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BRIEF COMMUNICATION

Induction of ageotropic response in lettuce radicle growth by epicuticular flavonoid aglycons of *Dittrichia viscosa*

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Abstract

Thirteen flavonoid aglycons, contained in the strongly allelopathic epicuticular exudates of *Dittrichia viscosa*, were investigated for their effects on lettuce seedling radicle growth. Concerning radicle length and mass, variable results were obtained, with most of the substances having no effect, some being inhibitory and some even promotive. Shoot mass was slightly reduced in four cases. Seed germination rates, root hair and lateral root formation were not affected either. Three of the compounds (namely quercetin 3,3-dimethylether, naringenin and eriodictyol) induced a strong ageotropic response in radicle growth.

Additional key words: allelopathy, eriodictyol, naringenin, quercetin 3,3-dimethylether.

Allelopathy is an interference mechanism through which plant secondary metabolites released from one plant exert an effect on other associated plants. These chemicals can be depleted from the leaves and dispersed to the soil by natural rain (Stephanou and Manetas 1997b), released from roots into the soil (Kato-Noguchi and Ino 2003), dispersed to the environment as volatile compounds, or added to the soil through litter decomposition (Reigosa *et al*. 1999).

Dittrichia viscosa (L.) W. Greuter [syn. *Inula viscosa* (L.) Aiton] is a Mediterranean ruderal aggressively occupying disturbed areas like roadsides and abandoned fields. Leaves and shoots of the plant bear abundant glandular hairs (Werker and Fahn 1981) excreting a complex, viscous mixture in which numerous sesquiterpene acids (Cecchereli *et al*. 1985) and flavonoid aglycones (Wollenweber *et al.* 1991) have been detected. This epicuticular exudate can be easily removed by washing the leaves or after natural or artificial rain (Stephanou and Manetas 1997a), giving the opportunity for an ecophysiological study of its function. Laboratory and field studies indicated a slight antitranspirant activity but no evidence for a UV-B radiation protective capability was obtained (Stephanou and Manetas 1995, 1997a). However, the exudate strongly inhibited seed germination of lettuce and *Malcomia maritima*, and retarded radicle growth in *Phlomis fruticosa*, the latter two species often found in the same habitat as *D. viscosa* (Stephanou and Manetas 1995, Levizou *et al*. 2002). The above results were evident in both filter paper and soil-based bioassays, but autotoxicity against the seeds of the mother plant was absent. An additional study on the seasonal variation in the concentration of epicuticular flavonoid aglycones in the leaves of *D. viscosa* revealed maxima during the dry summer and considerable drainage to the soil after the first heavy autumn rains, *i.e.* at the period of intensive germination of Mediterranean winter annuals (Stephanou and Manetas 1997b). Accordingly, an allelopathic effect on neighboring plants could be presumed on the basis of the above studies.

Apart from the suppression of seed germination and radicle growth, the crude epicuticular exudate from *D. viscosa* was recently shown to cause a variety of developmental perturbations in lettuce seedlings. Thus, the frequency of cell divisions in the root meristematic zone was reduced and the formation of root hairs was completely suppressed, but lateral roots were induced. In addition, statocytes in the root cap were rare and their

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polarity strongly perturbed, resulting in a considerable loss of the ability of primary roots to respond to gravity (Levizou *et al*. 2002). The multiplicity of the observed effects could be due to the complex nature of the epicuticular mixture and, accordingly, work with pure compounds is needed in order to approach the mode of action of *D. viscosa* epicuticular allelochemicals.

As a first step towards this goal, we investigated the effects of 13 flavonoid aglycones, similar to those excreted by *D. viscosa* glandular hairs (Wollenweber *et al*. 1991), on lettuce seed germination and seedling growth. Flavonoids have been frequently implicated in allelopathic reactions (Rice 1979), yet little is known for their mode of action (Berhow and Vaugh 1999).

Filter paper bioassays were performed in 6-cm sterile Petri dishes. The aglycon flavonoid compounds were dissolved in a small volume of pure methanol and evenly applied on the filter paper to give final concentrations of 0.3, 0.6 and 1.2 mM. After solvent evaporation, 1.5 cm^3 of distilled water were added. Seeds of *Lactuca sativa* (cv. Romana), were washed for 3 h in running tap water. This pretreatment afforded seeds devoid of fungal spores (personal observation). Germination took place in a growth chamber under irradiance of 35 μmol(PAR) m^{-2} s^{-1} (quantum sensor *LI-185*, *LI-COR*, Lincoln, USA), a temperature of 17 °C and photoperiod of 12 h. Twenty seeds were evenly spaced on filter paper in the dishes with 6 replications per compound and concentration. After 5 d, germination was completed and the experiment terminated. Loss of radicle orientation was quantified as the number of radicles that had lost contact with the surface of Petri dish (Levizou *et al*. 2002). Subsequently, the seedlings were layered on an appropriate dark background, scanned with a *Hewlett-Packard* scanner *Scanjet 6200C* (Houston, USA) and root length was measured with the *Image Tool 1.25* program. Then, the seedlings were divided into root and shoot and dried in an oven at 80 °C for 24 h for dry mass determination. Significance of differences between treatments and concentrations of active compounds were determined by applying a Student's *t*-test (*SPSS 9.01*, *SPSS Inc.*, Chicago, USA)

