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Inhibitive effects of barley (*Hordeum vulgare*) on germination and growth of seedling quack grass (*Agropyrum repens*)

Zoheir Y Ashrafi
Sedigheh Sadeghi
AND
Hamid R Mashhadi

Weed Science, Department of Weed Science, Faculty of Agronomy and Animal Science, University College of Agricultural and Natural Resource, Karaj Paradaise, University of Tehran, Tehran State, Iran
E-mail: z.y.ashrafi@gmail.com (corresponding author)
E-mail: hrmashhadi@ut.ac.ir
E-mail: sedsadeghi@ ut.ac.ir

ABSTRACT

Allelopathy is defined as the direct or indirect harmful or beneficial effects of one plant on another through the release of chemical compounds into the environment. Barley (*Hordeum vulgare*) contains water soluble allelochemicals that inhibit the germination and growth of other species. This characteristic could be used in weed management programmes. Greenhouse and laboratory experiments were conducted to determine (i) the effects of preceding crops, on quack grass (*Agropyrum repens*) germination and seedling growth (ii) and the effects of fresh barley residue incorporation, and (iii) barley leaf, stem, flower and root water extract concentrations on quack grass. Growth of quack grass, as indicated by plant height and weight, was significantly reduced when grown in soil previously cropped to barley compared with that cropped to quack grass. Soil incorporation of fresh barley roots and both roots and shoots reduced quack grass germination, plant height and weight when compared with a no-residue control. In bioassays, barley extracts reduced quack grass hypocotyl length, hypocotyl weight, radicle weight, seed germination, and radicle length by as much as 44, 57, 61, 68 and 79 %, respectively, when compared with water controls. Increasing the water extract concentrations from 4 to 20 g per 100 ml of water of all barley parts significantly increased the inhibition of quack grass germination, seedling length and weight. Based on 8-day-old quack grass radicle length, averaged across all extract concentrations, the degree of toxicity of different barley plant parts can be ranked in the following order of inhibition: leaves > flowers > mixture of all plant parts > stems > roots.

Keywords: allelopathy, barley, *Hordeum vulgare*, quack grass, *Agropyrum repens*, water extracts, inhibition, germination

YFIRLIT

Bygg (Hordeum vulgare) hindrar spírun og vöxt fræplantna af húsapunti (Agropyrum repens)

Allelopathy (návörn) er skilgreind sem bein eða óbein jákvæð eða neikvæð áhrif af einni plöntu á aðra með efnasamböndum sem plönturnar sleppa út í umhverfið. Bygg (*Hordeum vulgare*) inniheldur vatnsleysanleg

efnasambönd sem hindra spírun og vöxt annarra tegunda. Þennan eiginleika má nota í baráttunni við illgresi. Tilraunir voru gerðar í gróðurhúsi og á rannsóknastofum til að mæla áhrif á spírun og vöxt fræplantna af húsapunti (*Agropyrum repens*) af (i) gróðri síðasta árs, (ii) ferskum byggafgöngum og (iii) bygglaufi, stönglum, blómum og rótarsafaþykkni. Vöxtur húsapuntsins, mælt sem hæð og þungi, minnkaði raunhæft þegar hann óx í jarðvegi þar sem áður var ræktað bygg samanborið við jarðveg þar sem húsapuntur hafði vaxið. Í jarðvegi með ferskum byggrótum og stönglum dró úr spírun, hæð og þunga húsapunts samanborið við jarðveg án byggafganga. Í lívirkniprófun (bioassay) dró byggsafi úr lengd (44%) og þunga kímstönguls (57%), þunga (61%) og lengd kímrótar (79%) og spírun (68%) hjá húsapunti samanborið við vatnsviðmið. Ef styrkleiki vatnssafa af öllum bygghlutum var aukinn úr 4 í 20 g í 100 ml vatns uxu neikvæðu áhrifin á spírun og lengd og þunga kímplöntur húsapuntsins raunhæft. Miðað við lengd kímrótar húsapunts eftir 8 daga vöxt og meðaltal allra styrkleika má raða eitrunaráhrifum bygghluta þannig: laufblöð > blóm > blanda allra plöntuhluta > stönglar > rætur.

