International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 2 (4): 397-401, 2007
ISSN 1816-4897

Aspects of the Allelopathic Potential of Horseweed (*Conyza albida*)

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Abstract: The allelopathic effects of three plant tissues of *Conyza albida* (stems, leaves and inflorescences) on oat growth were further investigated using *in vivo* tests. Oat growth (fresh and dry weights of above and underground parts) was significantly inhibited from phytotoxic activity of upper leaves and inflorescence tissues of *C. albida* in pot experiments. The inhibition was significantly higher than in the case of stems. The inhibiting action of crude extracts and volatile compounds from young plants (rosette) were examined using two bioassay methods: (a) seed germination and radicle growth of oat and (b) fresh weight of duckweed plants. Both bioassayed species exhibited great phytotoxic response from the young plants, collected in winter, confirming the results of previous studies.

Key words: *Conyza albida*, horseweed,allelopathy, bioassay, oat, duckweed

INTRODUCTION

Horseweed (*Conyza albida* Willd. ex Sprengel) is an annual-biennial herb originating from South America. It is a widespread species found in many parts of the world. In Greece it is an well known species growing mainly in urban habitats and known since 1976. According to our observations it also presents a vigorous growth causing it to become a persistent weed problem in vineyards and orchards and recently vegetable gardens in many parts of Greece. It is a difficult noxious weed to control because it produces dense stands and can tolerate a variety of habitats and environmental conditions (Economou *et al*., 2002). Despite its invasiveness, the biology and ecology of this species is poorly documented (Thebaud and Abbott, 1995).

Allelopathy is one of the predominant forces in the development of plant communities and spatial patterns therein (Rice, 1984). Very few studies reported to date have assessed the allelopathic potential of *C. albida* (Economou *et al*., 2002). However, studies on species of the related genus *Erigeron* showed that cisdehydromatricaria ester and cis- and trans-matricaria esters were released (Putnam, 1988). These C_{10} polyacetylenes were discovered in soils in concentrations inhibitory to test plants and are probably allelopathic substances of ecological importance. Indeed, the potential for undesirable environmental contamination from herbicides is relatively high and these create a need for environmentally safe herbicides that are equally or more effective and selective than currently available synthetic herbicides (Putnam *et al*., 1983).

This study reports a preliminary investigation into the allelochemical characteristics of *C. albida*, supplementary of the study of Economou *et al*. (2002). The objective of this study was to evaluate the allelopathic activity: (a) of different horseweed tissues on oat seedling growth (b) of plant extracts and estimate the dose-response of oat radicle growth to varying concentrations and (c) to access the inhibitory action of volatile compounds on growth of oat radicle and duckweed fresh weight.

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MATERIALS AND METHODS

Pot Experiment

Plant material was collected from a natural population established in the Benaki Phytopathological Institute field in Kifisia, a suburb of Athens, at the reproductive stage during (1998, 1999). For the purposes of allelopathic experiments three tissue types were used: (i) inflorescences (ii) leaves and (iii) stems. Bioassay experiments were used to determine the inhibitory potential of each of these tissues on oat above- and underground growth in glasshouse pot studies. Oat (*Avena sativa*) was included in this study since it has been used extensively in allelopathy research as the receiver plant to test compounds released by a donor plant. Oat seeds germinate evenly, resulting in a uniform and rapid plant growth that enables qualification of biological response in plants. In addition, oat biotest is considered as a sensitive and easily facilitated method (Rice, 1984). Six seeds of oat were placed and grown in 10 cm diametric plastic pots containing 20 g freeze tissues per 200 g of vermiculite. The seeds were placed 2 cm deep in 20 test pots. The pots were watered daily with equal volumes of deionised water. All pots were watered to maintain adequate moisture and artificial light was supplied. Temperatures ranged from 18 to 25°C and daylength averaged 15 h. The upper and underground growth of oat were measured after two weeks and used as an index of allelochemical activity. Seedlings were collected two weeks after planting and the average shoot fresh and dry weight per pot was determined. The experimental design was a randomized block with four replicates for each treatment and control.

Plant Extracts Bioassay

The phytotoxicity of plant extracts was quantified with an *Avena sativa* seed bioassay. The plant material was collected from the University of Athens Campus at the rosette (February) stage during 1998. The air-dried aerial parts were cut into small pieces and extracted successively with methanol. Aqueous dilutions of the dried residues of crude extracts were bioassayed on filter paper in plastic Petri plates and the two most effective dilutions were identified and further investigated. Six oat seeds were placed onto two layers of 9 cm filter paper in Petri plates treated with 3 mL of test solution, exposed to vapour the methanol, wetted with 6 mL of distilled water, covered and incubated at 25°C in the dark.

