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A calorimetric study of the allelopathic effect of cnicin isolated from *Centaurea diffusa* Lam. on the germination of soybean (*Glicine max*) and radish (*Raphanus sativus)*

Fanny I. Schabes, E. Elizabeth Sigstad *

Instituto de Química Orgánica, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471, CP T 4000 INI, Tucumán, Argentina

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Abstract

This work concerns the application of isothermal calorimetry to measure the effect of 'cnicin' on the germination of the soybean (*Glycine max* (L.) Merr., cv. A7636 RG) and the radish (*Raphanus sativus* L., cv. Sparkler). The sesquiterpenelactone, cnicin was isolated from a highly invasive plant, the diffuse knapweed *Centaurea diffusa* Lam. Calorimetric experiments were performed with seeds on wetted filter paper disks or in agar, both containing varying concentrations of cnicin. Results indicate that this substance blocks the water uptake by roots inhibiting subsequent seedling growth but has no effect during germination.
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Keywords: Isothermal calorimetry; Allelopathy; *Centaurea diffusa*; Cnicin; *Glycine max*; *Raphanus sativus*

1. Introduction

Many chemical substances derived from plants seem to be potentially selective for weed control in certain conventional cultivation systems or of minimum tillage. They can be used as models for a type of totally new biorational synthetic herbicides. Allelochemicals – a class of biologically synthesized chemicals used by terrestrial plants in their fight against insect herbivores – are also important in plant–plant interactions (allelopathy). Thus there is an increasing interest in using this type of substances to control pests and weeds because they are considered environmentally more benign and secure; due to their mode of action, they are more specific in general [1–4].

Centaurea diffusa Lam., the diffuse knapweed, is a weed belonging to the Asteraceae family, recently introduced in Argentina and much earlier in North America from its origin in East Europe/Asia Minor. It contains a high concentration of a sesquiterpenelactone, called cnicin. This allelopathic compound acts more on the roots of other plants than on their stems or leaves and suppresses the growth of nearby native species, apparently in a competition for water and nutrients. Knapweed caused great economical damage in the US during the 1980's due to its allelopathic effects on cultivated species [5,6]. It is well known nowadays that non-native plants constitute a significant threat to native species and natural communities [7]. Therefore, is of great interest to elucidate the mechanisms of action that these species use to invade a field.

Preliminary studies with solutions of 500 and 1000 mg dm^{-3} of a chloroform extract of *C. diffusa* indicated such an effect on germinating quinoa seeds (*Chenopodium quinoa* Willd.). The seedling length and mitotic index were found to be reduced compared with the control at 24 h [8]. Moreover, the honeyweed *Leonurus sibiricus* experienced reduced germination (40%) and seedling length (93%) when exposed to a 1000 mg dm^{-3} solution [9]. As the *exo*-methylene-γ-lactone cnicin, isolated from the $CHCl_3$ extract of the aerial parts of *C. diffusa* [10], is the major metabolite, it was interesting to see if this sesquiterpenelactone had any effect on the germination of other species, as well as to elucidate its mechanism of action. In this sense, the germination of soybean (*Glycine max* (L.) Merr., cv. A7636 RG) and radish (*Raphanus sativus* L., cv. Sparkler), both dicotyledonous species, was studied using isothermal calorimetry.

* Corresponding author at: Paraguay 668, CP T 4107 DDN, Yerba Buena, Tucumán, Argentina. Tel.: +54 381 4247752x7070; fax: +54 381 4248169.
E-mail addresses: esigstad@arnet.com.ar, lizzie@fbqf.unt.edu.ar (E.E. Sigstad).

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2. Experimental

2.1. Plant material

Seeds of radish (*R. sativus,* L. cv. Sparkler) were obtained from the local market and seeds of soybean (*Glycine max* (L.) Merr, cv. A7636 RG) were obtained from the Agro-Industrial Experimental Station 'Obispo Colombres', in Tucumán. The mean weights per seed were 19.3 ± 3.8 mg or 144.8 ± 37.8 mg for radish (two seeds) or soybean (one seed), respectively; their water content was $5.7 \pm 0.1\%$ and $11.0 \pm 0.2\%$, respectively, as determined by drying at 95 °C until constant weight. The sesquiterpenelactone cnicin was obtained from the Natural Products group of the Organic Chemistry Institute of the Faculty of Biochemistry, Chemistry and Pharmacy, National University of Tucuman, Argentina. The isolation and structure of cnicin have been reported elsewhere [10].

2.2. Preparation of cnicin stock solution

Twenty-five milligrams of cnicin were dissolved in 2.5 cm^3 DMSO and diluted to 25 cm^3 with deionised water. Aliquots were taken to produce 10, 50 and 100 mg dm^{-3} solutions of cnicin containing 1% DMSO and used in the germination experiments on filter paper disks. For experiments with agar, 25 mg cnicin were dissolved in 2.5 cm^3 DMSO from which aliquots were taken to produce agars containing 10 and 100 mg dm^{-3} cnicin and 1% DMSO.

