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Induction of Resistance against the Leafminer, *Liriomyza trifolii*, by Jasmonic Acid in Sweet Pepper

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Sweet pepper (*Capsicum annuum*) leaves at the mature stage have strong ovipositional deterrence against *Liriomyza trifolii* (Burgess) (Diptera, Agromyzidae), whereas the cotyledons are fiercely attacked by the fly. Treatment of the cotyledons with 50 μM and 100 μM of a jasmonic acid (JA) solution caused the plant to acquire strong oviposition deterrence against the leafminer. An HPLC analysis of the JA-treated cotyledons revealed the inducible accumulation of a compound. Based on spectroscopic analysis and chemical methods, the induced compound was identified to be caffeoylputrescine (CP). The accumulated amounts of CP in the cotyledons treated with 0, 10, 50 and 100 μM of JA were 6.0, 43.0, 105 and 140 μg/g fr. wt., respectively. Treatment of the cotyledons with CP resulted in a significant decrease in the number of punctures made by *L. trifolii*, indicating that the JA treatment enhanced the deterrence against the leafminer by inducing CP accumulation.

Key words: caffeoylputrescine; jasmonic acid; *Liriomyza trifolii*; *Capsicum annuum*; ovipositional deterrent

Liriomyza trifolii (Burgess) (Diptera, Agromyzidae), a leafminer native to North America, causes serious damage to a wide variety of vegetables and nursery crops in greenhouses and open fields throughout the world.[1)] This leafminer is a small fly, measuring 2 mm in length, with yellow marking on a black body. The female flies make many punctures on the leaf surface by their ovipositor and lay eggs in some of those punctures.[2)] First instar larvae, after hatching from the eggs within 2 days, feed on mesophyll leaf tissue and form a mine. After passing through three instar stages in a week, the last instar larvae emerge from the leaves and pupate on the leaves or on the ground. Adult flies emerge from the pupa within a week. The high frequency of mines made by the larvae can reduce both the plant growth and crop yield. Furthermore, in leafy vegetables and ornamental plants, some crops could lose marketable worth even by only a single mine. To overcome these problems, an excess application of pesticides against the American serpentine leafminer, *L. trifolii* has been used, but it has acquired high resistance to various pesticides. As the leafminer has a short life cycle and high resistance to chemicals, it is getting difficult to control them by using pesticides. So, a pest management technique without depending on chemical pesticides would be essential. One of the answers is the utilization of natural chemical products. With this aim, we have already studied the resistance of the sweet pepper to this leafminer and found that resistance developed at the mature-stage of the plant.[3)] It was elucidated that this resistance was mainly attributable to the much higher content of luteolin 7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside in the mature-stage plant than at its younger stage.[4,5)]

Induced resistance against pathogens has recently been investigated in many plant species,[6)] and research on its application to crop protection has also been conducted.[7)] This resistance is caused by an accumulation of secondary metabolites, expression of pathogenesis-related genes and proteins, generation of reactive oxygen species and other unknown processes.[8,9)] The inducible resistance by insect feeding has also been studied by many groups.[10,11)] It has been demonstrated that jasmonic acid (JA) played an important role as a signaling cure.[12–15)] Constable and Ryan[16)] have revealed that the polyphenol oxidases, which are considered to be part of the anti-herbivore defense mechanism, were induced by an exogenous treatment with methyl jasmonate (MeJA)

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Abbreviations: JA, jasmonic acid; MeJA, methyl jasmonate; CP, caffeoylputrescine; pCP, *p*-coumaroylputrescine; FP, feruloylputrescine

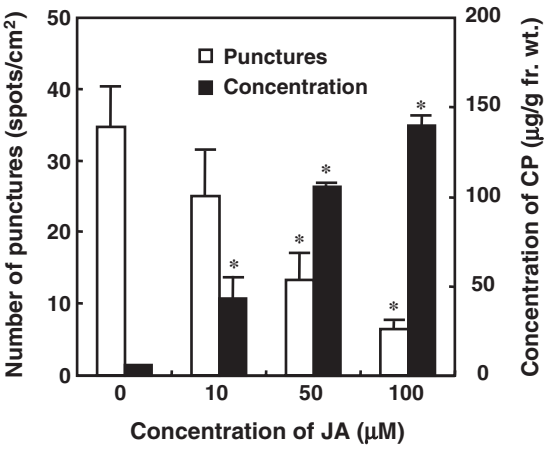

Fig. 1. Effects of the Concentration of JA on the Resistance of Cotyledons of the Sweet Pepper against Oviposition of the Leafminer (*L. trifolii*) and Accumulation of CP.

