



## RESEARCH PAPER

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## Allelopathic effects of downy brome extracts on germination and seedling growth in wheat genotypes

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**Key words:** Allelopathy, germination, seedling, downy brome, wheat.

<http://dx.doi.org/10.12692/ijb/4.9.177-182>

Article published on May 10, 2014

### Abstract

Phytotoxicity of downy brome (*Bromus tectorum*) extracts on bread wheat (*Triticum aestivum*) and durum wheat (*Triticum durum*) was investigated. Water extracts of downy brome were bioassayed on germination and seedling growth of both wheat species to: (i) test the heterotoxicity of downy brome on wheat, (ii) study the dynamics of allelopathic potential over four growth stages and (iii) identify the most allelopathic plant part of downy brome. Whole downy brome plants were extracted at growth stage 4 (stems not developed enough), whilst for the following growth stages roots, stems, and leaves were extracted separately. Seedling growth bioassays demonstrated that the two wheat species responded differently to the allelopathic potential of downy brome 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. The allelopathic potential of downy brome plant parts were not stable over its life cycle for either bread or durum wheat. It appeared that potential increased near physiological maturity. Leaves and roots were the most phytotoxic downy brome plant parts for bread and durum wheats, respectively. Results suggested that the response by durum wheat and bread wheat varied depending on the source of allelochemicals (plant part) and the growth stage of the downy brome plant. Consequently, downy brome should be considered a depressive prior crop for both durum wheat and bread wheat in a field cropping sequence.

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## Introduction

Allelopathy as mechanism of plant interference in agroecosystems offers an opportunity to manage weeds in a crop sequence (Nasir and Moinuddin, 2009; Vahedi, 2012), but could also adversely affect crop fields (Bertoldi *et al*, 2012; Sohrabi *et al*, 2014) and influence choice of rotation. Previous studies have shown that sorghum (*Sorghum bicolor*) vegetation possess a variety of potent inhibitors such as dhurrin, a cyanogenic glycoside (Gaudet and Keddy, 1988; Steckel *et al*, 1997) and phenolics (Hassan *et al*, 2012) which are potentially allelopathic to weeds (Alsaadawi *et al*, 2007; Saberi *et al*, 2013) with a maximum of inhibitory activity at harvest (Ahamd *et al*, 2000; Bahatti *et al*, 2000). The same results were reported for sudex, a hybrid of sorghum and sudangrass (*Sorghum Sudanese*) (Moosavi *et al*, 2011). This was not the case for all grasses, some exhibited higher toxicity to wheat seedling growth when their residues were still green (Nandal *et al*, 1992; Turk and Tawaha, 2003).

Bioassays of germination, radicle growth and coleoptile growth are used to test the allelopathic potential of crop species (Spiters *et al*, 1989; Han *et al*, 2008). The allelopathic potential can be observed in the form of autotoxicity as in case of alfalfa (*Medicago sativa* L.) (John and Nelson, 1998; Hedge and Miller, 1990) or heterotoxicity as in the case of tall fescue (*Festuca arundinacea* L.) (Bertoldi *et al*, 2012). Since the allelopathy of small grain cereals has been little studied, the present work aimed to : (i) test the heterotoxicity of downy brome on bread wheat and durum wheat varieties, (ii) study changes in allelopathic potential over four growth stages on both durum wheat and bread wheat and (iii) identify the most allelopathic plant part.

## Material and methods

### *Growth of downy brome plants*

Downy brome (*Bromus tectorum*) was sown on April 10, 2013 at the experimental station of Miandoab Azad University. The sandy-clay soil was alkaline with a pH of 7.9 and 1.6% of organic matter. From soil preparation to harvest, standard cultural practices for

the semiarid zone were applied. Plants were watered whenever severe wilting was observed. Whole downy brome plants were pulled out of the field at four growth stages (stage 1= leaf sheaths lengthening; stage 2= last leaf just visible; stage 3= in boot; stage 4= grain development) (Cheema *et al*, 2003; Xuan *et al*, 2005). For the stage 4, plants were sampled in late June 2013.

### *Preparation of water extracts*

Downy brome plants were gently washed with distilled water, dried between two paper towels and then separated into roots, stems and leaves. All plant components were chopped into 1 cm long pieces and dried at 50 °C for 24 h. An ungrounded 2.5 g dried portion of each plant component was extracted in 50 ml cold distilled water. Plants were extracted in a 500 ml flask on a horizontal shaker for 24 h at 200 rpm. Extracts were passed through cheese cloth and stored at less than 5 °C until bioassay. At stage 1, downy brome stems were not developed enough. So, whole plants were extracted as one unit (including roots) following the same technique described for plant components.

