the inducer. Maximum concentration of the two furanocoumarins (40–70 fold increase) is reached after 4–6 days. This pronounced effect of volatile MeJa on the physiological status of the celery leaf may be considered as representative of a build-up of chemical defenses, in an undamaged plant, after receipt of airborne signals from damaged or undamaged, but fragrant plants.
Key words. Induced defence; methyl jasmonate; jasmonic acid; furanocoumarins (mode of induction); *Apium graveolens*; xanthotoxin; bergapten.

The understanding of chemical communication pathways is of particular interest in the context of mutualistic interactions between plants[1], and also in the more complex tritrophic interactions between plants, insects and parasitoids. Due to recent analyses of volatiles from damaged plant tissues there is considerable evidence that volatile 'alarm' signals are induced by the feeding activities of herbivores[2,3]. The 'alarm blends' may be composed of chemical cues which are responsible for the stimulation of biochemical changes in neighbouring plants that could negatively influence the feeding and the growth of phytophagous insects[4,5]. The effect may be considered as a prophylactic build up of chemical barriers to prevent overexploitation of the plant population by herbivores[6]. For example, volatiles from *Aspergillus flavus*-infected cotton leaves (*Gossypium hirsutum* L.) can stimulate the biosynthesis of phoroglucinol-reactive compounds in undamaged cotton leaves sharing the same enclosure[7]. In a similar fashion, C_6–C_{10} alkenals and alkanals have been found to trigger the production of sesquiterpenoid naphthol phytoalexins and the coumarin scopoletin in the developing cotton boll[8]. If tomato plants are exposed to volatiles from the sagebrush *Artemisia tridentata*, the level of proteinase inhibitors increases rapidly[9], indicating some degree of interspecific communication. In the latter case, methyl jasmonate is responsible for the induced defence reaction.

The above examples suggest that defence responses in undamaged plants may be more generally triggered by volatiles which are systemically released from damaged or infested plants. Other inducible defensive substances include a wide range of low-molecular-weight secondary metabolites such as flavonoids, guaianolides, anthraquinones and various classes of alkaloids. The production of all of these could be triggered in a rather unspecific fashion by the addition of micromolar quantities of methyl jasmonate to cell cultures of selected mono- and dicotyledonous plants[10,11]. Furanocoumarins are characteristic plant constituents of Rutaceae and Umbelliferae, toxic for many animal species, which are often stored at rather low levels within the plant[12]. Herbivory by insects[13], or infection with pathogens[14], is known to induce their biosynthesis as a typical defence response, i.e. as phytoalexins. The toxicity of furanocoumarins for humans can lead to severe problems, for example in celery harvesting, as they can cause blistering, erythrema and dermatitis[15]. There is also evidence that furanocoumarins may intercalate with DNA in the dark and form photoadducts upon irradiation[16].

Here we address the question as to whether the biosynthesis of furanocoumarins in celery plants may be inducible by a plant-to-plant transfer of volatile chemical signals. It will be shown that, at least in principle, methyl jasmonate vapour is able to trigger the biosynthesis of furanocoumarins in healthy, undamaged celery plants at low ng levels.

Materials and methods

Chemicals. The furanocoumarins xanthotoxin, isopimpinellin, psoralen and bergapten were purchased from Sigma (Dreieich, Germany). Methyl jasmonate (MeJa)

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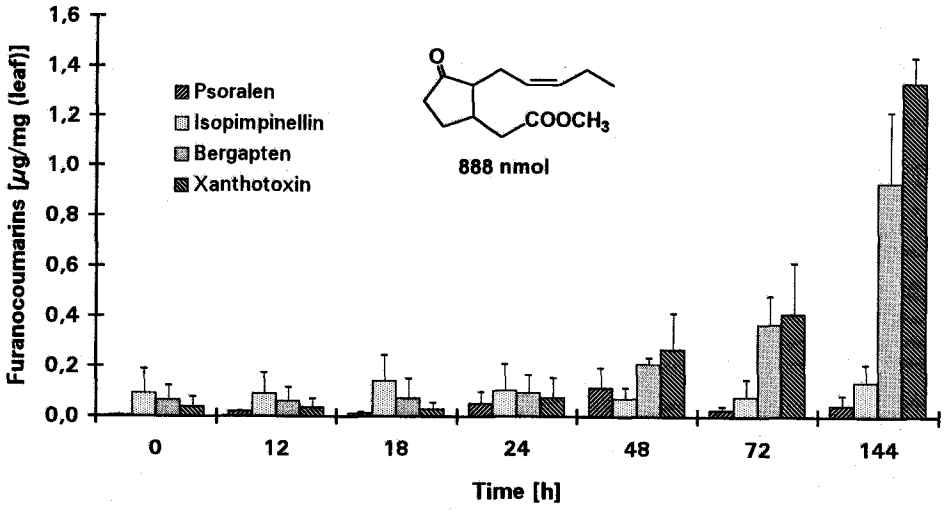