The numbers of ovipositional punctures are represented by the unshaded bar as the mean ± S. E. ($n = 6$) and the concentration of CP is represented by the shaded bar as the mean ± S. E. ($n = 4$). Data were analyzed by the Mann-Whitney U-test ($P < 0.05$).

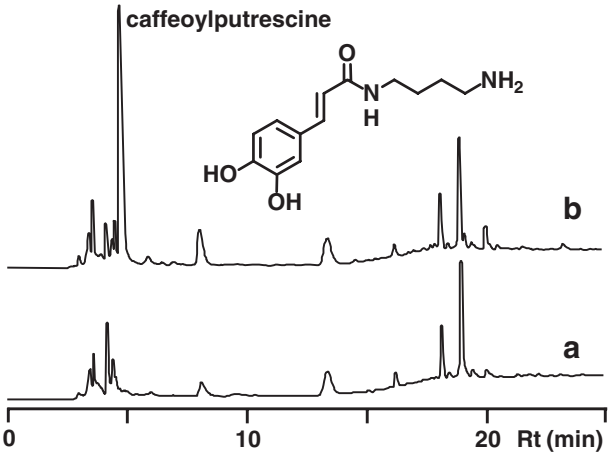

Fig. 2. HPLC Chromatograms of Extracts from the Untreated Cotyledon (a) and 100 μM JA-Treated Cotyledon (b).

in various plants including tomato (*Lycopersicon esculentum*), tobacco (*Nicotiana tabacum*), soybean (*Glycine max*) and alfalfa (*Medicago sativa*). Especially in the tomato plant, the resistance induced with JA or its methyl ester (MeJA) has been well investigated. The induced resistance factors against the noctuid caterpillar, *Spodoptera exigua*, have been related to the increased of enzyme activities including polyphenol oxidases, lypoxygenase and peroxidase,[17,18] the enhanced protease inhibitor activities[17] and the recruiting parasitoid wasp.[19] However a chemical cure for inducible resistance caused by the exogenous JA/MeJA treatment against insects has been rarely proved successful in any plant species. We report here the induced resistance of sweet pepper against the leafminer by a jasmonic acid treatment.

Results

The effect of JA on the resistance of cotyledons of sweet pepper (*Capsicum annuum* L. var. *grossum* Stendt) against the leafminer (*L. trifolii*) was investigated by using 3-week-old seedlings. Cotyledons were excised by a shape razor, and floated in solutions of JA at various concentrations for 48 hours. The cotyledons were then exposed to female flies in a 20 ml-glass vial for 24 hours. Many feeding and oviposition punctures (34.8 ± 5.5 marks/cm^2) made by the leafminer were observed on the surface of cotyledons treated with water (Fig. 1). The numbers of punctures were fewer on the cotyledons treated with the JA solution. The numbers of punctures on the cotyledons treated with the JA solutions of 50 μM and 100 μM were 13.4 ± 3.9 and 6.6 ± 1.1 marks/cm^2, respectively. The flies made fewer punctures (25.1 ± 6.4 marks/cm^2) even on the cotyledons treated with the 10 μM JA solution than with the water-treated (control) leaves, although the difference was not statistically significant.

The cotyledons were extracted with 90% methanol-water, and each extract was analyzed by reversed-phase HPLC in order to compare the amounts of secondary metabolites (Fig. 2). In the cotyledon extract treated with the 100 μM JA solution, a peak (compound **1**) at Rt 4.70 min was enhanced. However the induction of luteolin 7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside, which is an oviposition deterrent in mature leaves, was not observed. The peak was analyzed by LC–MS and UV without isolation to elucidate the structure of compound **1**. Compound **1** showed three characteristic absorbances at 234, 293 and 318 in its UV spectrum, and gave an $[M + H]^+$ ion at $m/z = 251$ and a characteristic fragment ion at $m/z = 163$ which indicated the presence of a caffeoyl moiety. Its high polarity and spectroscopic data suggested that the induced compound was caffeoylputrescine (CP). To confirm this, CP was synthesized by a condensation reaction between caffeic acid and putrescine by using dicyclohexylcarbodiimide as a condensing agent. The spectroscopic data for **1** were identical with those of the synthesized compound. Additionally, the identity of **1** was confirmed by co-chromatography with the synthetic compound by reversed-phase HPLC.

