

Effects of Downy Brome (*Bromus tectorum* L.) and Italian Ryegrass (*Lolium multiflorum* Lam.) on Growth Inhibition of Wheat and Weeds

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The objectives of this research were to determine the inhibitory effects of wheat and weeds by soil application and different extractions of downy brome (*Bromus tectorum*, DB) and Italian ryegrass (*Lolium multiflorum* Lam., IRG) shoot and roots, and to identify inhibition substances by fermentation extraction of IRG shoot and roots. Shoot fresh weight (SFW) of two wheat cultivars, Stephens and Tubbs 06, was reduced 28–53% and 53–55% by DB residues grown at 25, 35, and 45 d after seeding (DAS) in sandy loam soil under greenhouse conditions, respectively, compared with the control treatment. SFW of wheat cultivars Stephens and Tubbs 06 was reduced 30–48% and 34–45% by IRG residues grown at 25, 35, and 45 DAS in sandy loam soil under greenhouse conditions, respectively, compared with those of the control. In soil application (150 g m⁻² or 300 g m⁻²) of DB and IRG roots grown at 35 DAS, SFW of both wheat cultivars was reduced 33–52% compared with the control. However, the SFW of both wheat cultivars upon soil application of IRG and DB shoots was increased by increasing amounts of application. Common lambsquarters (*Chenopodium album* L.), pigweed (*Amaranthus alus* L.), spiny sowthistle (*Sonchus oleraceus* L.), white clover (*Trifolium repens*), barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.), and large crabgrass (*Digitaria ciliaris*) were inhibited 23–95% and 25–80% by soil application of IRG and DB roots, respectively, compared with the control. However, weed growth inhibition was less affected by these treatments. The shoot and root fresh weights of both wheat cultivars were inhibited by water extracts of IRG and DB shoots at 0.5%, 1%, 2.5%, 5%, and 10% concentrations, but not by water extracts of IRG and DB roots. Reduction of shoot and root fresh weight in both wheat cultivars was observed more in fermentation extracts of IRG shoots and roots than in water extracts of IRG roots. Phenol compounds hydrocinnamic acid, caffeic acid, p-coumaric acid, and ferulic acid were confirmed in fermentation extraction of DB and IRG shoot and roots by high performance liquid chromatography (HPLC); the contents of phenol compounds were greater in DB and IRG shoots than in roots. Shoot and root weight of both wheat cultivars was inhibited 42–69% by 0.5, 1, and 3 mM treatments of phenol compounds p-coumaric acid, ferulic acid, and caffeic acid. Therefore, retarded growth of wheat and weed may have been caused by the phenol compounds of DB and IRG.

Key Words: allelopathy, downy brome, growth inhibition, Italian ryegrass, phenol compound, weed, wheat

Abbreviations: DAS – days after seeding, DB – downy brome, IRG – Italian ryegrass, PH – plant height, SFW – shoot fresh weight

INTRODUCTION

Allelopathy occurs through the release of chemicals from one plant species affecting other species growing in its vicinity, usually to their detriment (An et al. 1998). Allelochemicals are released through volatilization, leaching from leaves, degradation of plant residues and root exudation (Pramanik et al. 2000). Plant residues in cultivated crop fields are an important resource not only as a source of significant quantities of nutrients for crop production but also as a source affecting soil physical and chemical properties. When plant residues are returned to the soil, crop production can have both positive and

negative effects through decomposition of plant residues (Kumar and Goh 1999). Incorporation of plant residues normally occurs just before the planting of subsequent crops. During plant residue decomposition, allelochemicals are released directly or indirectly into the soil through the action of microorganisms. The level of soil microbial activity is generally dependent on environmental features such as temperature, water, and nutrient content of the soil (Inderjit and Dakshini 1999). In addition, microorganisms play an important role in allelopathic activity of plant residues, either by increasing or decreasing their phytotoxicity (An et al. 2001; Blum 1998; Fischer 1986).

Plant residues of numerous weeds have allelopathic effects on the germination and growth of subsequent crops (Mersie and Singh 1987; Wilson 1981). Such effects are attributed to allelochemicals, which may result from microbial activity during decomposition (Inderjit and Dakshini 1999). Residues of *Medicago sativa* L. and *Brassica napus* L. used as cover crops inhibited growth of small-seeded plants in field studies, while *Secale cereale* L. residues showed no effects (Kruidhof et al. 2011). Laboratory bioassay results revealed that *S. cereale*, *Trifolium pretense* L. and *B. napus* extracts affected small-seeded weed species more than large-seeded crop species (Burgos and Talbert 2000; Liebmann and Sundberg 2006; Petersen et al. 2001).

