104. Changes in the Leaf Surface Chemistry of *Apium graveolens* (Apiaceae) Stimulated by Jasmonic Acid and Perceived by a Specialist Insect

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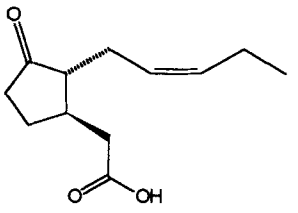
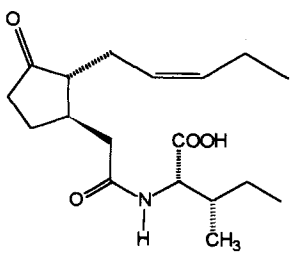
Treatment of celery leaves (*Apium graveolens*, cv. *secalinum*) with jasmonic acid (JA, **1**) or analogues of amino-acid conjugates of jasmonic acid such as the leucine conjugate with 1-oxoindan-4-carboxylic acid (IN-ILE, **2**) stimulated the biosynthesis of the furanocoumarins psoralen (**6**), xanthotoxin (**8**), bergapten (**7**), and isopimpinellin (**9**). Besides the increase of the compounds within the leaf, a significant amount (*ca.* 20%) of the total furanocoumarins was deposited on the surface of the leaf. The two monomethoxy furanocoumarins, **7** and **8**, began to increase steadily and simultaneously within the leaf and on the leaf surface *ca.* 40 h after the onset of the jasmonic-acid stimulus. Within the leaf, the ratio **7**/**8** was *ca.* 1.3:1.0, while among the surface lipids **8** dominated (**7**/**8** 0.8:1.0), indicating that the export of the compounds to the surface is not a simple diffusive translocation along the oil-ducts. Females of the carrot fly (*Diptera, Psilidae*) responded with an increased oviposition to the altered leaf surface chemistry of the JA-treated celery plants. The effect shown for total leaves was corroborated by surrogate leaves treated with leaf-surface extracts of JA-induced leaves. Based on the known stimulation of oviposition by furanocoumarins, we conclude that the enhanced amount of furanocoumarins on the surface can explain the insects' preference for the JA-stimulated plants. This is the first report of a JA-induced change of the surface chemistry of a plant and an increase of the acceptability of treated leaves for a specialist insect, like the carrot fly.

1. Introduction. – Jasmonic acid (JA, **1**) and certain amino-acid conjugates of jasmonic acid, such as 'jasmonoyl-isoleucine' (JA-ILE, **2**), represent a rather new family of highly potent plant stress hormones [1–3]. They induce the expression of specific genes that code for proteins (*J*asmonate *I*nduces *P*rotein, JIPs) causing a number of effects such as chlorosis [4] and plant senescence [5]. Other notable effects mediated by **1**, are the tuber induction of potato [6] or the rapid-coiling response of the touch-sensitive tendrils of *Bryonia dioica* [7]. Moreover, as a family of 'stress hormones', JA (**1**) and its amino-acids conjugates are generally involved as signal transducers in the plant defence against pathogens and herbivores [8] [9].

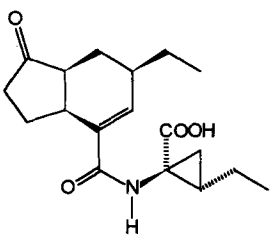
Other well-established JA-controlled chemical defences are the production of high-molecular-weight proteinase inhibitors [10], the biosynthesis of proteinaceous antifungal compounds (*e.g.*, the pathotoxic thionines [11]) and some hydroxyproline-rich proteins for cell wall strengthening [12]. Besides macromolecules, JA (**1**) also stimulates the biosynthesis of low-molecular phytoalexins like alkaloids [13], terpenoids, and, as an important aspect for plant protection, the emission of volatiles [14] [15]. Similar effects can be triggered by application of the phytotoxin coronatin [14] [16] and the methyl ester



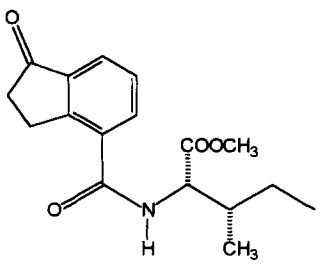
1, jasmonic acid (JA)