Figure 1. Time course of the stimulation of furanocoumarin biosynthesis by MeJa in leaves of *A. graveolens*. Leaves were exposed for increasing times to vapours of racemic MeJa (0.9 µmol) released from a filter paper fixed above the leaf in a closed system. Bars represent the mean concentrations of the furanocoumarins from leaf extracts at the intervals indicated. Standard deviations from n = 5 experiments are given.

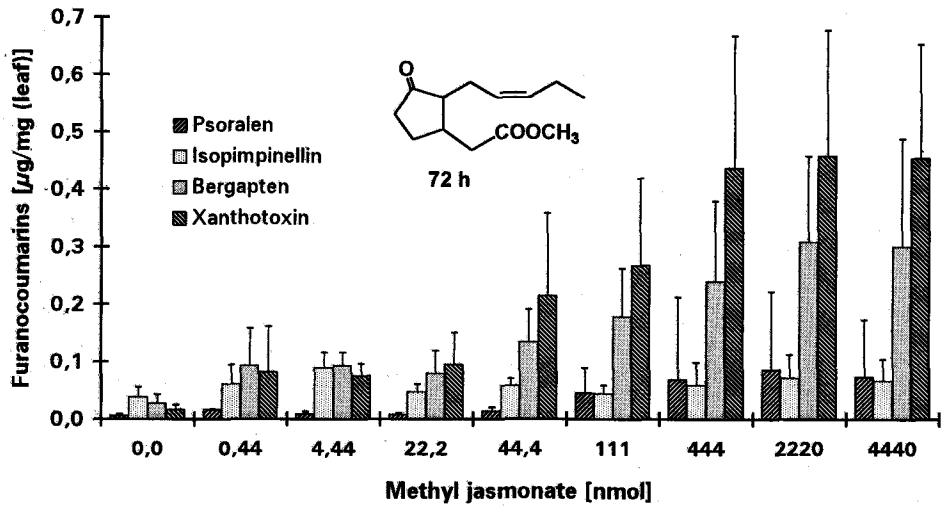


Figure 2. Stimulation of furanocoumarin biosynthesis by exposing detached leaves of *A. graveolens* to increasing amounts of airborne MeJa released from a filter paper fixed above the leaf in a closed system. Bars represent the mean concentrations of the furanocoumarins from leaf extracts after 72 h of exposure. Standard deviations from n = 5 experiments are given.

was kindly provided by Dr. R. Kaiser, Givaudan Co. (Dübendorf, Switzerland). Free jasmonic (Ja) acid was prepared from MeJa as described[9].

Plant material. Leaves from fully grown celery plants (*Apium graveolens*, cv. *secalinum*) grown in a greenhouse were used for all experiments.

Induction experiments. A freshly detached celery leaf in a small vial with tap water was enclosed in a desiccator (2700 ml). Methyl jasmonate was applied as a solution in pentane onto a small piece of filter paper. Following evaporation of the solvent, the filter paper was fixed as a 'dispenser' below the cap of the desiccator, thus avoiding any direct contact between the solvent and the leaf. The leaf was exposed to various concentrations (fig. 2) of airborne MeJa for the times given in figure 1. Control experiments were carried out using a similar enclosure without MeJa dispensers. Experiments were repeated at least five times, and the data are presented as mean values compared to the corresponding blank. Standard deviations are given in the figures. Induction experiments with jasmonic acid were performed, using the same experimental set-up, by supplying different concentrations of Ja through the cut stems for the whole period of the experiment.

Sample preparation. Pre-treated and untreated leaves were dried for 24 h at 50°. Samples of known weight

were ground in ethanol (2 ml) for 10 min with an Ultraturrax at 24000 U min^{-1}. Extraction of the furanocoumarins was completed by refluxing the solution for 30 min. After filtration and removal of solvents in vacuo, the residue was dissolved in ethyl acetate (150 µl). Following centrifugation (2400 U min^{-1}), the resulting clear solution was used without further purification for HPLC analysis.