The effect of JA concentration on the induction of CP was also investigated (Fig. 2). The cotyledons were treated with JA for 48 h. The accumulation of CP was enhanced with the increasing concentration of JA: the amounts of CP in the cotyledons treated with 0, 10, 50 and 100 μM were 6.0 (0.13), 43 (0.90), 105 (2.19) and 140 (2.91) μg/g fr. wt. (μg/cm^2), respectively (Fig. 1).

To address the significance of the induction of CP, we treated cotyledons with CP, and analyzed its effect on puncturing by the flies. The numbers of ovipositional punctures on the cotyledons treated with 0 and 0.25 mM were 30.9 and 28.8 marks/cm^2, respectively, (Fig. 3)

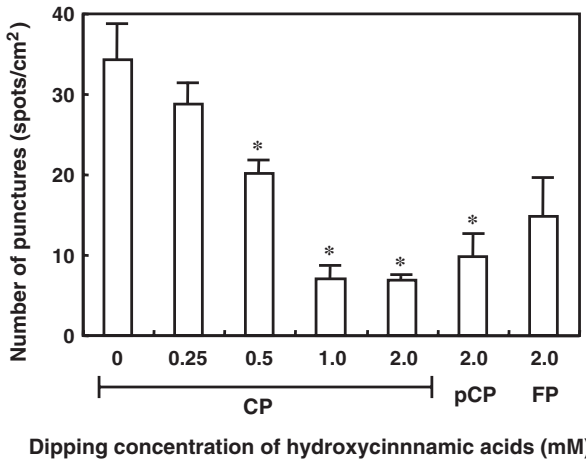

Fig. 3. Effects of the Dipping Concentration of Hydroxycinnamic Acids (CP, pCP and FP) on the Resistance of Cotyledons of the Sweet Pepper against Oviposition of the Leafminer Based on the Dipping Method.

Each value is expressed as the mean ± S. E. ($n = 6$ for the control and $n = 5$ for a treated sample). Data were analyzed by the Mann-Whitney U-test ($P < 0.05$).

and thus treating with CP at 0.25 mM was not sufficient to induce ovipositional deterrence. At higher concentrations, the numbers of punctures were reduced with increasing CP concentration. The numbers of punctures on the cotyledons treated with CP at 0.5, 1.0 and 2.0 mM were 20.1, 7.1 and 6.9 marks/cm^2, respectively.

The presence of fueruoylputrescine (FP) and *p*-coumaroylputrescine (pCP) has been reported, in other cultivars of the sweet pepper[20] so, we also examined their oviposition deterrence. The numbers of punctures made by the leafminer on cotyledons treated with a 2.0 mM solution of synthetic FP and pCP were 14.9 and 9.9 marks/cm^2, respectively. A significant difference in the number of punctures was only detected with the pCP treatment.

Discussion

Treatment of cotyledons of the sweet pepper with JA at concentrations higher than 50 μM reduced the number of punctures made by female flies as traces of oviposition or feeding. HPLC analysis of the cotyledons revealed that the accumulated concentration of CP in the JA-treated cotyledons was 20-fold higher than that in the water-treated cotyledons. The treatment by CP of the cotyledons effectively prevented the leafminer from making punctures, and we thus conclude that the JA treatment induced the resistance to *L. trifolii* in the cotyledons of sweet pepper by accumulating CP. Deterrence was observed at a dose of more than 0.81 μg/cm^2 on the surface of a cotyledon dipped into a 0.5 mM CP solution in a dipping test and 2.2 μg/cm^2 with a cotyledon treated with a 50 μM JA solution in an induction test. As the effective doses in both tests could not be directly compared, it is thought that another ovipositional deterrent(s) might have been induced by the JA treatment.