2, JA-ILE



3, coronatin



4, IN-ILE

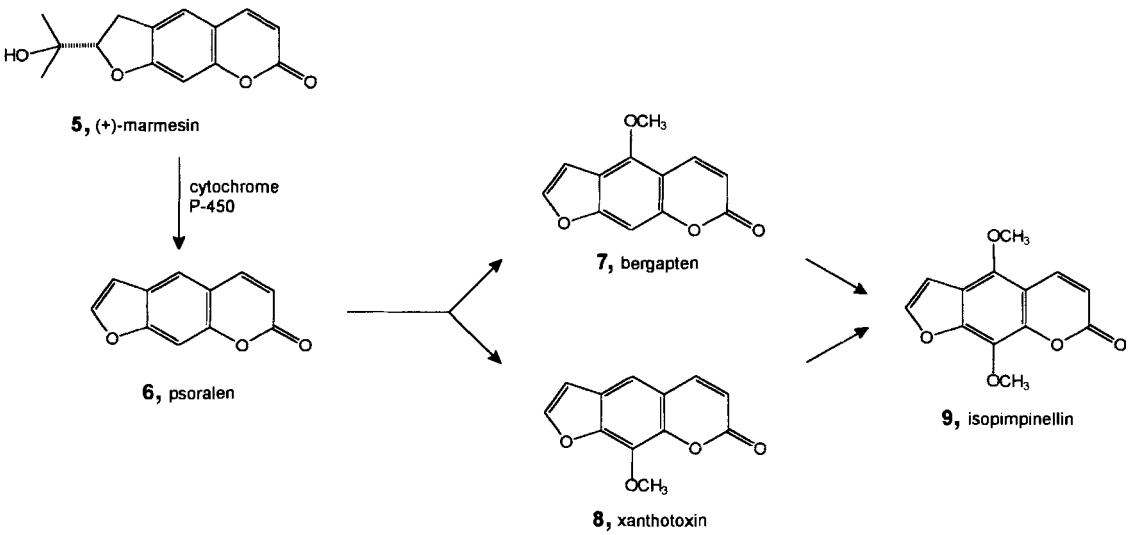
of IN-ILE (**2**) [2] [17], which, according to current knowledge, have to be considered as structural analogues of the naturally occurring amino-acid conjugate JA-ILE [1].

One of the most prominent and best studied pathways towards low-molecular defences is the phenylpropanoid pathway which starts with the induction of its key enzyme, the phenylalanine ammonia lyase (PAL) [18]. Typical late metabolites of this pathway are the (iso)flavonoids, anthocyans, chalcones, and the phototoxic furanocoumarins.

Furanocoumarins represent a large class of compounds, and they occur widespread in Rutaceae and Apiaceae. The contact with leaves containing furanocoumarins causes blistering and erythema, accompanied by allergic reactions, known as occupational diseases during harvesting of celery, parsley, or parsnip [19]. Usually, the level of furanocoumarins is low, but, after infection or damage of the plants by microorganisms (fungi, bacteria) or herbivores, a dramatic increase (10–100-fold) of these compounds is observed within the following 2–4 days [20–22]. Abiotic stress factors like UV light may have a similar effect [23].

The late events of the biosynthesis of the (bis)methoxy furanocoumarins bergapten (**7**), xanthotoxin (**8**), and isopimpinellin (**9**) begin with an oxidative dealkylation of (+)-marmesin (**5**) (*Scheme*). The removal of the side chain is achieved by a JA-inducible cytochrome P-450 enzyme [24] and generates psoralen (**6**), which, in turn, serves as the precursor for the methoxylated furanocoumarins **7** to **9** [25]. Although the induction of furanocoumarin biosynthesis by abiotic stresses like, *e.g.*, UV light apparently does not proceed *via* the jasmonate signalling pathway, it is clear by now, that the damage by pathogens or herbivores, at least in most cases, involves jasmonate as a signal transducer [26].

Scheme



So far as known, the various defence reactions, stimulated by JA (**1**), may alter the chemical composition of a plant leaf at several different levels. *1*) The plants chemical composition is changed *within* the cellular tissue, by inducing the biosynthesis of high- and low-molecular compounds protecting against biting insects. *2*) The chemical composition of the gas phase *above* the leaf surface is changed owing to the induced biosynthesis and emission of volatiles, which, eventually, attract the natural enemies of the herbivore [27] or act as deterrents. Thus, the first barrier, presented to an approaching herbivore by a damaged and already systemically induced plant, is the cloud of volatiles in the surrounding gas phase. Next, the chemical composition of the leaf surface may be expected to have a negative impact on an insect. If the volatiles and the leaf-surface chemicals successfully deter the attacking herbivore, the plant is not damaged at all. Hence, it follows that a damage-induced change of the chemical composition of a leaf surface, *i.e.*, the presentation of phytoalexins on the surface, should provide an effective shield against attacking insects. To test the influence of a JA stimulation on the surface chemistry of a plant, we choose celery plants (*A. graveolens*) as a model system, since its leaves, like related Apiaceae, possess oil-duct cells which may be somehow involved in an export of the compounds to the leaf surface [28].

The essence of the current work is twofold: *1*) It will be shown for the first time that stimulation of celery leaves (*A. graveolens*) with JA (**1**) or the *N*-(1-oxoindan-4-carbonyl)-isoleucine methyl ester (IN-ILE, **4**), a likely mimic of JA-ILE (**2**), not only enhance the internal level of the furanocoumarins **6**–**9**, but also cause a massive transport of the compounds to the leaf surface. *2*) Bioassays with females of the carrot fly (*Psila rosae*), which are stimulated by furanocoumarins to lay eggs on a leaf surface [29] [30], clearly demonstrate that the insects recognize the JA-induced changes of the leaf surface chemistry and prefer JA-treated plants over untreated leaves for oviposition.