### *Growth medium for bioassays of downy brome extracts*

Water extracts of the whole plant of downy brome at stage 1 and roots, stems and leaves for stages 2, 3 and 4 were tested for phytotoxicity to seed germination, radicle growth and coleoptile growth of 4 varieties of bread wheat ('Alvand', 'Shahriar', 'Omid' and 'Roshan') and 4 varieties of durum wheat ('Gerdish', 'Azar2', 'Karim' and 'Razzek'). For the bioassays, molten agar was amended with 20 ml extract of each plant part to make a water-extract-agar medium (1.2%). The medium of 1.2% distilled water-agar was used as a control.

### *Germination bioassays*

For germination bioassays, seeds of wheat were surface sterilized with a 5% aqueous solution of sodium hypochlorite for 1 min, rinsed 5 times with distilled water and dried between two paper towels. Surface sterilized seeds were placed in a 10×150 mm

Petri Dish (PD) containing 15 ml of water extract agar as growth medium and incubated for 35 h at 25 °C. Seeds were classed as germinated when the radicle extended 2 mm out of the seed coat.

#### Radicle and coleoptile growth bioassays

Radicle and coleoptile growth bioassays were determined using a Test Tube (TT) technique and per-germinated seeds. Surface sterile seeds were per-germinated on a 10×50 mm PD between two filter papers moistened with 1.2 ml of distilled water. Test tubes were covered with cotton to solidify. Then, seedlings with radicle 3mm long were transplanted into tubs. After 60 h incubation at 25 °C, lengths of both the coleoptile and central radicle of each wheat seedling were measured.

#### Experimental design and statistical analysis

Germination and seedling growth bioassays were conducted in a complete Randomized Design (CRD) with four replication. A non-amended treatment was included as a control. For germination bioassays, 25

seeds were placed in a PD. Each experimental unit consisted of two PD. For radicle or coleoptile bioassays, an average across a cluster of 10 growth TT with one pre-germinated seed each was used as a single observation for each treatment. Analysis of variance was conducted using SAS and Fisher's protected LSD at the 0.05 level of probability (Yazdi *et al*, 1998).

## Results

#### Germination bioassays

Extract of downy brome plants at stage 1 did not significantly affect seed germination of either durum or bread wheat varieties. When plant components (roots, stems and leaves) of downy brome were extracted separately at stages 2, 3 and 4 and bioassay on 'Azar 2' (durum) and 'Omid' (bread), both characterized by sensitive radicle growth (Tab.1), germination bioassays again did not appear to be a sensitive test for allelopathic effects. Therefore no data is presented from the germination bioassays.

**Table 1.** Radicle growth (mm) of four varieties of Bread wheat and four varieties of Durum wheat, treated with water extracts\* of downy brome.

Treatment	Bread wheat				Durum wheat			
	Alvand	Shahriar	Omid	Roshan	Gerdish	Azar2	Karim	Razzek
Control	5.97 a	5.43 a	7.86 a	5.75 a	6.21 a	6.97 a	4.34 a	5.29 a
Extract	5.03 a	3.63 b	3.1 b	4.19 b	5.78 a	3.35 b	3.76 a	4.41 a
LSD (0.05)	1.17	1.35	0.91	1.86	0.71	1.20	1.09	1.32

\* Extracted at stage 1 (stage 1= leaf sheaths lengthening).

#### Seedling growth bioassays

Extracts of whole downy brome plants at stage 1 significantly affected radicle growth of just one durum wheat variety 'Azar 2'. However, with bread wheat, three varieties 'Shahriar', 'Omid' and 'Roshan' had reduced radicle growth (Tab.1). 'Omid' was the most sensitive bread wheat variety with radicle and

coleoptile growth inhibited [Inhibition = (Control – Treatment) / Control × 100] by 60% and 49% respectively and 'Azar 2' was the most sensitive variety of durum wheat variety with radicle and coleoptile growth inhibited by 51% and 46% respectively (Tab.1, 2).

**Table 2.** Coleoptile growth (mm) of four varieties of Bread wheat and four varieties of Durum wheat, treated with water extracts\* of downy brome.

Treatment	Bread wheat				Durum wheat			
	Alvand	Shahriar	Omid	Roshan	Gerdish	Azar2	Karim	Razzek
Control	5.72 a	4.54 a	5.20 a	3.82 a	3.48 a	4.67 a	2.72 a	3.60 a
Extract	5.19 a	4.23 a	2.61 b	2.94 a	3.25 a	2.51 b	2.48 a	2.97 a
LSD (0.05)	1.04	1.38	1.52	0.97	0.63	1.25	1.20	1.48

\* Extracted at stage 1 (stage 1= leaf sheaths lengthening).