Italian ryegrass (*Lolium multiflorum* Lam., IRG) is a troublesome weed in cereal crops such as wheat and barley (*Hordeum vulgare* L.) worldwide (Paynter and Hills 2009; Trusler et al. 2007). IRG densities of 100 plants m^{-2} reduced wheat yield by 30%. Downy brome (*Bromus tectorum*, DB) is the most prevalent annual grass weed in winter wheat fields (Douglas et al. 1990) partly due to its ability to germinate successfully over a wide range of moisture and climatic conditions, quickly establish an extensive fibrous root system, and respond dramatically to nitrogen (Douglas et al. 1990). DB is a serious competitor of winter wheat. DB densities of 132 plants m^{-2} have been found to reduce winter wheat yield by 40% (Rydrych 1974; Stahlman and Miller 1990). Since a large amount of DB and IRG residues was incorporated directly into the soil, it was presumed that the decomposed DB and IRG residues might exhibit allelopathic effects on the subsequent crop, resulting in stunted growth. Thus, the objectives of this research were (1) to determine the inhibitory effects of wheat and weeds by soil application and different extracts of DB and IRG shoot and roots, and (2) to identify inhibition substances by fermentation extraction of DB and IRG shoot and roots.

MATERIALS AND METHODS

Effects of Downy Brome and Italian Ryegrass Residues on Wheat Growth

Experiments were conducted in a greenhouse (Suncheon, South Korea) using sandy loam collected from a wheat field. Soils in pots (72 cm^2) were sieved by passing them through a 4-mm size mesh. Seeds of DB and IRG alone were planted in the pots. One week after seeding, 15 plants were left in each pot. All above-ground parts of DB and IRG at 25, 35, and 45 d after seeding (DAS) were removed by cutting them with scissors at the soil level from pots. Soils from the pots were then homogenized and returned to the pots. Then, seeds of wheat cvs. Stephens and Tubbs 06 (5 seeds per cultivar) were

subsequently planted in each pot. Plant height (PH) and shoot fresh weight (SFW) were measured at 14, 21, and 30 DAS.

To confirm the effect of wheat growth inhibition by DB and IRG shoots or roots grown at 35 DAS, the shoots or roots were harvested and dried for 7 d at room temperature. The dried shoots or roots of 150 g or 300 g m^{-2} were applied to the soils. Five seeds of two wheat cultivars (Stephens and Tubbs 06) were planted in each pot. PH and SFW were measured at 30 d after treatment (DAT) for root application and at 7, 14, and 28 DAT for shoot application.

Effects of Downy Brome and Italian Ryegrass Residues on Weed Control

To investigate the effects of DB and IRG shoots or roots on weed control, 150 g or 300 g m^{-2} of dried shoots or roots of DB and IRG were applied in every pot. Thereafter, seeds of weeds, common lambsquarters (*Chenopodium album* L.), pigweed (*Amaranthus alus* L.), spiny sowthistle (*Sonchus oleraceus* L.), and white clover (*Trifolium repens*) for broad leaf weeds, and barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.) and large crabgrass (*Digitaria ciliaris*) for grass weeds were sown. SFW was measured 30 DAT.

Effects of Extracts of Downy Brome and Italian Ryegrass Shoots or Roots on Wheat Growth

Water, ethanol, and fermentation extracts were used in petri dish bioassay to determine inhibition of shoot and root fresh weight in the two wheat cultivars. Twenty grams (20 g) of each plant species were ground and homogenized in 400 mL distilled water and ethanol for 24 h. Another 20 g of the ground plant species was put in 400 mL distilled water and stored at room temperature for 20 d under dark conditions for fermentation extract.

Each extract was filtered with miracloth and then concentrated under reduced pressure; the pellet was completely evaporated using a vacuum dryer (Hanbaek Scientific Co. Korea). Each extract was dissolved in distilled water to ensure that the final concentration was 20%. The extracts were centrifuged at 10,000 g for 15 min and the supernatants were filtered by using a 0.45 μm syringe.