HPLC analysis of furanocoumarins. The analysis of the furanocoumarins followed the procedure of Berenbaum et al[17]. The sample (20 µl) was loaded onto a normal phase silica column (LiChrospher Si 60, 5 µm, 25 cm × 0.4 mm, E. Merck, Darmstadt, Germany), and the compounds were eluted with a solvent mixture of cyclohexane, di-*i*-propylether, *n*-amylalcohol and water (15:4:1, v:v:v + 0.01% water) at a flow rate of 0.7 ml min^{-1}. The addition of water is essential to improve the separation by minimising peak tailing of the compounds. Detection and integration were achieved with a Knauer/Barspec rapid scanning UV-monitor at 254 nm using the Knauer HPLC software package version 2.22.

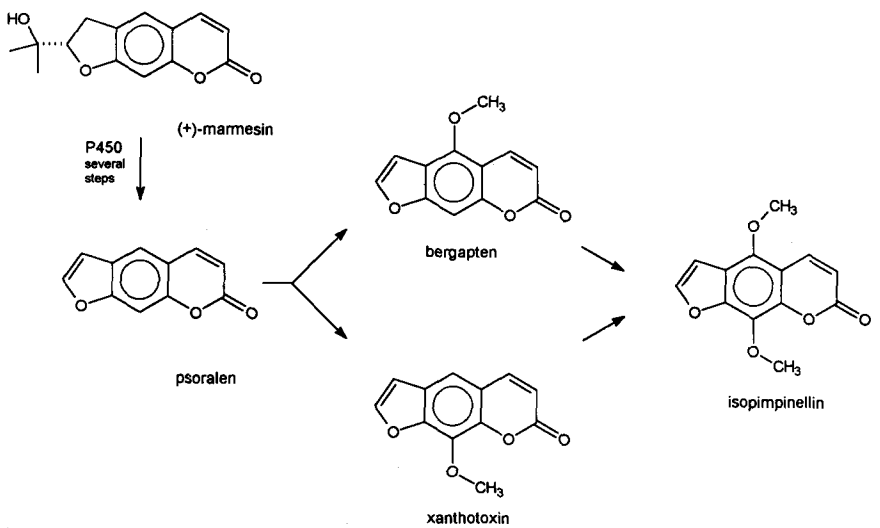
Results

Induction experiments with methyl jasmonate vapour. To study the inductive effect of volatile MeJa on the biosynthesis of furanocoumarins in celery plants, freshly detached leaves were exposed to MeJa vapour released from a filter paper. The time course and the dose-response profiles of the induction process were documented by quantifying the furanocoumarins in exposed leaves by HPLC. While the concentration of psoralen, the precursor of all other furanocoumarins[18] in scheme 1, remained fairly constant over the whole period of the experiment (7 days), the amount of two isomeric methoxy derivatives, xanthotoxin and bergapten, started to increase steadily and simultaneously after about 24 h following introduction of MeJa (fig. 1). After about 5–6 days the level of xanthotoxin and bergapten approached a maximum, corresponding to an average increase in furanocoumarin concentration of between 40–70 fold. The amount of the bismethoxylated isopiminellin was only moderately enhanced (ca. 2–4 fold).

Figure 2 shows that vapour phase concentrations of MeJa in the range of 0.44–22 nmol occasionally caused weak induction of furanocoumarin biosynthesis, but the effects occurred irregularly. At concentrations above 22 nmol the induction became reproducible and increased steadily in a dose-dependent manner. At concentrations of MeJa above ca. 400 nmol the maximum level of furanocoumarin biosynthesis was reached. The analyses were carried out 72 h after introduction of the MeJa stimulus, the time at which induced biosynthesis of the furanocoumarins approached its maximum (fig. 1).

Induction of furanocoumarin biosynthesis by jasmonic acid. In order to compare the stimulatory power of airborne MeJa and free jasmonic acid, experiments similar to those already described were carried out by supplying solutions of free jasmonic acid through the cut stems to the plant leaves. At regular intervals, given in figures 3 and 4, the leaves were assayed for furanocoumarin content. The time course and dose-dependency of induction followed the same pattern as observed for gas phase MeJa application.