2. Results. – 2.1. *Stimulation of Phytoalexin Biosynthesis and HPLC Analysis of Furanocoumarins.* The signal compounds JA (**1**) and the *N*-(1-oxoindan-4-carbonyl)isoleucine methyl ester (**4**) (1.0 mM each) were dispersed in tap water and sonicated until a clear emulsion resulted. Freshly detached celery plantlets (*A. graveolens*, cv. *secalinum*; *ca.* three month old) were placed into the above emulsions, and, then, the contents of the furanocoumarins was followed as a function of time (*cf. Figs. 1* and *2*, and *Exper. Part*). Brief dipping of the undamaged leaves (2 × 30 s) in CH_2Cl_2 removed all non-polar lipids including the furanocoumarins from the surface. After removal of the surface chemicals, the leaf was dried, and, then, the internal pool of furanocoumarins was extracted with AcOEt from the leaves after grinding. Individual compounds were separated by HPLC on SiO_2 [31] and quantified by their UV absorption.

According to *Fig. 1*, the stimulation of the celery leaves with JA (**1**) causes a massive increase of the furanocoumarin contents within 3–4 days after the onset of the JA stimulus. As already published previously, the increase becomes manifest after *ca.* 2 days and increases continuously until day 4 or 5 [32]. Due to competing senescence effects, the time course could not be followed beyond this time. Within this time, however, the amount of the individual furanocoumarins **7**–**9** increased more than tenfold. For example, the average amount of the major furanocoumarin bergapten (**7**) was found in untreated leaves at 20–30 μg g^{-1} dry weight, while, 96 h after treatment with JA, the amount of the same compound had increased to 300 μg g^{-1} dry weight. The increase rate of **6**, **8**, and **9** followed clearly the same trend.

Besides the increase of the furanocoumarin contents within the leaf tissue, the analysis of the surface-washings indicated a significant transport of these compounds to the leaf surface (*Fig. 2*). On average, the amount of the surface-deposited furanocoumarins was about one fifth of that of the leaf tissue. The overall time course, *i.e.*, the time of the

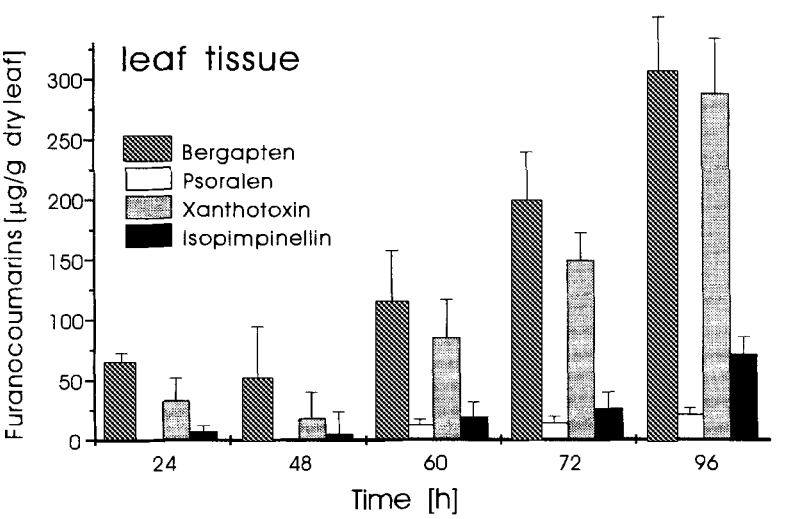


Fig. 1. *Time course of the JA-induced biosynthesis of furanocoumarins in celery leaves.* The bars represent the average concentration of individual furanocoumarins corrected for the amount of furanocoumarins from non-stimulated plants of the same treatment time. Given are standard deviations resulting from five independent experiments.

beginning increase, and the concentration profile of the surface material resembled the concentration-time course found for the induction process within the leaf. Interestingly, the ratio of the two methoxylated furanocoumarins bergapten (**7**) and xanthotoxin (**8**) was not identical within and above the leaf. Within the leaf **7** was the dominant furanocoumarin (**7**/**8** = 1.29 ± 0.23, t = 72 and 96 h, n = 5), but on the leaf surface **8** (**7**/**8** = 0.78 ± 0.12, t = 72 and 96 h, n = 5) always prevailed. This reverse ratio remained constant over the whole induction period and may indicate that the export of the furanocoumarins from the inner tissue to the surface cannot be regarded as a simple diffusive translocation of the compounds through the oil-ducts.

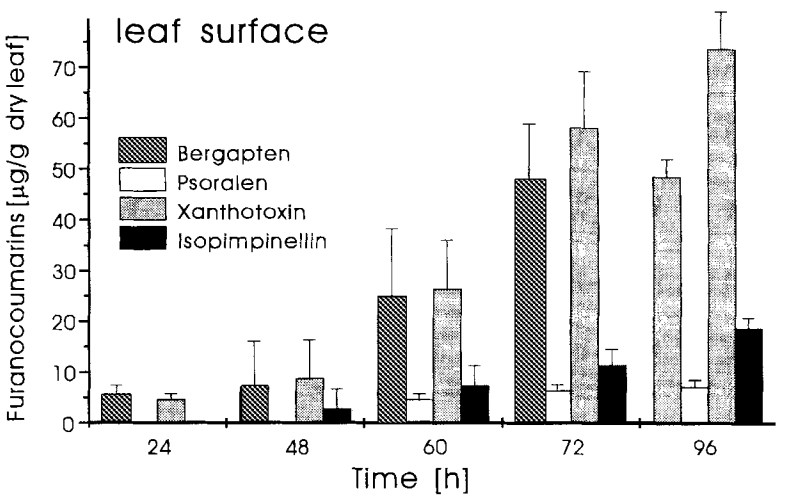


Fig. 2. *Time course of the JA-induced accumulation of furanocoumarins on the surface of cellery leaves.* The bars represent the average concentration of individual furanocoumarins corrected for the amount of furanocoumarins from non-stimulated plants of the same treatment time. Given are standard deviations resulting from five independent experiments.