Pure flavonoid aglycons of *D. viscosa* were kindly donated by Prof. E. Wollenweber (6-methoxy-kaempferol, 6-methoxy-luteolin, kaempferol-7-methyl-ether, quercetin 3,3-dimethylether), Prof. E. Skaltsa (aromadendrin-7-methylether) or purchased from *Extrasynthase*, Genay Cedex, France (apigenin, eriodictyol, isorhamnetin, luteolin, narigenin, rhamnetin, taxifolin, quercetin dihydrate).

Some flavonoid aglycones had no effect on the measured parameters at the concentrations used (*i.e* luteolin, 6-methoxy-luteolin, aromadendrin-7-methyl-ether). When an effect was observed with the rest of flavonoids, a concentration-dependent response was generally lacking. For example, maximum suppression of root growth by rhamnetin occurred at 0.6 mM with less effect at 1.2 mM and no effect at 0.3 mM (Fig. 1). Variability related to the applied allelochemical concentration and zig-zag profiles of dose/response curves are rather common in allelopathy investigations (Reigosa *et al*. 1999). In a summary of the observed responses (Table 1) only statistically significant differences are presented, with the corresponding effective concentrations in parenthesis. The effects in root length were generally slight, with four compounds being inhibitory by up to 30 % and three being promotive by up to 40 %. The effects on root mass were slighter but equally variable, with three compounds being inhibitory and one promotive. Shoot mass was also slightly inhibited by four compounds.

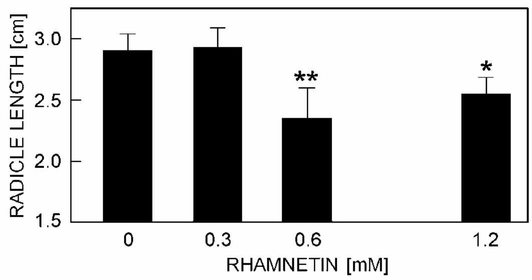

Fig. 1. Lettuce radicle length versus rhamnetin concentration. Data are means ± SD from six independent replications (Petri dishes) with 20 seeds per dish. The differences significant at $P < 0.05$ (*) and $P < 0.005$ (**).

Three of the compounds (*i.e*. eriodictyol, naringenin, quercetin 3,3-dimethylether) induced a strong ageotropic response with a high frequency of roots oriented towards the dish cap. The effect was particularly pronounced and concentration-dependent in the case of eriodictyol, where at a concentration of 1.2 mM more than 60 % of the radicles showed loss of normal gravitropic orientation.

Finally, the tested compounds had no effect on seed germination rate, formation of root hairs and induction of lateral roots.

It is evident from our results that the pure flavonoid aglycons contained in the epicuticular exudate of *D. viscosa* do not cause the multiplicity of effects obtained with the corresponding crude epicuticular preparations (Levizou *et al.* 2002). Thus, seed germination and formation of root hairs and lateral roots were not affected, while the effects on root elongation were subtle and variable. Work is needed with the rest of compounds found in *D. viscosa* epicuticular excretions (*i.e*. sesquiterpene acids, Ceccherelli *et al.* 1985) in order to assess a particular allelochemical effect to a specific compound. However, some of the tested flavonoids were strongly effective in causing a loss of orientation in lettuce radicles. Some of these compounds are known to act as polar auxin transport inhibitors altering normal auxin concentrations in roots and inducing ageotropic responses (Jacobs and Rubery 1988, Brunn *et al.* 1992). Since allelopathic flavonoids can be detected in soils associated with donor plants (Inderjit and Dakshini 1992), their alleged perturbation of gravitropic growth may suppress their ability for resource acquisition and change the competitive interactions with the donor plants.