The bioassay used 9-cm-diameter petri dishes, containing 5 seeds, as experimental units arranged in a completely randomized design with three replications. The seeds were placed in petri dishes between two sheets of filter paper (Whatman #2). Eight milliliters (8 mL) of plant extracts at concentrations of 0, 0.5, 1, 2.5, 5, and 10% were applied to each petri dish. The petri dishes were incubated in the dark for 3 d and in the light for 3 d in a growth chamber (25°C temperature with a light intensity

of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The shoot and root fresh weights of each cultivar were measured at 7 DAT.

Phenolic Compound Analysis by HPLC

Phenolic compound standards, hydrocinnamic acid, *p*-coumaric acid, ferulic acid, and caffeic acid were purchased from Sigma Aldrich (St. Louis MO, USA) for standard curves. All standard calibration curves showed high degrees of linearity ($r^2 > 0.99$) (data not shown). Samples for HPLC analysis used fermentation extracts of the same as in the above section. The solutions of fermentation extracts were concentrated under reduced pressure and dissolved in 10 mL of methanol (HPLC grade). The suspension was filtered through a 0.45- μm syringe filter. The 10 μL filtrate was loaded on the HPLC system (Agilent 1200 series). Separation was achieved on a 205 \times 4.60 mm, Spherclone 5 μ ODS (2) column. The absorbance of each sample solution was measured by UV detector at 210 nm. The mobile phase was 0.1% glacial acetic acid in water (solvent A) and 0.1% glacial acetic acid in acetonitrile (solvent B). The gradient was 50 min, 0% to 40% (A); 2 min, 40% to 100% (B). Run time was 60 min using a flow rate of 1 mL/min.

Effect of Phenolic Compounds on Growth Inhibition of Wheat

For the seed bioassay, the phenolic compounds caffeic acid, *p*-coumaric acid, and ferulic acid at 0, 0.5, 1 and 3 mM were applied in order to confirm growth inhibition of wheat. The seed bioassay was the same as previously described for the effects of extracts of DB and IRG shoots or roots on wheat growth.

Statistical Analysis

All experiments were conducted two or three times with three replicates for each treatment. Data were analyzed using analysis of variance (ANOVA) in the Statistical Analysis Systems (SAS 2000) software (SAS Institute, Inc., Cary, North Carolina, U.S.A.). Means were separated using Duncan's multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Effects of Downy Brome and Italian Ryegrass Residues on Wheat Growth

Plant height (PH) of wheat cvs. Stephens and Tubbs 06 at 30 DAT was reduced 16–29% and 32–34%, respectively, by DB residues grown at 25, 35, and 45 DAS in sandy loam soil under greenhouse conditions compared with the control (Table 1). In addition, shoot fresh weight (SFW) of the wheat cultivars was reduced 28–53% and 53–55%, respectively, by DB residues grown at 25, 35, and 45 DAS in sandy loam soil under greenhouse conditions compared with the control. There were no significant differences in PH and SFW of wheat cultivars among DB residues grown at 25, 35, and 45 DAS. Except for 25 DAS, the PH of cvs. Stephens and Tubbs 06 was reduced 14–28% and 18–26%, respectively, by IRG residues grown at 35 and 45 DAS in sandy loam soil under greenhouse condition. In addition, SFW of the wheat cultivars was reduced 30–48% and 34–45%, respectively, by IRG residues grown at 25, 35, and 45 DAS in sandy loam soil under greenhouse conditions. The reduction levels of PH and SFW were similar to those between DB and IRG residues and between wheat cultivars.

Table 1. Effects of downy brome and Italian ryegrass residues grown at 25, 35, and 45 d after seeding on wheat