The resistance of the sweet pepper at the mature stage against *L. trifolii* has been attributed to the presence of luteolin 7-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside[4] 4-aminobutanoic acid, (2*S*,4*R*)-4-hydroxy-1-methyl-2-pyrrolidine carboxylic acid, 4-amino-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone[5] and phytol.[3] The plant has consistently produced defensive chemicals against *L. trifolii* at the mature stage, but at the cotyledon stage, the plant could not produce a sufficient amount of the chemical required to provide resistance to attack by this species. Furthermore, based on our preliminary experiments, the content of CP in the lower leaves of the sweet pepper at the mature stage was 36.0 μg/g fr. wt., although the plant had various contents of CP depending on the growth stage or region. Gerhard and Nikolaus[20] have also shown such variation in the contents of CP in banana pepper consistent with our results. As a defensive compound, CP was induced by the JA treatment at the cotyledon stage, so it is thought the sweet pepper has multiple defense systems.

JA was induced by feeding the herbivore caterpillar, tobacco hornworm (*Manduca sexta* L.) in tobacco plant,[21] the beet armyworm (*Spodoptera exigua* (Hübner)) in corn seedling[22] and the cabbage butterfly (*Pieris rapae* (L.)) in Arabidopsis.[23] Salicylate and JA were also accumulated by attacking spider mites, *Tetranychus urticae*, in lima bean leaves,[24] and these signaling compounds accumulated by the action of an attacking herbivore could induce resistance against insects or pathogens. It is not known whether or not oviposition of the fly would induce JA in a plant as far as we know, and the cotyledon of sweet pepper did not show any resistance to *L. trifolii* attack, so the induced amount of JA would have been negligable or nothing. Further studies concerning the diversity of endogenous JA and related compounds in the sweet pepper are needed.

The presence of CP was first reported from a callus tissue culture of *N. tabacum*,[25] and later from the apical leaves, anthers and ovaries of *N. tabacum*.[26] CP has also been found in several Solanceous species including *C. annuum*,[20] although its function has not yet been clear. On the basis of its chemical structure, CP is classified as a hydroxycinnnamic acid amide. This class of compound has been identified in many plant species, mainly *Poaceae* and *Solanaceae*. Some of them, namely *p*-coumaroylagmatine in barely[27] and avenanthramide in oats,[28] have been shown to be toxic against pathogenic fungi. Furthermore, hydroxycinnnamic acid amides have been demonstrated to be induced by various exogenous stimuli. For example, avenanthramides were induced by inoculation with the fungus,[29] *p*-coumaroyloctopamine was induced by β-1,3-glucooligosaccharide in potato tuber disks,[30] clovamide and its derivatives were induced by JA in red clover[31] and caffeoylputrescine was induced by MeJA in tobacco.[32]

Based on their toxicity and inducibility by various exogenous stimuli, hydroxycinnnamic acid amides have generally been established as compounds involved in the inducible defense system against pathogens. In this study, we have revealed that caffeoylputrescine acted as an oviposition deterrent against *L. trifolii*, and this is the first report on the biological activity of a hydroxycinnnamic acid amide against insects. Henceforth, we should pay more attention to the possibility of the action of hydroxycinnnamic acid amides as defensive substances against insects and not only against pathogens. Hydroxycinnnamic acid amides should therefore be studied for their oviposition deterrent activity, insecticidal activity, feeding deterrent activity, repellent activity and growth regulator activity against various insect pests.

FP and pCP also decreased the number of punctures by *L. trifolii*, although their activity was weaker than that of CP. FP and pCP were not detected in the cotyledons treated with JA in our analysis. However, the presence of FP and pCP has previously been reported in different cultivars of the sweet pepper.[20] Thus, FP and pCP may also play an important role in the defense system against *L. trifolii* in other varieties of sweet pepper.

The inhibition by CP against *L. trifolii* was almost saturated when used above a concentration of 1.0 mM CP, while even a 2.0 mM solution of FP or pCP was exhibited weaker activity than that of a 1 mM CP solution against the flies. Tanaka *et al.*[33] have similarly reported that feruloylserotonin induced in witches' broom diseased bamboo along with *p*-coumaroylserotonin showed weaker antifungal activity than *p*-coumaroylserotonin. It thus, seems that substitution of the hydroxyl group in the benzene ring of hydroxycinnnamic acid amide might be important for this activity.