Based on the radicle bioassays, 'Azar 2' (durum) and 'Omid' (Bread) were selected as test varieties for further bioassays at stage 2, 3 and 4. Water extracts of plant components (roots, stems, leaves) of downy brome at stage 2, 3 and 4 showed a significant inhibitory activity on radicle growth of 'Omid' (Tab.3). The inhibitory activity was not stable over the life cycle regardless of the source of extract. The response of 'Azar 2' about radicle and coleoptile was very different than 'Omid' to downy brome extracts.

'Azar 2' radicle was stimulated when treated with stem extracts at stage 2 (Tab 3). Overall, inhibitory activity was greater in 'Omid' than in 'Azar 2' (Tab 3,4). Coleoptile growth of 'Azar 2' was unaffected at

stage 2, increased at stage 3 and reduced at stage 4 (Tab 4). 'Omid' was highly sensitive to water extracts with growth being reduced at all stages. As it happened to radicle growth, coleoptile growth of 'Azar 2' was significantly enhanced with all types of water extracts (roots, stems and leaves) at stage 3 (Tab 3,4). At stage 4, extracts of leaves and roots were the most phytotoxic to the growth of 'Azar 2' and 'Omid' coleoptiles, respectively. In contrast to 'Azar 2' was observed for radicle or coleoptile growths were always inhibited by water extracts of plant parts of downy brome at all stages 2, 3 and 4 with the radicle being more sensitive than the coleoptiles.

**Table 3.** Radicle growth (mm) of 'Omid' (Bread) and 'Azar2' (Durum) treated with water extracts of plant parts prepared from downy brome at stage 2\*, 3\* and 4\*.

Treatment	Stage 2		Stage 3		Stage 4	
	Omid	Azar2	Omid	Azar2	Omid	Azar2
Control	7.86 a	6.97 a	7.86 a	6.97 a	7.86 a	6.97 a
Root extract	3.50 b	4.74 b	2.35 b	2.92 b	1.33 b	2.50 b
Leaf extract	2.81 c	4.49 b	1.87 c	1.60 c	1.25 b	1.38 d
Stem extract	3.73 b	7.25 a	1.61 c	2.89 b	1.06 b	1.94 c
LSD (0.05)	0.49	0.73	0.41	1.06	0.87	0.23

\*Stage 2= last leaf just visible; stage3= in boot; stage4= grain development.

### Discussion and conclusion

Germination bioassays of downy brome at four different phenological stages were not sensitive enough to detect the heterotoxicity potential of any plant component of downy brome. However, seedling growth bioassays were sensitive to allelopathic effects

with the radicle being relatively more sensitive than the coleoptile (Tab 1,2,3,4). Results of both types of bioassay are in agreement with the results reported by Hedge and Miller (1990) and Saberi *et al* (2013), respectively.

**Table 4.** Coleoptile growth (mm) of 'Omid' (Bread) and 'Azar2' (Durum) treated with water extracts of plant parts prepared from downy brome at stage 2\*, 3\* and 4\*.

Treatment	Stage 2		Stage 3		Stage 4	
	Omid	Azar2	Omid	Azar2	Omid	Azar2
Control	5.20 a	4.67 a	5.20 a	4.67 c	5.20 a	4.67 a
Root extract	3.97 b	4.34 a	2.84 b	6.35 b	1.43 bc	3.65 b
Leaf extract	3.23 b	3.91 a	2.06 bc	5.82 bc	1.78 b	1.50 d
Stem extract	2.16 c	4.25 a	1.69 c	7.91 a	1.22 c	2.78 c
LSD (0.05)	1.27	0.84	0.87	1.22	0.51	0.79

\* Stage 2= last leaf just visible; stage3= in boot; stage4= grain development.

Irrespective of the wheat species, radicle growth was generally reduced by downy brome extracts, except for stem extracts at stage 2 and root extracts at stage 3 which stimulated radicle growth of the durum

wheat 'Azar 2' (Tab 4). The allelopathic potential of a downy brome plant on wheat species varied according to the source of extracts as was found with sorghum (Alsaadawi *et al*, 2007; Hassan *et al*, 2012). The

sensitivity of the radicle was higher for bread wheat than durum wheat (Tab 3). This could be the case among varieties within the same species as reported by Xuan *et al* (2005) and Herro and Callaway (2003). In addition, the allelopathic potential of downy brome was unstable over the life cycle of the downy brome plant. This potential was at maximum near physiological maturity as was for sorghum plant (Weiget and Jolliffe, 2003; Nasir and Moinuddin, 2009). These results support the use of seedling bioassays as a tool to screen for tolerance or sensitivity of a crop species to the allelopathic potential of another crop species.

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