The level of the furanocoumarins steadily increased from about 24 h after stimulation and approached a maximum after about 4–6 days. Again, the levels of xanthotoxin and bergapten were most strongly enhanced. Isopimpinellin increased only moderately; the



Scheme 1. Biosynthesis of furanocoumarins from *A. graveolens*. Dealkylation of (*S*)-(+)-marmesin by a Cytochrome P450-type enzyme generates psoralen, the precursor of xanthotoxin, bergapten and the bismethoxylated furanocoumarin isopimpinellin[18].

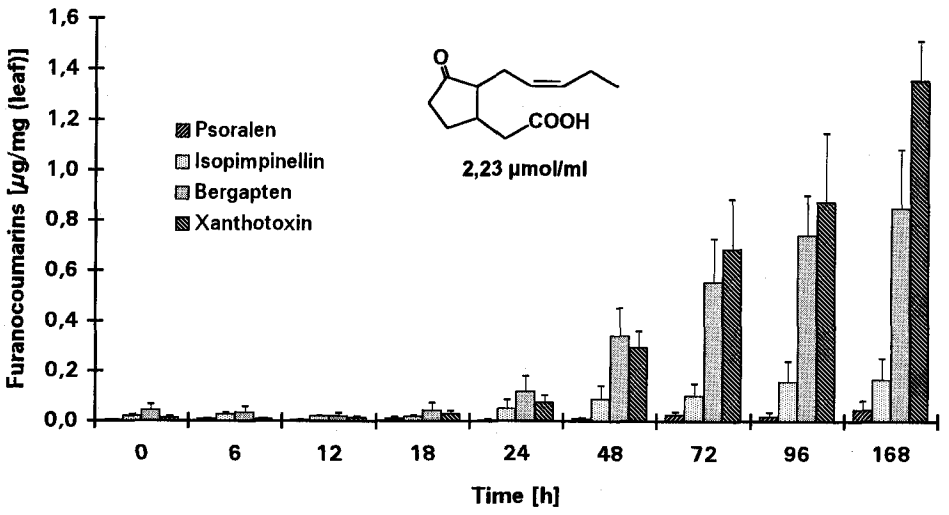


Figure 3. Time course of the stimulation of furanocoumarin biosynthesis in celery leaves after treatment with jasmonic acid. Leaves were fed through the cut stem with an aqueous solution (2.4 µmol ml^{-1}) of free jasmonic acid during the whole experiment. Bars represent the mean concentrations of the furanocoumarins from leaf extracts at the intervals given. Standard deviations from n = 5 experiments are given.

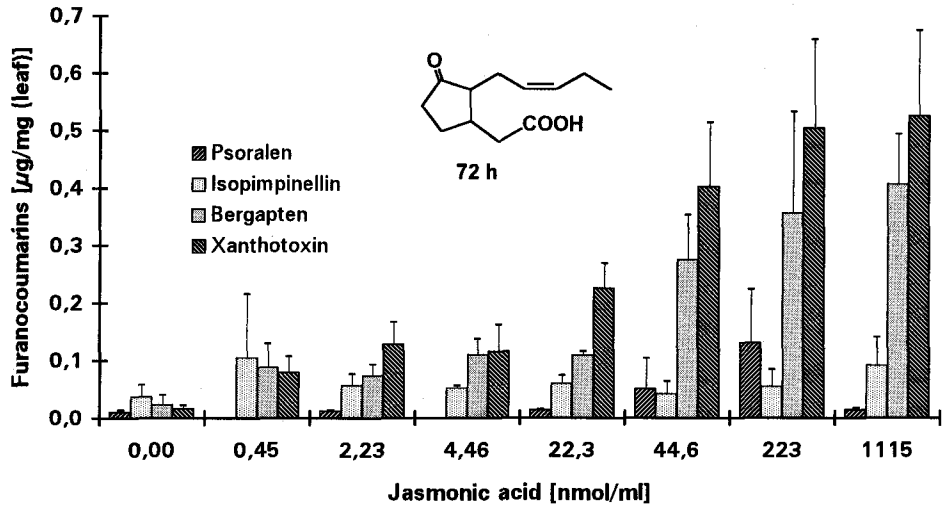


Figure 4. Stimulation of furanocoumarin biosynthesis in celery leaves by increasing amounts of jasmonic acid. Leaves of *A. graveolens* were supplied through the cut stem with increasing amounts of racemic jasmonic acid during the whole experiment. Bars represent the mean concentrations of the different furanocoumarins from leaf extracts after 72 h. Standard deviations from n = 5 experiments are given.

amount of psoralen remained more or less constant. Significant and reproducible stimulation was observed at concentrations of Ja greater than 20 nmol ml^{-1}. The stimulatory effect of Ja was dose-dependent and reached a maximum around 400 nmol ml^{-1} and, hence, compares well with the data obtained for the volatile MeJa.