Experimental

Plant materials. Sweet pepper (*Capsicum annuum* L. var. *grossum* Stendt cv. new Sakigake Number 2) and kidney bean (*Phaseolus vulgaris* L.) seeds were purchased from Maekawa-syubyo nursery (Japan). The sweet pepper seeds were sown on a tray (20 × 15 × 1 cm depth) containing nursery soil (Cell Compost, Mitsui-Nourin Co., Ltd., Japan) and maintained at 25 ± 2 °C with a 14 L-10 D photo period in a growth chamber. After 3 weeks, the seedlings, which consisted only of cotyledons, were used for bioassays. Three kidney bean seeds were sown in a vinyl pot containing nursery soil (Tsuchitaro, Mitsui-Nourin Co., Ltd., Japan) and maintained at 25 ± 5 °C with a natural photoperiod in a greenhouse. After the primary leaves had developed for 4 weeks, the seedlings were used to maintain the population of *L. trifolii*.

Elicitor treatment and chemical application. The elicitor treatment was done by the floating method. Cotyledons of the sweet pepper, which had been excised from the seedlings with a sharp razor blade, were floated on 10 ml of various concentrations of an aqueous jasmonic acid solution in a Petri dish (9 i.d. × 1 cm). Each container was maintained at 25 ± 2 °C with a 14 L-10 D photoperiod in a growth chamber for 24 h. After cleaning the surface of each cotyledon with water, the treated cotyledon was used for the bioassay or extraction.

Application of the chemicals was carried out by the dipping method, the cotyledons being dipped into various chemical-methanol solutions for 5 seconds. After the solvent had been evaporated by air drying at room temperature, the treated cotyledons were used for the bioassay. To estimate the dose of chemicals applied to a leaf in the dipping test, the applied solvent volume was measured by dipping ten cotyledons into a 0.25 mM CP methanol solution for 5 s, the consumption of the solvent then being measured and the area of the cotyledons recorded by a PC equipped with a scanner. The applied volume of solvent per cm^2 was then calculated and used to estimate the dose of various hydroxycinnnamic acid amides in the dipping test.

Analytical methods. The treated and untreated cotyledons of sweet pepper were extracted with methanol for 48 h, and each extract was directly analyzed by HPLC or LC–MS. HPLC analyses was carried out with an ODS column (Wakosil-II 5C18 HG, 150 × 4.6 mm i.d., Wako Pure Chemical Industry, Japan) with a 10ADvp system equipped with a diode array detector (SPD-M10Avp, Shimadzu Co., Ltd., Japan) under the following conditions: linear gradient (10% A/B for the first 5 min, then 10–30% for 5 min, and finally 30–90% A/B for 25 min) {solvent A, H_2O–AcOH (99:1, v/v); solvent B, acetonitrile–AcOH (99:1, v/v)}. LC–MS data were measured by a Perkin-Elmer-Sciex API-165 or API-3000 instrument (ion-spray voltage, 5 kV; orifice voltage, 30 V; nebulizer gas, air; curtain gas, nitrogen) connected to a Shimadzu 10A HPLC system equipped with the same ODS column.

NMR data were recorded with a JEOL JNM-AL400 spectrometer in DMSO-d_6 or with a Bruker Avance400 spectrometer in methanol-d_4, using TMS as an internal standard. Chemical shifts are represented as δ units, and the multiplicity of signals is abbreviated as singlet (*s*), doublet (*d*), triplet (*t*), quartet (*q*), multiplet (*m*). Coupling constants (*J*) are given in Hz.

Insect. L. trifolii was reared on the primary leaf of the kidney bean seedling. Nine seedlings were exposed to 15 female *L. trifolii* for 2 days in an acrel cage (30 × 25 × 28 cm) which was fitted with nylon mesh (0.5 mm) at the back and two sides. After removing the flies, the kidney bean seedlings were placed in a growth chamber maintained at 25 ± 2 °C with a 14 L-10 D photo period. The leaves were cut from the seedlings before the emergence of maggots, and kept in an ice cream cup (12 i.d. × 7 cm) under the same conditions. Adult flies were

collected everyday to arrange their age for bioassay and maintain the population.