INTRODUCTION

Allelopathy is defined as the direct or indirect harmful or beneficial effects of one plant on another through the release of chemical compounds into the environment (Rice 1984). Several phytotoxic substances causing germination and/or growth inhibitions have been isolated from plant tissues and soils. These substances, collectively known as allelochemicals, are usually secondary plant products or waste products of the main metabolic pathways of plants (Whittaker & Feeny 1977, Hall & Henderlong 1989, Chon & Kim 2002, Ashrafi et al. 2007). Barley (*Hordeum vulgare* (L.) Koch.) is well known for its allelopathic compounds. Several phenols and terpenes have been reported in various cultivars of barley (Spring et al. 1992, Macias et al. 2002, Ben-Hammouda et al. 2001, Ashrafi et al. 2008) and it is reported that this same cultivar of barley was autotoxic to other cultivars of barley, though not to itself. Leaves were the most important source of allelopathic substances. This same cultivar of barley was also found to be phytotoxic to durum wheat (*Triticum durum* Desf.) and bread wheat (*T. aestivum L.*). Seedling growth bioassays demonstrated that the two wheat species responded differently to the allelopathic potential of barley, with a greater sensitivity shown by the bread wheats. For both wheat species, radicle growth was more depressed than coleoptile growth, though stimulation of seedling growth was observed for durum wheat. Leaves and roots were the most phytotoxic barley plant parts for durum and bread wheats, respectively. Results suggested that the response by durum wheat and bread wheat varied depending on the source of allelochemicals (i.e. plant part) and the growth stage of the barley plant. Consequently, barley should be considered a depressive prior crop for both durum wheat and bread wheat in a field cropping sequence. However, studies with other species have reported that the response to allelochemicals may be dependent on concentration. Allelochemicals that inhibit the growth of some species at certain concentrations might in fact stimulate the growth of the same or different species at different concentrations (Narwal 1994). It is thus essential to identify concentrations at which each specific response occurs if allelopathic interactions are to be used in weed management programmes. In addition, various plant parts may vary in their allelopathic potential (Chon & Kim 2002, Economou et al. 2002, Ashrafi et al. 2007). Information about the allelopathic potential of the flora of Mediterranean regions remains scarce. The present study was conducted to determine the allelopathic potential of barley towards quack grass (*Agropyrum repens* (L.) Beauv.), a problematic weed in Mediterranean regions. The objectives were to determine the effects of (i) preceding crops on germination and seedling growth of quack grass, (ii) fresh barley residue incorporation on early growth of quack grass, and (iii) the effects of water extract concentration of various barley parts on quack grass seed germination and seedling growth.

MATERIALS AND METHODS

Greenhouse experiments

Effects of preceding crops

The effects of preceding crops were studied by growing barley and quack grass in soils from fields in central Iran (Tehran State) cropped in the previous season with either species, to assess the existence of long-term allelopathicity of barley. Ten quack grass seeds were planted in pots (150 mm wide and 150 mm high), each containing soil (loam) from adjacent fields previously cropped either to quack grass (quack grass soil) or barley (barley soil). Each treatment, quack grass grown in quack grass soil and quack grass grown in barley soil, was replicated eight times and arranged in a completely randomized design. A similar experiment was performed with barley, planting five seeds per replicate pot. Plants were grown at constant temperature (26ºC) with a 16-h light 8-h dark cycle for 35 days. At the end of the growth period, germination percentage, plant height and fresh weight were recorded.

Effects of fresh residue incorporation

The effects of incorporating fresh barley or quack grass whole plants or roots only on quack grass were studied to test for the existence of short-term barley allelopathicity. Treatments were designed in a 2×3 factorial assigned to a randomized complete block design with four replications. Treatment combinations included source of residues (barley or quack grass) and type of residues incorporated (whole plants, roots only) or no residue (control). Ten barley or quack grass plants were grown for 30 days in pots (170×165 mm) kept under greenhouse conditions. At the end of this period, whole plants or roots only were mixed into the soil in situ. Control treatments contained only soil. Four days after incorporation, 10 quack grass seeds were planted in each pot, including control pots. Germination, plant height and dry weight were recorded 30 days after planting.