Plant Residues	Wheat (cv. Stephens)				Wheat (cv. Tubbs 06)				
	Plant Height (cm)			Shoot FW (g/3 plants)	Plant Height (cm)			Shoot FW (g/3 plants)	
	14 DAT	21 DAT	30 DAT	30 DAT	14 DAT	21 DAT	30 DAT	30 DAT	
Downy brome	0 DAS	14.2 ^a	21.3 ^a	24.3 ^a	1.03 ^a	15.0 ^a	22.0 ^a	25.3 ^a	1.13 ^a
	25 DAS	10.3 ^b	17.0 ^b	20.3 ^b	0.74 ^b	10.0 ^b	16.3 ^b	16.7 ^b	0.61 ^b
	35 DAS	9.7 ^b	16.0 ^b	19.7 ^b	0.65 ^b	10.2 ^b	16.2 ^b	17.0 ^b	0.57 ^b
	45 DAS	10.3 ^b	15.7 ^b	17.3 ^c	0.48 ^c	11.3 ^b	15.7 ^b	16.7 ^b	0.50 ^b
Italian ryegrass	0 DAS	18.5 ^a	21.3 ^a	21.7 ^a	0.79 ^a	16.3 ^a	20.0 ^a	20.8 ^a	0.67 ^a
	25 DAS	15.0 ^{ab}	20.2 ^{ab}	20.0 ^{ab}	0.55 ^b	15.3 ^a	19.7 ^a	19.3 ^a	0.65 ^a
	35 DAS	14.2 ^b	20.2 ^{ab}	18.7 ^b	0.46 ^{bc}	13.7 ^{ab}	18.7 ^a	17.0 ^b	0.44 ^b
	45 DAS	13.5 ^b	18.0 ^b	15.7 ^c	0.41 ^c	11.3 ^b	15.0 ^b	15.3 ^b	0.37 ^b

Plants were 15 populations per pot (72 cm²).

DAT, days after treatment; DAS, days after seeding

Significant differences ($p < 0.05$) are indicated with different letter superscripts according to Duncan's Multiple Range Test.

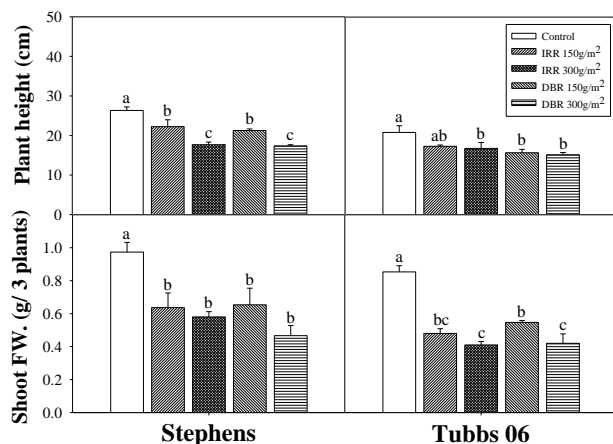


Fig. 1. Effects of Italian ryegrass (IRR) and downy brome roots (DBR) grown at 35 d after seeding with different application rates on the growth of wheat (cvs. Stephens and Tubbs 06) in sandy loam soil under greenhouse conditions. Parameters were measured at 30 d after treatment. Significant differences ($p \leq 0.05$) are indicated with different letters according to Duncan's Multiple Range Test.

or 300 g m⁻²) of IRR and DB roots grown at 35 DAS, SFW of both wheat cultivars was reduced by 33–52% compared with the control. There were no significant differences in PH and SFW between different plant species (IRR and DB), cultivars (Stephens and Tubbs 06), and application amounts (150 g m⁻² or 300 g m⁻²). However, SFW in soil application of DW and IRR roots showed more sensitivity than PH. Allelopathic effects of decomposed residues might be an important factor influencing growth, particularly seedling growth of subsequent crops. Microorganisms play an important role in the allelopathic activity of plant residues, either increasing or decreasing their phytotoxicity (An et al. 2001; Blum 1998). We assumed that the microbial activities in the pot bioassay were similar to those in natural field conditions because we used the same soil as that of the wheat field. PH of both wheat cultivars was not significantly different in soil application of IRR and DB shoots (Table 2). Furthermore, SFW of both wheat cultivars in soil application of IRR and DB shoots was increased by increasing amounts of application. This trend may be caused by organic matter

Table 2. Effects of Italian ryegrass and downy brome shoots with different application rates on wheat growth in sandy loam soil under greenhouse conditions.

Treatments	Wheat Cultivars							
	Tubbs 06				Stephens			
	Plant Height (cm)		Shoot FW (g/3 plants)		Plant Height (cm)		Shoot FW (g/3 plants)	
	7 DAT	14 DAT	14 DAT	28 DAT	7 DAT	14 DAT	14 DAT	28 DAT
Control	12.3 ^{ab}	20.0 ^b	0.57 ^b	1.14 ^c	11.8 ^a	21.2 ^a	0.57 ^b	1.10 ^c
IRS 150	11.1 ^{ab}	19.7 ^b	0.58 ^b	1.44 ^b	10.8 ^{ab}	20.2 ^a	0.56 ^b	1.70 ^b
IRS 300	10.3 ^b	20.1 ^b	0.61 ^b	1.95 ^a	9.5 ^{bc}	20.7 ^a	0.64 ^b	2.02 ^{ab}
DBS 150	12.6 ^a	22.3 ^{ab}	0.69 ^b	1.59 ^b	11.2 ^{ab}	21.4 ^a	0.61 ^b	1.65 ^b
DBS 300	10.3 ^b	24.5 ^a	1.11 ^a	2.03 ^a	8.6 ^c	22.8 ^a	0.80 ^a	2.18 ^a