The experimental design was a randomized block with four replicate plates for each treatment. Control solutions were prepared using mannitol-water adjusted to correspond to the osmotic potentials of the different extracts (Bell, 1974). Inhibitory concentrations were calculated after 7 days and used as an index of allelochemical activity. An analysis was conducted according to Finney (1962). A 5 mm radicle length was considered germinated. Growth was quantified by measuring the radicle length of germinated oat.

Allelopathic Characteristics of Volatile Compounds

The volatile compounds from the two most effective oil fractions of the fresh aerial parts of the above sample (rosette) were obtained by steam distillation for 3 h using a modified Clevenger apparatus (Hellenic Pharmacopoeia, 1989). The oils were dispersed as an emulsion in water using Tween 20. Seven concentrations were used. Dilutions were made with distilled water. Oat seeds were placed on filter paper in conical tapped vials (six seeds per vial) and were soaked with 3 mL of the tested water dilutions. The vials were wetted with distilled water and incubated in darkness at 25°C. Radicle elongation of oat was measured at 7 days, using the same approach and that outlined above for the germination bioassay with corresponding controls. Seeds that did not germinate were considered to have a radicle length at 0 mm. The experiment was repeated four times as described previously. Data were expressed as a percentage of radicle elongation in control vials. The dose needed to inhibit oat

radicle growth to 50% of control radicle growth (hereafter called the I_{50} value) was determined from dose-response bioassays (Finney, 1962; 1978). For all the measurements SPSS software was used (SPSS, 1997).

The same dilutions from the two oils were tested via another bioassay using as test plant a species of duckweed (*Spirodella polyrhiza* L.) and measuring the decrease of its fresh weight. This plant indicator has been used in several allelopathic studies, since the bioassay is sensitive and reliable especially at the first steps of a screening procedure. Moreover, duckweed species are highly sensitive to chemicals that inhibit the function of Photosystem II and their response by chlorosis is readily measurable through the drastic decrease in their fresh weight (Leather and Einhellig, 1985; Olofsdotter *et al*., 1995).

RESULTS AND DISCUSSION

Pot Experiment

The response of oat bioassay to the horseweed (*Conyza albida*) debris varied among the three types. The leaves and inflorescences showed a higher phytotoxic effect on the oat growth. The fresh and dry weight accumulation was significantly inhibited, more than the stems, when they were incorporated on the vermiculite surface (Table 1). Fresh and dry weights of above and underground oat parts were significantly inhibited from phytotoxins derived from horseweed leaves, such as in the measurements of Economou *et al*. (2002). Strongly inhibitory substances were released from horseweed debris derived from inflorescence tissues, too. The biological activity of compounds derived of *Conyza* species flowers has been also reported elsewhere (Peterson *et al*., 1989). The same plant tissues caused a greater inhibitory effect on underground oat growth reducing its biomass to a significant degree. These findings are in accordance with those of Putnam and Duke (1978), Rice (1984) and Economou *et al*. (2002) whose studies showed that quantities of allelochemicals within plants vary with plant tissue. Allelochemicals may be synthesized and stored in other tissues and then transported into new leaves and inflorescence. Alternatively, it may indicate transport of allelochemicals from root via phloem of the inner bark to the developing leaves (Heisey, 1990). Furthermore, as the radicles of newly germinated seeds are very susceptible to phytotoxins, it is possible that the wide distribution of *C. albida* is due to allelopathic potential of the species.

Plants Extract Bioassays

The tissue debris of horseweed was found to have an inhibitory effect on the oat growth bioassay and the crude plant extracts also demonstrated inhibition in oat seedling growth. In Table 2, it is shown the inhibitory effects of the two most effective samples (Ca2 and Ca7) on oat growth. There is a strong inhibition response of the two samples, as the I_{50} estimates (189 and 96 μg mL^{-1}) were about two to three times lower than the average I_{50} estimate of the rosette stage (324 μg mL^{-1}) and three to five times lower than the average I_{50} estimate (524 μg mL^{-1}) derived from the samples at the mature stage (Economou *et al*., 2002). The strong inhibitory action of Sample Ca7 was true even from the concentration of 153 μg mL^{-1}, as long as the oat radicle length was 73 % lower than the control.