Laboratory experiments

Preparation of extracts

Barley plants were collected from fields in central Iran (Tehran State) during the 2006–2007 growing season. Fresh barley plants were separated into leaves, stems, roots and flowers. Tissues from each plant part were soaked in distilled water for 24 h at 25ºC in a lighted room to give concentrations of 4, 8, 12, 16, and 20 g of tissue per 100 ml of water.

After soaking, solutions were filtered through four layers of cheesecloth and the filtrate was then centrifuged (1500 g) for 4 h. The supernatant was filtered again using a 0.2 mm Filter ware unit to give the final water extract. Ten-millilitre aliquots from each plant part extract were mixed together to constitute whole-plant extracts.

Seed bioassays

A hundred quack grass seeds were surface sterilized with water:bleach solution (10:1) and were placed evenly on filter paper in sterilized 9 cm Petri dishes. Ten millilitres of extract solution from each plant part was added to the Petri dishes and distilled water was used as a control. All Petri dishes were placed in a lighted room at 25ºC. Treatments (extracts from the various plant parts and the distilled water control) were arranged in a completely randomized design with four replications. After 7 days, germination was determined by counting the number of germinated seeds and expressed as total percentage. Radicle and hypocotyl lengths were determined after 78 days by measuring 24 representative seedlings. After measuring the radicle and hypocotyl lengths, the seedlings were separated into hypocotyl and radicle parts. The plants were then dried and their respective dry weights recorded.

Water uptake by seeds

One-gram samples of quack grass seeds were soaked for 4, 8, 12 and 16 h in barley leaf water extracts at concentrations of 4, 8, 12, 16 and 20 g per 100 ml of water. Distilled water was used as the control. Treatments were arranged

in a completely randomized design with four replications. After soaking, seeds were taken from the solution, blotted for 2 h and weighed. Wat-er uptake was calculated by subtracting the original seed weight from the final seed weight and expressed in millilitres.

Statistical analyses

All experiments were repeated twice and the pooled mean values were separated using least significant differences (LSD) at the 0.05 probability level following an analysis of variance, except for the experiment investigating the effects of preceding crops, for which t-tests were used. Statistical analyses were made with the MSTAT statistical program (Michigan State University, East Lansing, MI).

RESULTS AND DISCUSSION

Greenhouse experiments

Effects of preceding crop

Growth of barley, as indicated by the plant height and fresh weight of plants grown for 35 days, was significantly reduced in soil previously cropped to barley compared with that cropped to quack grass (Table 1). However, the preceding crop did not affect barley germination. In the case of quack grass, differences in germination percentage, plant height and fresh weight per plant caused by preceding crops were all significant. All variables were significantly lower when the preceding crop was barley than when it was quack grass. These results suggest that barley has a longterm potential to reduce the growth of plants from other (i.e. allelopathicity) or the same species (i.e. autotoxicity). Other species, e.g. alfalfa (*Medicago sativa* L.), have both allelopathic and autotoxic potentials (Chon & Kim 2002).

Effects of residue incorporation

Quack grass germination percentage, plant height and dry weight of plants grown for 35 days were all significantly lower with fresh barley or quack grass residue incorporation than the controls, suggesting the presence of short-term allelopathic and autotoxic effects (Table 2). However, germination and growth inhibition of quack grass were 16–28% greater with barley than with quack grass incorporation. Allelopathicity and autotoxicity were also greater when whole plants were incorporated than when roots only were incorporated. This response could be attributed to a greater contribution of allelochemicals from leaves or simply to the greater amount of residues incorporated with whole plants.

Table 1. Germination and growth of quack grass and barley 35 days after planting in soils previously grown with barley or quack grass.

	Barley			Quack grass		
Soil	Germination (%)	Plant height (cm)	Fresh weight per plant (g)	Germination (%)	Plant height (cm)	Fresh weight per plant (g)
Barley	68.0	6.1	0.5	81.3	22.0	0.77
Quack grass	64.1	7.3	0.12	94.0	29.6	1.24
t-test	ns	*	*	*	*	*

ns, not significantly different (P > 0.05). *Significantly different at P < 0.01.