2.3. Calorimetric determinations

A twin heat conduction calorimeter (a non-commercial isothermal-type instrument built at Lund University, Sweden, 25 °C, 8 cm^3 vessel, 7 cm^3 headspace, at least 10 experiments per treatment) was applied to these studies as described elsewhere [11–13]. One (1) seed of soybean or two (2) seeds of radish were used for each calorimetric curve. In a previous work we reported that the best conditions to germinate seeds in a calorimeter were with a solid medium of 1% agar for imbibition [12]. It was also stated that this setting was convenient to control the concentration of a metabolic effector. To confirm that conclusion, soybean seeds were germinated by either using filter paper disks (treatment 1, T1) or 1 cm^3 of 1% agar (T2) in this work. For T1, the seed was placed on a filter paper disk wetted with 0.2 cm^3 of 10, 50 or 100 mg dm^{-3} solution of cnicin and a further addition of 0.2 cm^3 after 9–10 h of imbibition. For T2, the seed was inserted into 1 cm^3 of 1% agar containing 100 mg dm^{-3} of cnicin. Radish seeds with their flat shape were placed over the agar layer containing 10 and 100 mg dm^{-3} cnicin. Control experiments were performed with either water or agar containing 1% DMSO. When agar was used, the ampoule was removed from the calorimeter after 9–10 h, opened, covered with polyethylene, left for 5 min to exchange gases with the surrounding atmosphere and returned to the calorimeter. After thermal equilibration for 30 min, the power (p)–time (t) curves of seed germination were recorded and further processed as reported elsewhere [12].

Table 1
Mean (±S.D.) values of specific enthalpy of imbibition, $\Delta_i h$, time of germination, Δt_g, and specific enthalpy of germination, $\Delta_g h$, as determined for soybean (S) and radish (R) seeds under different concentrations of cnicin

Seed/cnicin (mg dm^{-3})	$-\Delta_i h$ (J g^{-1})	Δt_g (h)	$-\Delta_g h$ (J g^{-1})
Soybean seeds			
S/0	29.2 ± 4.6	18.8 ± 3.7	81.9 ± 21.2
S/10	31.7 ± 4.7	22.9 ± 3.3	93.4 ± 20.1
S/50	31.0 ± 6.3	22.9 ± 3.7	117.4 ± 24.2[a]
S/100	29.9 ± 4.3	28.3 ± 3.5[a]	116.4 ± 10.9[a]
S/Agar/0	27.62 ± 3.3	20.1 ± 2.6	80.6 ± 12.5
S/Agar/100	23.3 ± 7.1	21.1 ± 7.0	65.2 ± 8.1[a]
Radish seeds			
R/Agar/0	57.3 ± 6.8	30.6 ± 6.2	252.1 ± 111.6
R/Agar/10	54.4 ± 14.6	29.9 ± 5.5	178.8 ± 88.0
R/Agar/100	64.3 ± 8.7	29.6 ± 5.1	146.1 ± 22.5[a]

[a] Significant differences against the control ($p < 0.05$).

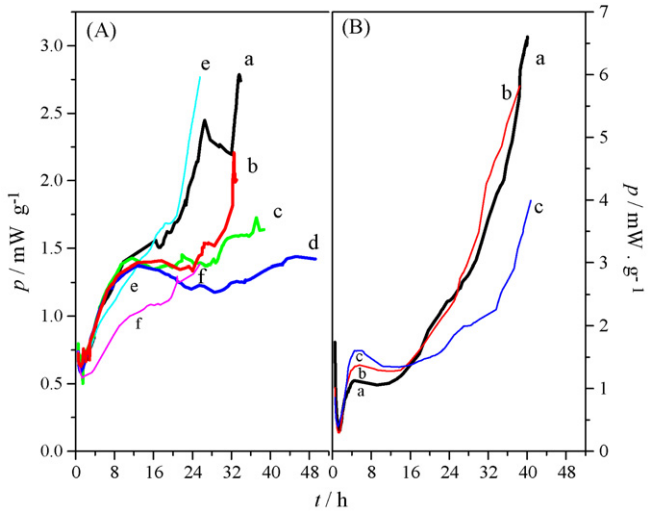

Fig. 1. The average specific thermal power (p)–time (t) curves of germination for (A) soybean seeds on filter paper disks wetted with 0.2 cm^3 and a further addition of 0.2 cm^3 after 9–10 h of: (a) distilled water; (b) 10, (c) 50 and (d) 100 mg dm^{-3} cnicin or inserted in 1 ml of 1% agar containing (e) distilled water or (f) 100 mg dm^{-3} cnicin and (B) radish seeds in 1 ml of 1% agar containing (a) distilled water, (b) 10 and (c) 100 mg dm^{-3} cnicin.

The enthalpy values shown in Table 1 were determined from the initial parts of the p–t curves in Fig. 1 as the area under the calorimetric curve for the indicated time period.

2.4. Germination in closed Petri dishes

Soybean seeds (10 × 3) were placed in Petri dishes (90 mm diameter) at 25 °C to germinate on filter paper disks wetted with 5 cm^3 water containing 1% DMSO (control) or 10, 50 and 100 mg dm^{-3} cnicin solutions with 1% DMSO. The percentage of germinated seeds, G (determined from the 30 seeds used for each treatment), root length, Lr, and seedling water content, WC, were determined at 72 h.

3. Results and discussion

The calorimetric experiments on germination had 10 replicates for each treatment and each concentration. Representative

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