DAT, days after treatment; FW, fresh weight

IRS, Italian ryegrass shoot at 35 d after seeding (DAS); DBS, downy brome shoot at 35 DAS

Significant differences ($p < 0.05$) are indicated with different letter superscripts according to Duncan's Multiple Range Test.

In some farming systems, the decomposition of the crop residue on the soil surface or incorporated into the soil has led to suppression of the germination and growth of neighboring weeds (Putnam and DeFrank 1983). It is possible that the crop residues resulted from the release of allelochemical compounds (Kruidhof et al. 2009). In our study, using soil application (150 g m⁻² or 300 g m⁻²) of IRR and DB roots grown at 35 DAS, PH of both wheat cultivars was reduced by 16–34% compared with the control (Fig. 1). In addition, in soil application (150 g m⁻²

and growth promotion substances in residues of IRR and DB shoot.

Effects of Downy Brome and Italian Ryegrass Residues on Weed Control

Common lambsquarters, pigweed, spiny sowthistle, white clove, barnyardgrass, and large crabgrass were controlled 64–88%, 92–95%, 79–88%, 23–54%, 64–86%, and 67–76%, respectively, by soil application (150 g m⁻² or 300 g m⁻²) of IRR roots (Table 3). However, the above weed species were not significantly controlled by soil

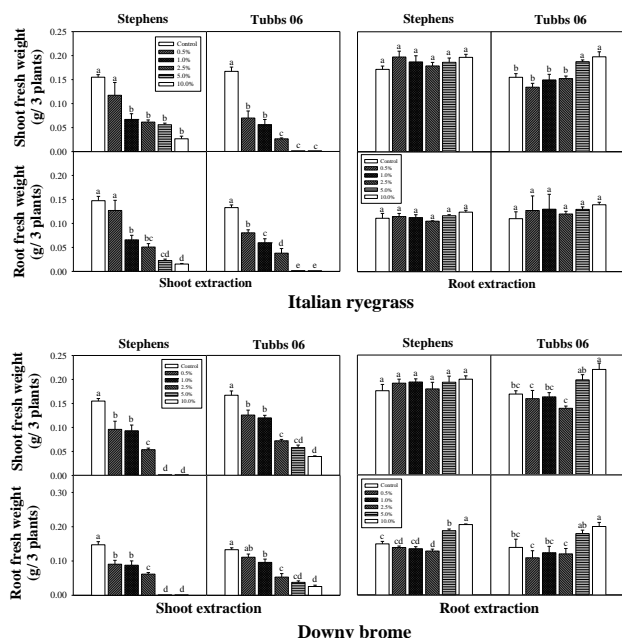


Fig. 2. Effects of water extraction of Italian ryegrass and downy brome shoot and roots on wheat (cvs. Stephens and Tubbs 06) shoot and root fresh weight in Petri dishes. Parameters were measured at 7 d after treatment. Significant differences ($p \leq 0.05$) are indicated with different letters according to Duncan's Multiple Range Test.