To test, whether or not the induction of furanocoumarin biosynthesis can be also induced by a JA amino-acid conjugate such as JA-ILE (**2**) (*vide supra*), additional induction experiments were carried out with the easily available IN-ILE (**4**). However, due to the limited solubility of the compound, stable test solutions at 1.0 µmol ml^{-1} were obtained only after addition of a detergent like *Triton X 100* prior to sonication. Control experiments were done with generally inactive 1-oxoindan-4-carboxylic acid (**10**) [17]. As shown in the *Table* the conjugate showed a significant induction of **8** and **7**. As expected, the effect of the free acid **10** was much lower. It was interesting to note that even *Triton X 100* effected a moderate induction of furanocoumarin biosynthesis, which probably is initiated by a nonspecific stress reaction of the plant. So it seems likely that even the effect of the free acid **10** is not caused by the inductive capability of **10** but by a nonspecific reaction.

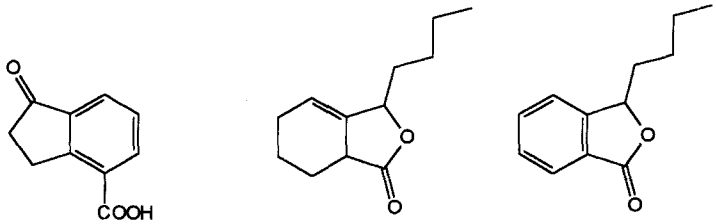
As yet, no attempt was made to discriminate between the surface lipids and leaf compounds after induction with IN-ILE (**4**). On average, the amount of total furanocoumarins was somewhat lower (*ca.* 60%) than after elicitation with JA (**1**).

Table. *Induction of Furanocoumarin Biosynthesis by IN-ILE* (**4**) *and 1-Oxoindan-4-carboxylic Acid* **10**. The amount of furanocoumarins is given in $\mu g\ g^{-1}$ dry weight of the leaves and represents the total amount (surface plus tissue) of induced compounds. Given are standard deviations resulting from five independent experiments.

Furanocoumarin	Inducing compound			
	IN-ILE (**4**) (1.0 mM)	Free acid **10** (1.0 mM)	*Triton X 100* (4.0 mg ml^{-1})	Tap water
Bergapten (**7**)	278 ± 59	113 ± 27	69 ± 10	31 ± 14
Psoralen (**6**)	21 ± 8	–	–	–
Xanthotoxin (**8**)	218 ± 88	42 ± 8	20 ± 4	6 ± 6
Isopimpinellin (**9**)	72 ± 12	14 ± 6	16 ± 6	3 ± 4

2.2. *GLC Analysis of Induced Surface Constituents.* If the surface extract was analyzed by GLC/MS, additional changes in the composition of the surface compounds became apparent. In agreement with the HPLC analyses, the surface washings from unstimulated leaves showed no furanocoumarins, while, after stimulation (96 h), the furanocoumarins became the dominant surface constituents with xanthotoxin (**8**) prevailing (*Fig. 3*).

The major surface lipid, however, was identified as sedanolide (**11**) representing an important aroma constituent of celery plants [33] [34]. Interestingly, this typical celery fragrance was present only in small amounts on the leaf surface of non-stimulated plants. Small amounts of the structurally and biosynthetically related butylphthalide (**12**) were also found only after JA treatment. β-Selinene and caryophyllene were identified as the major products of the JA-induced terpenoid biosynthesis. Detailed analyses of other lipid compounds were, as yet, not performed.



10, 1-oxoindan-4-carboxylic acid **11**, sedanolide **12**, butylphthalide

Fig. 4, presenting the oviposition data obtained with normal and JA-induced celery leaves, shows that, beginning on the fourth day of induction, carrot flies significantly preferred to lay eggs below induced leaves. This effect occurred in both independent experiments differing in the light conditions during the important 4 days (see *Fig. 2*) of the induction. Since such an induction caused also changes in color of the leaves (senescence), and since metabolic changes are likely, we compared in an additional experiment the effect of surrogate leaves treated with leaf-surface extracts of induced and control celery leaves. The results compiled in *Fig. 5* indicate that the flies clearly discriminate between the surrogate leaves treated with the induced and the control surface extract. Thus, purely chemical factors were responsible for the observed discrimination. We have shown earlier that furanocoumarins isolated from carrot leaves stimulate oviposition in