Table 1: Response of oat growth to vermiculite-incorporated plant tissues of *Conyza albida* debris

	Above ground		Underground	
Plant tissue	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
Inflorescences	77.51c	28.24b	15.36c	11.78b
Leaves	154.82c	66.15b	31.88c	18.67b
Stems	273.12b	82.78b	134.67b	38.22b
Control	652.08a	174.56a	322.45a	84.23a

Means followed by the same letter(s) within a row are not significantly different at p = 0.05 Fisher's least significant difference test

Table 2: Response of oat radicle elongation to the allelopathic component from *Conyza albida* most effective crude extracts

Sample Ca2		Sample Ca7	
Concentration (μg mL^{-1})	Radicle length (cm)	Concentration (μg mL^{-1})	Radicle length (cm)
Control	11.40 (0)	Control	10.51 (0)
36	10.93 (4)	38	8.30 (21)
72	10.50 (28)	76	6.27 (40)
144	9.55 (16)	153	2.85 (73)
288	2.67 (77)	306	1.98 (81)
575	0.99 (91)	612	0 (100)
1150	0 (100)	1224	0 (100)
2300	0 (100)	2448	0 (100)
I_{50} = 189 μg mL^{-1}		I_{50} = 96 μg mL^{-1}	

Numbers in parentheses indicate percent inhibition.

Table 3: Response of oat radicle elongation and duckweed fresh weight to the most effective volatile compounds obtained from *Conyza albida* tissues in vegetative stage (rosette)

	Cawoil8			Cawoil10	
Concentration (μg mL^{-1})	Radicle length (cm)	Duckweed fresh weight (mg)	Concentration (μg mL^{-1})	Radicle length (cm)	Duckweed fresh weight (mg)
Control	3.90 (0)	40 (0)	Control	6.18 (0)	40 (0)
38	2.46 (37)	40 (0)	34	1.00 (84)	20 (50)
75	0 (100)	20 (50)	69	0 (100)	20 (50)
150	0 (100)	20 (50)	138	0.90 (85)	20 (50)
300	0 (100)	30 (25)	275	0 (100)	30 (25)
600	0 (100)	30 (25)	550	0 (100)	30 (25)
1200	0 (100)	30 (25)	1100	0 (100)	20 (50)
2400	0 (100)	30 (25)	2200	0 (100)	20 (50)
I_{50} = 47 μg mL^{-1}			I_{50} = 26 μg mL^{-1}		

Numbers in parentheses indicate percent inhibition

Even if Sample Ca7 was significantly more effective than Ca2, for both samples, radicle elongation was reduced with increasing extract concentrations (Table 2). Indeed, it is well known that the magnitude of phytotoxic activity is dependent upon the concentration and chemical stability of the active compounds (Einhellig, 1986).

Allelopathic Characteristics Of Volatile Compounds

Placing emulsions of the oils obtained from the potted vial with germinating oat seeds resulted in a considerable decrease in seedling growth compared to the control seedling. The most effective samples (Cawoil8 and Cawoil10) were identified and isolated (Table 3). The same results are in accordance with the duckweed bioassay, as long as the emulsions of the oils obtained were tested for its influences on the duckweed fresh weight as well. The oil obtained from the most effective winter samples of our study caused a strong inhibition response, as the I_{50} estimates (47 and 26 μg mL^{-1}) were more than five times lower than the I_{50} estimate (316 μg mL^{-1}) derived from the samples at the mature stage (Economou *et al*., 2002). It is also noticeable that only 69 μg mL^{-1} of the most effective oil sample of the rosette stage (Cawoil10) could totally inhibit oat growth (100 %), while the corresponding value reported by Economou *et al*. (2002) for the mature stage of *C. albida* was 3166 μg mL^{-1} (Table 3).

CONCLUSIONS

Present results support the assumption of Economou *et al*. (2002) that phytotoxicity from horseweed in the natural ecosystems and agroecosystems could occur from compounds that escape from the plant by volatility. Nowadays, allelopathy is important in research involving sustainable

agriculture, also referred to as organic, low input, biodynamic or resource concerning. The allelopathic plant products are known to offer a vast array of secondary compounds which have the potential role of use directly as herbicide substitute or as structural leads for new synthetic herbicides. In order to try for a sustainable agriculture, the need for new herbicides is inevitable and the contribution of allelopathic plants on that could be very important.

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