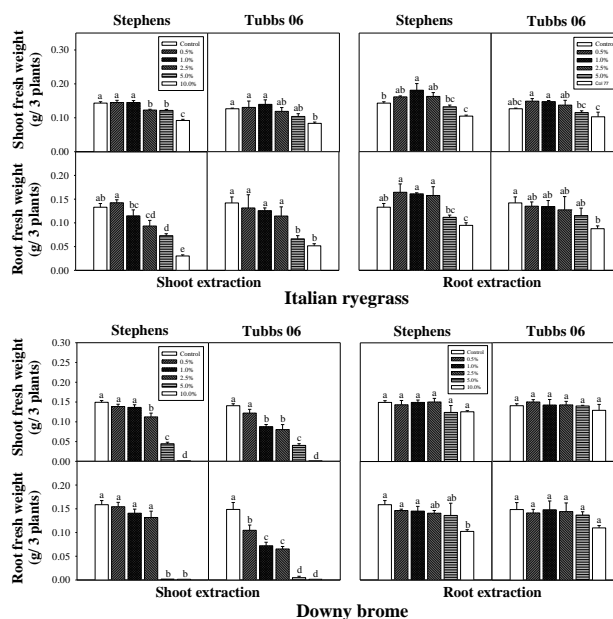


Fig. 3. Effects of ethanol extraction of Italian ryegrass and downy brome shoot and roots on wheat (cvs. Stephens and Tubbs 06) shoot and root fresh weight in Petri dishes. Parameters were measured at 7 d after treatment. Significant differences ($p \leq 0.05$) are indicated with different letters according to Duncan's Multiple Range Test.

application (150 g m^{-2} or 300 g m^{-2}) of IRG shoots. Furthermore, SFW of pigweed and large crabgrass were increased 31% and 81%, respectively, by soil application

(300 g m^{-2}) of IRG shoots. Common lambsquarters, pigweed, white clove, barnyardgrass, and large crabgrass were controlled 74–78%, 80–86%, 39–55%, 51–66%, and 25–48%, respectively, by soil application (150 g m^{-2} or 300 g

Table 3. Effects of Italian ryegrass and downy brome shoot and roots with different application rates on weed control in sandy loam soil under greenhouse conditions.

Treatment (g/m^2)	Shoot Fresh Weight (g/pot)					
	Broad Leaf Weed				Grass Weed	
	Common Lambsquarters	Pigweed	Spiny Sowthistle	White Clove	Barnyardgrass	Large Crabgrass
Control	0.352 ^{bc}	0.510 ^{bc}	0.105 ^b	0.560 ^{ab}	0.230 ^a	0.902 ^{bc}
IRR 150	0.126 ^{de}	0.043 ^e	0.022 ^c	0.433 ^{bcd}	0.083 ^b	0.300 ^{de}
IRR 300	0.042 ^e	0.023 ^e	0.012 ^c	0.257 ^d	0.033 ^b	0.217 ^e
IRS 150	0.338 ^{bc}	0.450 ^{cd}	0.092 ^b	0.472 ^{bc}	0.317 ^a	1.143 ^b
IRS 300	0.206 ^{cd}	0.667 ^a	0.067 ^b	0.361 ^{cd}	0.350 ^a	1.640 ^a
DBR 150	0.092 ^{de}	0.100 ^e	-	0.342 ^{cd}	0.113 ^b	0.677 ^{cd}
DBR 300	0.077 ^{de}	0.067 ^e	-	0.252 ^d	0.077 ^b	0.473 ^{de}
DBS 150	0.466 ^b	0.393 ^d	0.152 ^a	0.726 ^a	0.237 ^a	1.010 ^{bc}
DBS 300	0.759 ^a	0.600 ^{ab}	0.185 ^a	0.577 ^{ab}	0.230 ^a	1.630 ^a

Shoot fresh weight was measured at 30 d after treatment

IRR, Italian ryegrass root at 35 d after seeding (DAS); IRS, Italian ryegrass shoot at 35 DAS; DBR, downy brome root at 35 DAS; DBS, downy brome shoot at 35 DAS

Significant differences ($p < 0.05$) are indicated with different letter superscripts according to Duncan's Multiple Range Test.

m²) of DB roots. However, the above weed species were not significantly controlled by soil application (150 g m⁻² or 300 g m⁻²) of DB shoots. Furthermore, SFW of common lambsquarters, spiny sowthistle, and large crabgrass were increased 115%, 76%, and 80%, respectively, by soil application (300 g m⁻²) of DB shoots. This result was in agreement with the above study on increase of wheat growth by soil application of IRG shoots. Plant residues of many weeds show allelopathic effects on the germination and growth of subsequent crops (Mersie and Singh 1987). *Vulpia (Vulpia myuros)* residue displays phytotoxicity to germination, coleoptile and root growth of wheat (An et al. 1997). Plant residues in cultivated crop fields are an important resource not only as a source of significant quantities of nutrients for crop production but also as a source affecting soil physical and chemical properties. When plant residues are returned to the soil, crop production can have both positive and negative effects through decomposition of plant residues (Kumar and Goh 1999). However, the cover crop residue alone cannot lead to effective weed control; many studies indicate the need to provide additional weed control measures for reducing the competitive effects of the weeds toward the crops (Teasdale and Mohler 2000).