Table 1. Effects of *D. viscosa* epicuticular flavonoid aglycons on growth and graviperception of lettuce seedlings [% change]. Effects were either positive (+), negative (-) or non significant (ns) at all three concentrations used. Ageotropic denotes the percentage of seedlings showing loss of normal root orientation in the presence of the corresponding allelochemical. No ageotropic response was observed in controls. The effective concentrations are shown when the responses were different. Data are means from six replications (Petri dishes) with 20 seeds per dish. The asterisks denote significant differences at $P < 0.05$ (*) and $P < 0.005$ (**).

Flavonoid	Conc. [mM]	Root length [%]	Root mass [%]	Shoot mass [%]	Ageotropic [%]
Apigenin	0.6	–13.89*	ns	ns	ns
Luteolin		ns	ns	ns	ns
6-Methoxy-luteolin		ns	ns	ns	ns
Kaempferol-7-methyl ether	0.6	ns	–12.47*	ns	ns
6-Methoxy-kaempferol	1.2	–29.99**	–21.82*	ns	ns
Quercetin dihydrate	0.6	+39.31**	ns	ns	ns
	1.2	+31.10*	+19.82*	–11.53*	ns
Rhamnetin	0.6	–18.97**	ns	–13.09*	ns
	1.2	–12.18*	ns	ns	ns
Isorhamnetin	0.6	–19.86*	ns	ns	ns
	1.2	ns	ns	–14.10*	ns
Quercetin 3,3-dimethyl ether	0.6	ns	ns	ns	+33.24*
	1.2	ns	ns	ns	+32.37*
Taxifolin	0.3	ns	–20.42*	ns	ns
	0.6	+28.53*	ns	–13.95*	ns
Naringenin	0.6	ns	ns	ns	+24.47**
Eriodictyol	0.3	+13.63	ns	ns	+43.29**
	0.6	ns	ns	ns	+50.18**
	1.2	ns	ns	ns	+62.16**
Aromadendrine-7-methylether		ns	ns	ns	ns

References

Berhow, M.A., Vaughn, S.F.: Higher plant flavonoids: biosynthesis and chemical ecology. - In: Inderjit, Dakshini, K.M.M., Foy, C.L (ed.): Principles and Practices in Plant Ecology: Allelochemical Interactions. Pp. 423-438. CRC Press, Boca Raton 1999.

Brunn, S.A., Muday, G.K., Haworth, P.: Auxin transport and the interaction of phytotropins. - Plant Physiol. **98**: 101-107, 1992.

Ceccherelli, P., Curini, M., Marcotullio, M.C., Menghini, A.: Sesquiterpene acids from *Dittrichia viscosa*. - Phytochemistry **24**: 2987-2989, 1985.

Inderjit, Dakshini, K.M.M.: Formononetin 7-O-glucoside (ononin), an additional growth inhibitor in soils associated with the weed *Plucea lanceolata* (DC) C.B. Clarke (Asteraceae). - J. chem. Ecol. **18**: 713-718, 1992.

Jacobs, M., Rubery, P.H.: Naturally occurring auxin transport regulators. - Science **241**: 346-349, 1988.

Kato-Noguchi, H., Ino, T.: Rice seedlings release allelopathic substances. - Biol. Plant. **46**: 157-159, 2003.

Levizou, E., Karageorgou, P., Psaras, G.K., Manetas, Y.: Inhibitory effects of water soluble leaf leachates from *Dittrichia viscosa* on lettuce root growth, statocyte development and graviperception. - Flora **197**: 152-157, 2002.

Reigosa, M.J., Sanchez-Moreiras, A., Gonzalez, L.: Eco-physiological approach in allelopathy. - Crit. Rev. Plant Sci. **18**: 577-608, 1999.

Rice, E.L.: Allelopathy - an update. - Bot. Rev. **45**: 15-109, 1979.

Stephanou, M., Manetas, Y.: Allelopathic and water conserving functions of leaf epicuticular exudates in the Mediterranean shrub *Dittrichia viscosa*. - Aust. J. Plant Physiol. **22**: 755-759, 1995.

Stephanou, M., Manetas, Y.: Ultraviolet-B radiation effects on the Mediterranean ruderal *Dittrichia viscosa*. - Plant Ecol. **128**: 109-112, 1997a.

Stephanou, M., Manetas, Y.: Seasonal variations in UV-B absorbing capacity and allelopathic potential of *Dittrichia viscosa*. - Can. J. Bot. **75**: 1371-1374, 1997b.

Werker, E., Fahn, A.: Secretory hairs of *Inula viscosa* (L.) Aiton – development, ultrastructure and secretion. - Bot. Gaz. **142**: 461-476, 1981.

Wollenweber, E., Mayer, K., Roitman, J.N.: Exudate flavonoids of *Inula viscosa*. - Phytochemistry **30**: 2445-2446, 1991.