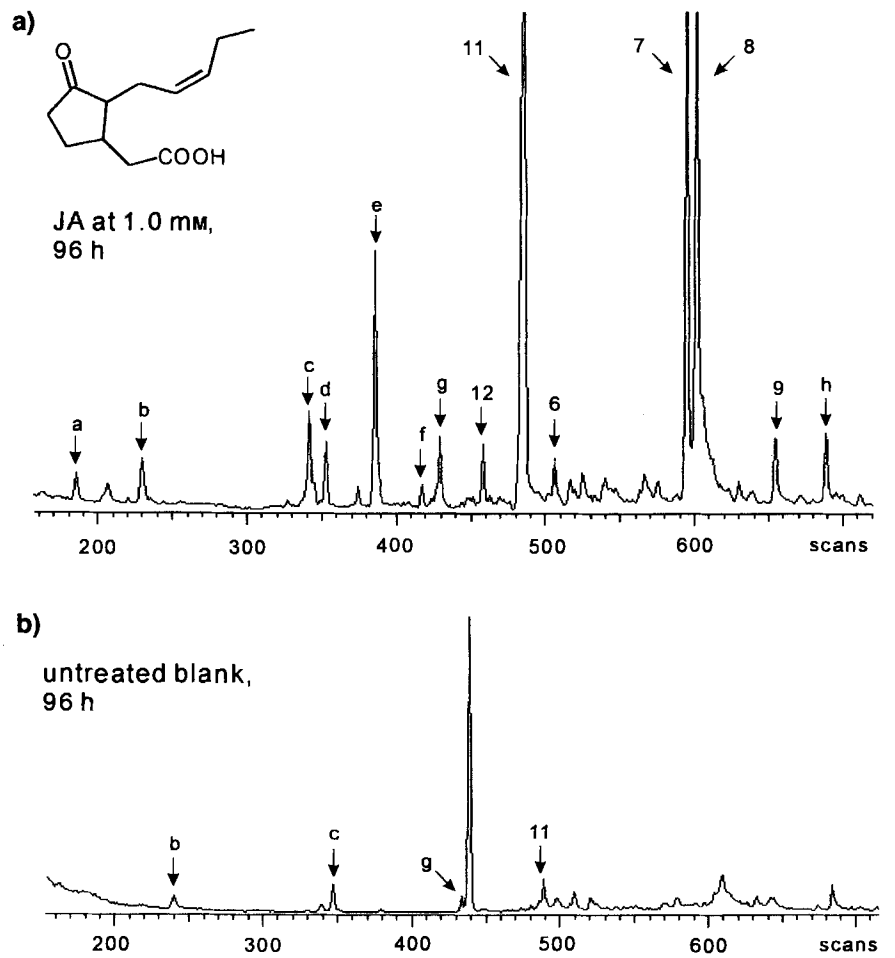


Fig. 3. *GLC Profile of surface compounds from celery leaves.* The profile represents the surface lipids 96 h after stimulation with JA (**1**). The surface washing (1.0 µl, *cf. Exper. Part*) was injected onto a fused silica column coated with *DB 1* (15 m × 0.25 mm). Compounds were separated under programmed conditions (50° for 2 min, then to 280° at 20° per min), and the eluting compounds were analyzed by a *Finnigan Ion Trap, ITD 800*. Identification of compounds: *a*) 3,6,6-trimethylcyclohex-2-en-1-one; *b*) tetradecene; *c*) hexadecene; *d*) caryophyllene; *e*) β-selinene; *f*) pentylcyclohexadiene; *g*) octadecene; **12**, butylphthalide; **11**, sedanolide; **6**, psoralen; **7**, bergapten; **8**, xanthotoxin; **9**, isopimpinellin, *h*) alkyl phthalate. No attempt was made to determine the C=C bond positions of the alkenes.

the carrot fly [29], and that they are perceived by the olfactory receptors located on the antennae [35]. Thus, it seems most likely that the stimulating factors of the induced celery leaf-surface extract are furanocoumarins. However, it is possible that JA induction does change also other leaf-surface compounds influencing the oviposition behavior. This assumption is supported by the observation that the difference between induced and control surrogate leaves increased markedly during the experiment (after the second day; see *Fig. 5*). A possible explanation might be that JA did not only induce stimulants