Bioassay. The degree of ovipositional deterrence was measured by counting the number of leaf punctures made by the female flies. The treated cotyledon was put into a 20 ml-glass vial which had a small sheet of moistened filter paper inside. Five 2-day-old female flies were released into the vial. After screwing on a lid, the vial was then placed in a growth chamber maintained at $25 \pm 2\,^{\circ}C$ with a 14 L-10 D photo period for 24 h. The number of punctures made on a leaf surface was counted under a microscope, and the area of the leaf was measured by a PC equipped with a scanner. Data were statistically analyzed by using the Mann-Whitney U-test ($P < 0.05$).

Preparation of the hydroxycinnnamic acid amides. Caffeoylputrescine was synthesized according to the method of Tebayashi *et al.*[31] A mixture of caffeic acid (5 mmol), 1,4-butandiamine and dicyclohexylcarbodiimide (5.5 mmol) was stirred in dry pyridine (20 ml) at room temperature for 24 h. After removing the pyridine *in vacuo*, the residue was dissolved in MeOH, and the solution was added to a 2 M KOH water solution (200 ml). The mixture was stirred at room temperature for 6 h under N_2. After the mixture had been neutralized with AcOH, the solvent was evaporated *in vacuo*, the residue was dissolved in few drops of MeOH solution and filtered to remove dicyclohexylurea and salts. The mother liquor was purified with an ODS column (Chromatorex DM1020T, Fuji Silysia Chemical, Japan) and by reversed-phase preparative HPLC (Capcellpak C18 AG120, 250 × 10 mm id, Shiseido, Japan) using MeOH–water solvent system to yield caffeoylputrescine (35.3%). CP: LC–MS, m/z (rel. int.): 250$[M + H]^+$ (42), 163[caffeoyl]$^+$ (100); UV, λ_{max} nm: 234, 293, 318. ^{1}H-NMR (DMSO-d_6) δ: 1.5–1.8 (4H, *m*), 2.94 (2H, *t*, J = 7.4 Hz), 3.32 (2H, *t*, J = 7.4 Hz), 6.43 (1H, *d*, J = 15.6 Hz), 6.75 (1H, *d*, J = 8.1 Hz), 6.89 (1H, *dd*, J = 2.0, 8.1 Hz,), 6.99 (1H, *d*, J = 2.0 Hz), 7.38 (1H, *d*, J = 15.6 Hz). ^{13}C-NMR (DMSO-d_6) δ: 26.0, 27.6, 39.5, 40.4, 115.0, 116.5, 118.2, 122.1, 128.2, 142.4, 146.4, 148.9, 169.5.

pCP and FP were synthesized by using the foregoing protocol. pCP: LC–MS, m/z (rel. int.): 235$[M + H]^+$ (100), 218$[M - NH_2]^+$ (12), 147[*p*-coumaroyl]$^+$ (24); UV, λ_{max} nm: 225, 293, 308. ^{1}H-NMR (CD_3OD) δ: 1.6–1.8 (4H, *m*), 2.93 (2H, *t*, J = 7.2 Hz), 3.33 (overlapping with CD_3OD), 6.42 (1H, *d*, J = 15.7 Hz), 6.80 (2H, *d*, J = 8.6 Hz,), 7.40 (2H, *d*, J = 8.6 Hz), 7.45 (1H, *d*, J = 15.7 Hz). ^{13}C-NMR (CD_3OD) δ: 26.3, 27.5, 39.6, 40.4, 116.8, 118.2, 127.5, 130.5, 141.9, 160.7, 169.4. FP: LC–MS, m/z (rel. int.): 265$[M + H]^+$ (100), 248$[M - NH_2]^+$ (7), 177[feruloyl]$^+$ (24); UV, λ_{max} nm: 220, 240 (sh), 290, 317. ^{1}H-NMR (CD_3OD) δ: 1.50–1.65 (4H, *m*), 2.69 (2H, *t*, J = 7.0 Hz), 3.30 (overlapping with CD_3OD), 3.86 (3H, *s*), 6.39 (1H, *d*, J = 15.6 Hz), 6.87 (1H, *d*, J = 8.3 Hz), 6.90 (1H, *dd*, J = 8.3, 1.8 Hz), 7.02 (1H, *d*, J = 1.8 Hz,), 7.39 (1H, *d*, J = 15.7 Hz).). ^{13}C-NMR (CD_3OD) δ: 27.9, 30.6, 40.2, 42.0, 56.2, 112.2, 114.9, 118.9, 121.0, 129.5, 142.1, 150.2, 151.8, 169.2.