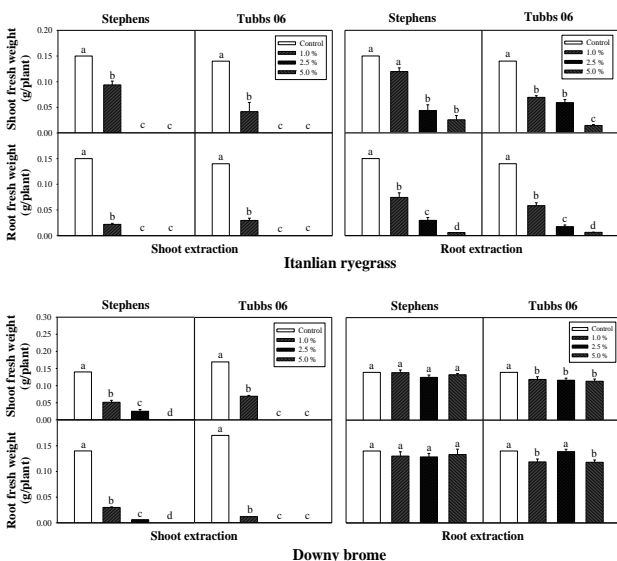


Fig. 4. Effects of fermentation extraction of Italian ryegrass and downy brome shoot and roots on wheat (cvs. Stephens and Tubbs 06) shoot and root fresh weight in Petri dishes. Parameters were measured at 7 d after treatment. Significant differences ($p \leq 0.05$) are indicated with different letters according to Duncan's Multiple Range Test.

From this perspective, cover crops can be a part of an integrated weed management strategy which combines the cover crop effect with mechanical or chemical means in order to obtain satisfactory weed control.

Effects of Extracts of Downy Brome and Italian Ryegrass Shoots and Roots on Wheat Growth

Shoot and root fresh weights of both wheat cultivars were inhibited by water extracts of IRG shoots at 0.5, 1, 2.5, 5, and 10% concentrations (Fig. 2). The levels of inhibition were increased with increasing extract concentrations. However, the shoot and root fresh weights of both wheat cultivars were not inhibited by water extracts of IRG roots. In addition, SFW of Tubbs 06 was increased 21–28% by water extraction of IRG roots at 5% and 10% compared with the control. Similar to the IRG study, shoot and root fresh weights of both wheat cultivars were inhibited by water extracts of DB shoots at 0.5, 1, 2.5, 5, and 10% concentrations, but not in water extracts of DB roots. Furthermore, the shoot and root fresh weights of both wheat cultivars were increased by water extracts of DB roots at 5% or 10% concentrations. Shoot and root fresh weights of wheat (cv. Stephens) were significantly inhibited by ethanol extract of IRG shoots at 2.5, 5, and 10% concentrations (Fig. 3). However, shoot and root fresh weights of Tubbs 06 were inhibited by ethanol extract of IRG shoots at only 5% or 10% concentrations.

Shoot and root fresh weights of both wheat cultivars were inhibited by ethanol extract of IRG roots at a concentration of only 10%. Shoot and root fresh weights of both wheat cultivars were inhibited by ethanol extract of DB shoots at 1, 2.5, 5, and 10% concentrations, but not in DB roots except for root fresh weight in cv. Stephens at 10% concentration. Shoot and root fresh weights of both wheat cultivars were completely inhibited by fermentation extract of IRG shoots at 2.5% and 5% concentrations (Fig. 4). Shoot and root fresh weights of both wheat cultivars were also 82–100% inhibited by fermentation extract of DB shoots at 2.5% and 5% concentrations. Shoot and root fresh weights of both wheat cultivars were 80–96% inhibited by fermentation extract of IRG roots at 2.5% and 5% concentrations. However, inhibition of shoot and root fresh weights of both wheat cultivars by fermentation extract of DB roots was below 15% even at 5% concentration. Reduction of shoot and root fresh weight in both wheat cultivars was observed more in fermentation extract of IRG roots than in water and ethanol extracts of IRG and DB roots and fermentation extract of DB roots. For the extracts from IRG and little barley, it was estimated that tissue concentrations of approximately 5.0 g L⁻¹ resulted in a 50% reduction in seed germination and seedling growth for alfalfa and IRG. Shoot extracts of *L. rigidum* displayed more consistent effects than root extracts on target species (San Emeterio et al. 2004).