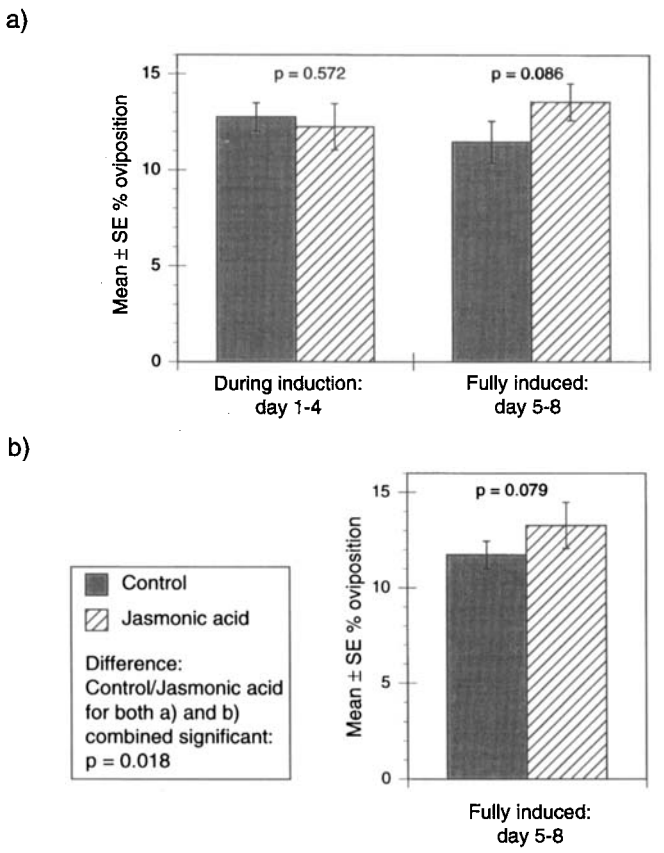


Fig. 4. *Oviposition of the carrot fly below celery leaves.* The egg counts are expressed as percent of the total oviposition per day. The petioles of the leaves were either immersed in tap water or 1.0 mM JA. In *a*) the flies were exposed to the two different treated leaves from the beginning of the experiment. In *b*) the oviposition experiment started after the leaves were induced in the greenhouse. *Mann-Whitney U test* for the differences of the control and JA-treated leaves in the first days of the induction in *a*) is not significant ($p = 0.572$). The same difference for the combined data of *a*) and *b*) (day 5 to 8) is significant at $p = 0.018$.

(furanocoumarins) but also inhibitory compounds in celery leaves which might be labile or volatile so that their effect gradually disappears during experimental periods of more than 2 days as has been observed earlier with other celery leaf extracts [36].

3. Discussion. – In many plants, a small, local damage by a herbivore results in a systemic build-up of many different chemical defences (*cf. Introduction*) which render the leaves of the remaining plant unpalatable for subsequent nonadapted herbivores [26]. At the same time, the emission of plant-typical volatiles starts, and the infested plant becomes attractive for the natural enemies of the herbivore. The effect is a reduction of the herbivore population and an increased resistance of the plant against future attacks. As shown in previous studies [8] [9], most of these defences, including the volatile emission [14] [15], are under the control of the octadecanoid signalling cascade which

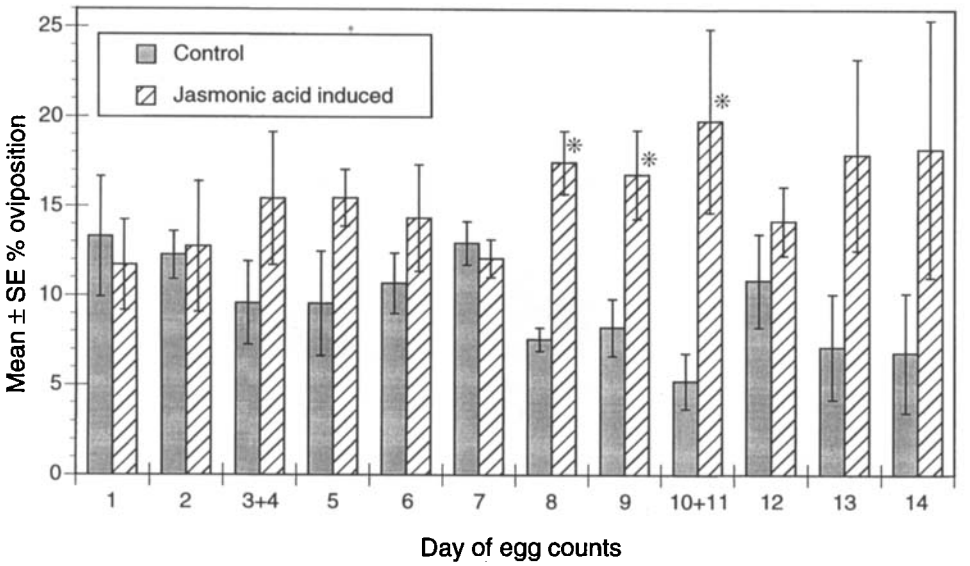


Fig. 5. *Oviposition of the carrot fly below surrogate leaves.* The wax-covered green paper leaves were sprayed with leaf surface extracts (CH_2Cl_2) of JA-induced plants and control plants. The egg counts are expressed as percent of the total oviposition per day. *Mann-Whitney U test* for the comparison of all 48 counts significant at $p < 0.0001$; for single days marked * significant at $p < 0.05$.

finally yields JA and/or certain amino-acid conjugates of JA as the effective signal transducers. Some responses to abiotic stresses such as osmotic stress are also controlled by JA [37].

The data presented clearly demonstrate that not only the plant interior but also the surface chemistry of a plant may be under control of signals from the jasmonate family. *Ca.* 2 days after the onset of a JA stimulus, the internal level of the furanocoumarins of celery leaves begins to raise, and at the same time significant amounts of the same compounds are deposited in the leaf surface. This coincidence indicates that the export of the compounds proceeds faster than the induction of the *de novo* biosynthesis. Since the ratio bergapten (**7**)/xanthotoxin (**8**) is different within the leaf tissue and on the leaf surface, the export of the furanocoumarins cannot be regarded as a simple translocation of the compounds along the oil-ducts. As the export could involve glycosides, the furanocoumarins, or their biosynthetic precursors, such enzymes apparently exert a significant control on the composition of the surface-deposited compounds. This is supported by the constant ratio of furanocoumarins within and above the leaf, independent of the time course of the induction process and the increasing concentration of the furanocoumarins within the leaf.