References

1) Spencer, K. A., Agromyzidae (Diptera) of economic importance. In "Series Entomologica" Vol. 9, Dr. W. Junk, The Hague, p. 418 (1973).
2) Minkenberg, O. P. J. M., and Van Lenteren, J. C., The leafminers *Liriomyza bryoniae* and *L. trifolii* (Diptera: Agromyzidae), their parasites and host plants: a review. *Agricultural University Wageningen Papers*, **86**, 1–50 (1986).
3) Kashiwagi, T., Mikagi, E., Mekuria, D. B., Boru, A. D., Tebayashi, S.-I., and Kim, C.-S., Ovipositional deterrent on mature stage of sweet pepper, *Capsicum annuum*, against *Liriomyza trifolii* (Burgess). *Z. Naturf.*, **60c**, 739–742 (2005).
4) Kashiwagi, T., Horibata, Y., Mekuria, D. B., Tebayashi, S.-I., and Kim, C.-S., Ovipositional deterrent in the sweet pepper, *Capsicum annuum*, at the mature stage against *Liriomyza trifolii* (Burgess). *Biosci. Biotechnol. Biochem.*, **69**, 1831–1835 (2005).
5) Dekebo, A., Kashiwagi, T., Tebayashi, S.-I., and Kim, C.-S., Nitrogenous ovipositional deterrents in the leaves of sweet pepper (*Capsicum annuum*), at the mature stage against the Leafminer, *Liriomyza trifolii* (Burgess). *Biosci. Biotechnol. Biochem.*, **71**, 421–426 (2007).
6) Clarke, D. D., "Active Defence Mechanisms in Plants," ed. Wood, R. S. K., Plenum Press, New York, p. 381 (1982).
7) Ku'c, J., Concepts and direction of induced systemic resistance in plants and its application. *Eur. J. Plant. Pathol.*, **107**, 7–12 (2001).
8) Sticher, L., Mauch-Mani, B., and Me'traux, J. P., Systemic acquired resistance. *Annu. Rev. Phytopathol.*, **35**, 235–270 (1997).
9) Hutcheson, S. W., Current concepts of active defense in plants. *Annu. Rev. Phytopathol.*, **36**, 59–90 (1998).
10) Kennedy, G. G., Tomato, pests, parasitoids, and predators: tritrophic interactions involving the genus *Lycopersicon*. *Annu. Rev. Entomol.*, **48**, 51–72 (2003).
11) Kessler, A., and Baldwin, I. T., Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, **291**, 2141–2144 (2001).
12) Ziegler, J., Keinänen, M., and Baldwin, I. T., Herbivore-induced allene oxide synthase transcripts and jasmonic acid in *Nicotiana attenuata*. *Phytochemistry*, **58**, 729–738 (2001).
13) Tscharntke, T., Thiessen, S., Dolch, R., and Boland, W., Herbivory, induced resistance, and interplant signal transfer in *Alnus glutinosa*. *Biochem. Syst. Ecol.*, **29**, 1025–1047 (2001).
14) De Bruxelles, G. Z., and Roberts, M. H., Signals regulating multiple responses to wounding and herbivores. *Crit. Rev. Plant Sci.*, **20**, 487–521 (2001).
15) Thaler, J. S., Stout, M. J., Karban, R., and Duffey, S. S., Jasmonate-mediated induced plant resistance affects a community of herbivores. *Ecol. Entomol.*, **26**, 312–324 (2001).