Table 2. Quack grass seed germination, plant height and weight 35 days after planting as affected by species and tissues incorporated into soil.

	Species incorporated		
Tissue incorporated	Barley	Quack grass	LSD (0.05)
Germination (%)			
None (control)	91.0	96.5	4.8
Roots only	63.1	71.8	5.6
Whole plant	44.7	66.0	4.3
LSD (0.05)	5.6	4.7	
Plant height (cm)			
None control)	41.1	38.7	ns
Roots only	22.3	24.3	2.4
Whole plant	14.0	17.6	3.0
LSD (0.05)	4.6	3.8	
Plant dry weight (g)			
None (control)	1.42	1.35	ns
Roots only	0.77	1.2	0.17
Whole plant	0.62	0.87	0.22
LSD (0.05)	0.21	0.17	

LSD, least significant differences; ns, not significantly different (P > 0.05).

Laboratory experiment

Germination

Extracts from fresh barley leaves, stems, flowers, roots and their mixture greatly inhibited quack grass seed germination at all concentrations compared to water control (Table 3).

Germination reductions ranged between 12 and 67%. The degree of inhibition increased for all tissues with an increase in extract concentration from 4 to 20 g per 100 ml of water. Plant parts varied in their allelopathicity to quack grass germination. Leaf extracts had the greatest allelopathic potential at all concentrations and stems had the lowest. Leaf extracts reduced germination by 34, 48, 53, 59 and 64% at concentrations of 4, 8, 12, 16 and 20 g per 100 ml of water, respectively. These results are in accordance with other studies which reported that allelopathicity may vary among plant parts (e.g. Chon & Kim 2002, Economou et al. 2002) and in accordance with the data of Turk and Tawaha (2002), who reported that barley leaves had the greatest inhibitory effect on lentil (*Lens culinaris* Medik.).

Seedling length

All extracts, except that of stems, significantly reduced hypocotyl length at all concentrations compared to water controls (Table 4). Reductions ranged between 7 and 46 %. Hypocotyl length was not affected by stem extracts at any concentration. For all other extracts, allelopathicity increased with increases in concentrations. At all concentrations, reduction was greatest with leaf extracts compared to extracts from other parts. Radicle length appeared more sensitive to allelochemicals than was hypocotyl length. These results are in agreement with the finding that water extracts of allelopathic plants generally have more pronounced effects on radicle, rather than hypocotyl, growth (Turk & Tawaha 2002). This may be attributable to the fact that radicles are the first to come in contact with allelochemicals. Extracts from all plant parts caused a marked reduction in radicle length of quack grass seedlings, ranging between 11 and 55% compared to water controls. Again, allelopathicity increased with an increase in extract concentration of all plant parts and was greatest with leaf extracts. Radicle length inhibition was lowest with root extracts. Besides the inhibition of radicle elongation, many of the extracts also altered radicle morphology, producing distorted and twisted radicles compared to control seedlings. Allelochemicals also affect root morphology in the alfalfa autotoxic

Table 3. Effect of the concentrations of water extracts made from various barley plant parts on the germination of quack grass seeds.

	Concentration (g per 100 ml of water)					
	4	8	12	16	20	LSD (0.05)
Tissues extracted	Germiniation (%)					
Leaves	55	48	40	35	31	3.0
Stems	88	80	80	72	68	2.8
Flowers	65	56	51	50	45	3.9
Roots	77	70	66	67	67	2.0
Mixture	70	67	60	65	54	3.1
LSD (0.05)	4.0	4.4	3.2	4.0	4.8	

LSD, least significant differences. Water control = 98. The mixture consisted of equal parts of leaf , stem, flower and root extracts

Table 4. Effects of the concentration of water extracts made from various barley plant parts on the hypocotyl and radicle length of 7-day-old quack grass seedlings.