The constant but different ratio bergapten (**7**)/xanthotoxin (**8**) within and above the leaf provides also good evidence that the surface washing does not extract, at least not at a significant level, compounds from the inner leaf tissue.

Beside the export of the furanocoumarins, the stimulation of the biosynthesis of the sedanolide (**11**) is noteworthy. Although this compound lends the plant its typical 'celery odor' for humans, it was present only in rather low concentrations on the surface of

non-induced leaves (*Fig. 3,b*). However, 4 days after the onset of the JA stimulus it became one of the major compounds of the surface lipids, indicating that the biosynthesis of the phthalides is, at least in celery plants, under the control of the octadecanoid signalling pathway. However, nothing is yet known about the mode of their transport to the leaf surface.

In agreement with previous studies on the induction of the biosynthesis of volatile compounds in plants, not only JA but also the synthetic conjugate IN-ILE (**4**) activates the biosynthesis of the furanocoumarins. The reason for the somewhat lower efficiency of this synthetic elicitor is not known, but it might be attributed to the different solubility and transport properties of free JA and the amino-acid conjugate **4**.

As indicated by the choice tests, the females of the carrot fly clearly recognize the compounds which are exported to the leaf surface after stimulation with JA (**1**). Their preference for pre-damaged and systemically induced plants (by herbivory or JA) may be interpreted as choice of a better protected plant as the food source for the offspring. As the emerging larvae feed on the roots of the plant they live in a completely dark environment, and, hence, the phototoxicity of the furanocoumarins is not a relevant factor for these herbivores. On the other hand, if the aerial parts of the plant have already built up a systemically induced resistance, they may deter foraging insects or prevent the infection by microorganisms by exposing significant amounts of the phototoxic furanocoumarins on the surface. In consequence, the plant may be significantly less attacked, thus leaving the plant to the larvae of the carrot fly as the only, or at least one of only few herbivores. This view is supported by the fact that nonadapted herbivores that feed on artificially wounded or herbivore-damaged plants often exhibit reduced population growth, survival, growth rates, adult weight, and adult fecundity [38–40].

Several questions arise from the results presented in this work. It is, as yet, largely unknown which and to which extent plants respond to a microbial or herbivore attack by modification of their surface chemistry. As the insect damage is usually mediated *via* the octadecanoid signalling cascade, the exogenous application of, *e.g.*, JA or IN-ILE now provides the plant scientists with the unique chance to study the induced transport of compounds to the leaf surface without relying on the ill-defined general set-up of a microbialor insect attack. Thus, more exact kinetics of the export process will become available using 'jasmonates' for the stimulation. It will be interesting to see which other compound classes may serve as toxic surface defences or as compounds protecting the plant against evaporation of water (dry stress) and other stresses, which are JA-mediated. In this way, plant-surface chemistry, the first line of defence for the plant, may soon become an important piece in our understanding of induced resistance. Moreover, it is to be expected that the damage-induced changes of the surface chemistry of a plant will significantly influence the feeding behavior of insects as well as other activities like, for example, the egg-laying and hatching both of which are essential for the survival of the species. Well-designed experiments based on the use of 'jasmonates' could shed light on the radition and the impact of induced changes of the surface chemistry of plants in the environment.

Experimental Part

General. The furanocoumarins xanthotoxin, isopimpinellin, and bergabten were purchased from *Sigma*, Dreieich, Germany. Jasmonic acid (**1**) was prepared from methyl jasmonate (*Givaudan-Roure*, Zürich, Switzerland) according to standard procedures [41]. IN-ILE was synthesized as described in [17]. TLC: with silica-gel plates

Polygram Sil G_{F254}, from *Merck*. GLC: *Carlo Erba*, Series *4100*, equipped with a fused silica capillary coated with *SE 30* (10 m × 0.31 mm) from *Macherey & Nagel* (Düren, Germany). HPLC: *Spark Holland* autosampler, model *Marathon*, combined with a high-pressure gradient system, consisting of two *Knauer* pumps *64* and a *Knauer* spectrophotometer *256* adjusted to 254 nm. *Knauer* HPLC software *Euro-Chrom* served for aquisition and quantification of the data. Silica gel, *Si 60* (0.200–0.063 mm, *E. Merck*, Darmstadt, Germany) was used for chromatography. GC/MS (70 eV): *Finnigan ITD 800* coupled with a *Carlo Erba* GC *6000*, model *Vega* or *Fisons MD 800* GLC/MS system, equipped with a fused silica column (*DB 1*, 15 m × 0.25 mm). HR-MS: *Kratos MS 50*.

Induction Experiments, General Plants. Young celery plants (*Apium graveolens*, cv. *secalinum*) were bought from the local market and grown in 6-cm^2-diameter pots filled with potting soil. The plants were kept at 23° and illuminated (*ca.* 270 μE m^{-2} s^{-1}, 14 h light and 10 h dark) using daylight fluorescence tubes. Plants used for induction experiments were at the four-to-six true-leaf stage when used for induction experiments.