16) Constabel, C. P., and Ryan, C. A., A survey of wound- and methyl jasmonate-induced leaf polyphenol oxidase in crop plants. *Phytochemistry*, **47**, 507–511 (1998).
17) Thaler, J. S., Stout, M. J., Karban, R., and Duffey, S. S., Exogenous jasmonates simulate insect wounding in tomato plants (*Lycopersicon esculentum*) in the laboratory and field. *J. Chem. Ecol.*, **22**, 1767–1781 (1996).
18) Thaler, J. S., Induced resistance in agricultural crops: effects of jasmonic acid on herbivory and yield in tomato plants. *Environ. Entomol.*, **28**, 30–37 (1999).
19) Thaler, J. S., Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature*, **399**, 686–688 (1999).
20) Gerhard, L.-M., and Nikolaus, A., The distribution of hydroxycinnamoylputrescines in different organs of *Solanum tuberosum* and other solanaceous species. *Phytochemistry*, **32**, 551–556 (1993).
21) Ziegler, J., Keinänen, M., and Baldwin, I. T., Herbivore-induced allene oxide synthase transcripts and jasmonic acid in *Nicotiana attenuata*. *Phytochemistry*, **58**, 729–738 (2001).
22) Schmelz, E. A., Alborn, H. T., Banchio, E., and Tumlinson, J. H., Quantitative relationships between induced jasmonic acid levels and volatile emission in *Zea mays* during *Spodoptera exigua* herbivory. *Planta*, **216**, 665–673 (2003).
23) De Vos, M., Van Zaanen, W., Koornneef, A., Korzelius, J. P., Dicke, M., Van Loon, L. C., and Pieterse, C. M. J., Herbivore-induced resistance against microbial pathogens in Arabidopsis. *Plant Physiol.*, **142**, 352–363 (2006).
24) Arimura, G.-I., Ozawa, R., Nishioka, T., Boland, W., Koch, T., Kühnemann, F., and Takabayashi, J., Herbivore-induced volatiles induce the emission of ethylene in neighboring lima bean plants. *Plant J.*, **29**, 87–98 (2002).
25) Mizusaki, S., Tanabe, Y., Noguchi, M., and Tamaki, E., *p*-Coumaroylputrescine, caffeoylputrescine and feruloylputrscine from callus tissue culture of *Nicotiana tabacum*. *Phytochemistry*, **10**, 1347–1350 (1971).
26) Buta, J. G., and Izac, R. R., Caffeoylputrescine in *Nicotiana tabacum*. *Phytochemistry*, **11**, 1188–1189 (1972).
27) Stoessl, A., and Unwin, C. H., The antifungal factors in barley. V. Antifungal activity of the hordatines. *Can. J. Bot.*, **48**, 465–470 (1970).
28) Mayama, S., Tani, T., Matsuura, Y., Ueno, T., and Fukami, H., The production of phytoalexins by oat (Avena sativa) in response to crown rust, *Puccinia coronata* f. sp. avenae. *Physiol. Plant Pathol.*, **19**, 217–226 (1981).
29) Miyagawa, H., Ishihara, A., Nishimoto, T., Ueno, T., and Mayama, S., Induction of avenanthramides in oat leaves inoculated with crown rust fungus, *Puccinia coronata* f. sp. avenae. *Biosci. Biotechnol. Biochem.*, **59**, 2305–2306 (1995).
30) Miyagawa, H., Ishihara, A., Lim, C.-H., Ueno, T., and Furuichi, N., Induction of N-p-coumaroyloctopamine in potato tuber disks by β-1,3-glucooligosaccharide. *J. Pest. Sci.*, **23**, 49–53 (1998).
31) Tebayashi, S.-I., Ishihara, A., Tsuda, M., and Iwamura, H., Induction of clovamide by jasmonic acid in red clover. *Phytochemistry*, **54**, 387–392 (2000).
32) Keinänen, M., Oldham, N. J., and Baldwin, I. T., Rapid HPLC screening of jasmonate-induced increases in tobacco alkaloids, phenolics, and diterpene glycosides in *Nicotiana attenuata*. *J. Agric. Food Chem.*, **49**, 3553–3558 (2001).
33) Tanaka, E., Tanaka, C., Mori, N., Kuwahara, Y., and Tsuda, M., Phenylpropanoid amides of serotonin accumulate in witches' broom diseased bamboo. *Phytochemistry*, **64**, 965–969 (2003).