Discussion

MeJa and Ja and its biosynthetic precursors have been previously recognised as potent endogenous signal transducers in elicitor-induced cell cultures or wounded plants[9–11,19]. Therefore, the pronounced stimulatory effect of MeJa or Ja on the biosynthesis of typical phytoalexins, such as the furanocoumarins in celery plants, clearly fits into this scheme. Unlike the previously reported case of stimulation of the coiling of the touch sensitive tendrils of *Bryonia dioica*[20], where MeJa had a far superior activity compared to Ja, in the present study, both jasmonates, MeJa and Ja, stimulated the biosynthesis of the furanocoumarins in a comparable manner. Similar dose-response curves and saturation effects were observed for either airborne MeJa or aqueous solutions of Ja. In contrast to the

increasing amounts of bergapten and xanthotoxin, the level of psoralen, which serves as the precursor to all furanocoumarins, stayed fairly constant. This corresponds to previous observations with parsley cell cultures (*Petroselinum crispum*). Here, a transient accumulation of psoralen occurred for a short time after treatment with a *Phytophtora megasperma* derived elicitor. Later, psoralen diminished in correlation with increasing amounts of bergapten, xanthotoxin and isopimpinellin[21].

The lag phase of about 24 h between the application of the (Me)Ja-stimulus and the increase in furanocoumarin concentration is comparable to that found in several jasmonate-triggered systems: for example, induction of the biosynthesis of a number of hydroxylated and methoxylated benzo[c]phenanthridine alkaloids in cell suspension cultures of *Eschscholtzia californica*[10]. Like the furanocoumarins in parsley or celery, these alkaloids are produced in response to an attacking pathogen[23]. Yet another example is the enhanced activity of polyphenol oxidase (PPO) in tomato plants which were either overproducing systemin or had been artificially treated with MeJa vapour[24]. PPO oxidises a wide range of plant phenolics to produce substances that are effective against pathogens and herbivores[25]. Since a related enzymatic activity, the 4-coumarate-CoA-ligase, is required to produce umbelliferone[26], the precursor to (+)-marmesin (cf. scheme 1), it is highly likely that in both plants functionally related genes are activated via the octadecanoid signal transduction pathway.

In view of the various reports on systemic responses of non-infested plants towards elicitors transported through the gas phase from infested plants,[6] the current work provides another example that MeJa is, indeed, a powerful volatile signal. It is able to induce furanocoumarin biosynthesis in celery plants at very low concentrations in the gas phase (cf. fig. 2). Since the generally more active (+)-*epi*-jasmonate[22] is present at rather low levels (ca. 5%) in the equilibrium mixture of synthetic MeJa or Ja, the actual threshold concentration for this isomer might be even lower than the 20–40 nmol found in this work. It must be stressed, however, that the observed stimulation of the biosynthesis of the furanocoumarins by airborne MeJa is achieved with enclosed plants under controlled laboratory conditions. Up to now, it is unclear how these findings apply to field situations, where air currents and masking odours may complicate things.

Independent of these principal questions, an interesting practical application of volatile MeJa could be seen. A prophylactic build-up of chemical defences in crop plants endangered by a herbivore might be provoked by simple treatment with MeJa vapours released from dispensers. Furthermore, since Ja and MeJa also elicit the emission of other (bioactive) volatiles[27] in undamaged, uninfested plants, a complex scenario of plant-plant and/or plant-insect interaction via the gas phase becomes apparent.

Acknowledgements. Financial support by the Deutsche Forschungsgemeinschaft (Bonn) and the Fonds der Chemischen Industrie (Frankfurt am Main) is gratefully acknowledged. We also thank BASF (Ludwigshafen) and Bayer AG (Leverkusen) for generous supplies of chemicals and solvents. MeJa was kindly provided by Dr. R. Kaiser, Givaudan Company, Dübendorf, Switzerland. We are grateful to Dr. N. Oldham for proof-reading the manuscript.

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