	Concentration (g per 100 ml of water)					LSD (0.05)
	4	8	12	16	20	
Tissues extracted	Hypocotyl length (cm)					
Leaves	3.6	3.5	3.2	3.0	2.6	0.3
Stems	5.1	4.8	4.7	4.5	4.3	ns
Flowers	4.1	3.9	3.6	3.3	2.9	0.3
Roots	4.8	4.5	4.2	3.7	3.3	0.4
Mixture	4.6	4.1	3.6	3.3	3.0	0.2
LSD (0.05)	0.2	0.3	0.3	0.2	0.2	
Radicle length (cm)						
Leaves	3.6	3.1	2.8	2.6	2.5	0.3
Stems	5.1	4.8	4.5	4.1	3.8	0.4
Flowers	4.2	3.8	3.6	3.3	3.0	0.3
Roots	5.6	5.2	4.8	4.5	4.3	0.2
Mixture	4.5	4.2	3.8	3.5	3.1	0.3
LSD (0.05)	0.2	0.2	0.3	0.1	0.3	

LSD, least significant differences; ns, not significant. Water control hypocotyl = 4.6. Water control radicle = 5.7. The mixture consisted of equal parts of leaf , stem, flower and root extracts.

response (Jennings & Nelson 2002).

Seedling weight

All barley extracts caused a marked reduction in quack grass hypocotyl dry weight at all concentrations compared to water controls, ranging between 30 and 77% (Table 5). For all tissues, hypocotyl dry weight also decreased as the extract concentration increased. Leaf extracts were again the most inhibitory at all concentrations compared with the water controls, and reduced hypocotyl dry weight by 58, 64, 68, 72 and 76% at concentrations of 4, 8, 12, 16 and 20 g per 100 ml water, respectively. The response of quack grass radicles was similar to that of hypocotyls, although inhibition was somewhat lower; barley extracts cause weight reductions ranging between 5 and 58%.

Table 5. Effects of the concentration of water extracts made from various barley and plant parts on the hypocotyl and radicle dry weight of 7-day- old quack grass seedlings.

	Concentration (g per 100 ml of water)					LSD
	4	8	12	16	20	(0.05)
Tissues extracted	**Hypocotyl weight (cm)**					
Leaves	0.63	0.58	0.55	0.50	0.45	0.05
Stems	1.40	1.33	1.30	1.27	1.23	0.06
Flowers	1.10	1.00	0.97	0.94	0.91	0.04
Roots	1.20	1.03	0.99	0.95	0.93	0.03
Mixture	0.90	0.86	0.84	0.81	0.78	0.04
LSD (0.05)	0.04	0.05	0.04	0.03	0.04	
	Radicale wight (mg)					
Leaves	0.51	0.47	0.45	0.41	0.38	0.03
Stems	0.73	0.70	0.67	0.64	0.61	0.05
Flowers	0.64	0.61	0.58	0.55	0.54	0.05
Roots	0.86	0.82	0.79	0.75	0.73	0.04
Mixture	0.77	0.74	0.70	0.67	0.65	0.03
LSD (0.05)	0.03	0.03	0.04	0.06	0.03	

LSD, least significant differences. Water control hypocotyl = 1.90. Water control radicle = 0.95. The mixture consisted of equal parts of leaf, stem, flower and root extracts.

Table 6. Water uptake by quack grass seeds soaked in barley leaf water extract at different concentrations.

	Concentration (g per 100 ml of water)						
Soaking time (h)	**0**	**4**	**8**	**12**	**16**	**20**	**LSD (00.5)**
4	1.38	0.91	0.80	0.71	0.59	0.50	0.02
8	1.24	0.88	0.82	0.74	0.68	0.52	0.04
12	1.33	0.94	0.89	0.81	0.65	0.61	0.03
16	1.54	0.95	0.88	0.84	0.62	0.63	0.05
LSD (0.05)	0.08	0.6	0.08	0.03	0.02	0.04	

LSD, least significant differences.

Water uptake by seeds

Increasing the concentration of water leaf extracts significantly inhibited water uptake by quack grass seeds (Table 6). For all soaking times, the greatest inhibition in water uptake when compared with the water control occurred at the 20 g per 100 ml of water concentration, averaging 57%. These results suggest that the allelopathicity of barley may be mediated in part through a regulation of water uptake and inhibition of seeds. This could be due to a reduction of seed protease activity, which plays a key role in protein hydrolysis during germination and which is to a large extent related to water imbibition and water uptake of seeds (Rice 1984).

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