Test Compounds. Jasmonic acid (JA, **1**), was dispersed in tap water by sonication (1.0 mmol). In the case of the *1-oxoindan-4-carboxylic acid* (**10**) and its amino-acid conjugate IN-ILE (**4**), *Triton X 100* (*ca.* 40 mg/10.0 ml) had to be added to facilitate emulsification. All compounds gave stable emulsions which could be used for the time of the induction experiments (max. 4 d) without noticeable decomposition. Freshly detached leaves were immediately placed into the emulsions for the intervals given in *Figs. 2* and *3*, and in the *Table*. Control experiments were generally done using tap water without additives. When *Triton X 100* was added for solubilization of the test compounds, tap water containing *ca.* 4.0 mg of the emulsifier per ml was used for the control experiments. All induction experiments were repeated five times to secure reproducibility.

Collection of Surface Compounds. The non-polar surface compounds were obtained by brief dipping (2 × 30 s) of the induced, but undamaged leaves into CH_2Cl_2 (*ca.* 8 ml) [29]. Control plants were treated in the same way. During dipping, the leaves were gently moved with forceps carefully avoiding any mechanical damage of the leaf which could falsify the result by extraction of furanocoumarins from the inner leaf tissue. Contact of the cut with the solvent was avoided for the same reason. Following filtration and controlled removal of the solvent *in vacuo* (*ca.* 300 Torr), the residue was dissolved in AcOEt (150 μl). The resulting soln. is ready for HPLC analysis.

Extraction of Furanocoumarins from the Leaf Tissue. Following removal of the surface compounds, the leaf was dried for 24 h at 50°. The dry leaf was crushed and grinded in AcOEt (15 ml) using an ultraturrax (10 min at 20000 rpm). The greenish suspension was stirred for 24 h at r.t. to complete the extraction. The suspension was filtered, the solvent removed *in vacuo* (*ca.* 120 Torr), and the residue redissolved in AcOEt (150 μl). The resulting soln. was used for HPLC analysis.

Induction Experiments with 1-Oxoindan-4-carboxylic Acid **10** *and IN-ILE* (**4**). Induction experiments were carried out as described above. The induced furanocoumarins were extracted from the pre-treated leaves without previous removal of the surface compounds. Leaf material was dried (24 h at 50°), grinded, and extracted with AcOEt as described above. The HPLC analysis of these extracts, therefore, represents the sum of surface- and tissue-derived furanocoumarins.

HPLC Analysis of the Furanocoumarins. The analysis of the furanocoumarins was performed according to a protocol of *Berenbaum et al.* [31]. The sample (20 μl) was separated on SiO_2 (*LiChrospher Si 60*, 5 μm, 25 cm × 0.4 mm, *E. Merck*, Darmstadt, Germany) using an isocratic solvent cocktail consisting of cyclohexane, $(i\text{-}Pr)_2O$, pentan-1-ol, and H_2O (15:4:1, *v*/*v*/*v* 0.01% H_2O) at 1.0 ml min^{-1} for elution. Compounds were detected and quantified by their UV absorption at 254 nm. The addition of H_2O was essential to minimize peak tailing. The quantification of the furanocoumarins was done relying on calibration curves with authentic reference compounds.

MS Analysis of Surface Compounds. The solns. of the surface material (AcOEt) from the washings were directly analyzed by GLC/MS by injecting (1.0 μl) onto a fused silica column coated with *DB 1* (15 m × 0.25 mm). Compounds were separated under programmed conditions (50° for 2 min, then to 280° at 20° per min), and the eluting compounds were analyzed by MS (*Finnigan Ion Trap ITD 800*). Transfer line: 250°. Scan range: 35–350 Da s^{-1}. Authentic references were used for the unequivocal identification of all furanocoumarins **6**–**9** and sedanolide (**11**). The structures of the terpenoids β-selinene and caryophyllene were attributed by library search (*Wiley*) without additional proof.

Oviposition Assays. The carrot flies used originated from a continuous culture [42] in the Wädenswil laboratory. For the oviposition assays, we used 0.34 m^3 screened cages and artificial oviposition devices allowing the test of cut leaves immersed with cut petioles in soln. [43] [44]. The effect of jasmonate-induced leaves was tested in two independent experiments: *1*) the cut leaves were immersed into 1.0 mM JA dissolved in tap water or into pure tap water (control) and exposed to the flies in cages (reduced light intensity) in climate controlled rooms; *2*) the cut leaves were induced using 1.0 mM JA in the greenhouse with optimal lighting for plant growth during 4 d. Subsequently, the induced and control leaves were tested still with the petioles in the respective soln. in the oviposition assay as described above.

Leaf surface extracts (CH_2Cl_2) at 2.4-g leaf equivalents dissolved in 2.0 ml of solvent were sprayed on surrogate leaves and attached to the surface of the screen of the devices as described above [44]. In all experiments, four induced leaves or treated surrogate leaves, and four control leaves and surrogate leaves were arranged in alternate sequences in a circle on the floor of the cage. After 1–2 d of oviposition, all the eggs were counted and removed from the oviposition devices and put back into the cage in reversed positions. The egg counts of control and treatment for each day were transformed in percentages of the total egg count and analyzed using the *Mann-Whitney U test*.

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