Shoot extracts of the same genera *L. rigidum* used in our study stimulated shoot growth, but most studies

Table 4. Contents of phenolic compounds (mg/g) in fermentation extracts of shoots and roots of Italian ryegrass (IRG) and downy brome (DB).

Phenolic Compounds	IRG Shoot	IRG Root	DB Shoot	DB Root
Caffeic acid	41.13 ± 11.8	20.01 ± 5.8	466.31 ± 31.6	26.06 ± 2.8
<i>p</i> -Coumaric acid	6.83 ± 0.4	1.59 ± 0.5	4.92 ± 1.4	N. D
Ferulic acid	85.49 ± 2.6	378.31 ± 4.0	132.95 ± 14.5	N. D
Hydrocinnamic acid	51.85 ± 7.2	12.54 ± 2.7	181.45 ± 3.3	33.76 ± 4.8
Total	185.3	412.5	596.8	279.6

*N. D, Not detected

showed inhibition (Mandal 2001; Rice 1984; Tefera 2002). Lickfeldt et al. (2001) found that leaf extracts from perennial ryegrass inhibited germination and root growth of radish, IRG, lettuce, large crabgrass, and white clover in petri dishes. Our study demonstrated that IRG and DB residues displayed allelopathic activity and influenced the emergence and seedling growth of wheat and several weeds. The effects varied depending on the tissue and extract concentration, the target species and the growth parameters measured. Phenol compounds hydrocinnamic acid, caffeic acid, *p*-coumaric acid, and ferulic acid were confirmed in fermentation extraction of IRG and DB shoot and roots by HPLC analysis (Table 4). The contents of the phenol compounds were greater in IRG roots than in IRG shoots. It may be especially noted that the content of ferulic acid in IRG root was much higher than in IRG shoot. In contrast, the contents of phenol compounds were greater in DB shoots than in DB roots. Thus, total phenol contents were related to inhibition effect on wheat growth. The quantity of total phenolics was higher in the leaves of all the weeds compared with their roots (Chauhan and Chauhan 2014). Shoot and root weights of both wheat cultivars were inhibited 42–69% by 0.5, 1, and 3 mM treatments of phenol compounds *p*-coumaric acid, ferulic acid, and caffeic acid (Fig. 5). Therefore, retarded growth of wheat and weed may have been caused by the phenol compounds of DB and IRG.

CONCLUSION

Shoot fresh weight (SFW) of wheat cultivars Stephens and Tubbs 06 was reduced 28–53% and 34–55%, respectively, by DB and IRG residues grown at 25, 35, and 45 days after seeding (DAS) under greenhouse conditions, compared with the control. In addition, SFW of both wheat cultivars in soil application (150 g m⁻² or 300 g m⁻²) of IRG and DB roots grown at 35

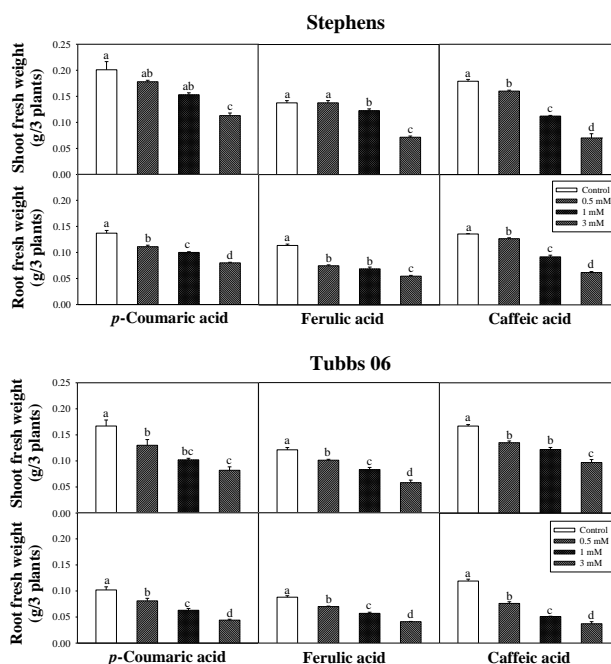


Fig. 5. Effect of phenolic compounds on shoot and root weights of wheat (cvs. Stephens and Tubbs 06). Parameters were measured at 7 d after treatment. Significant differences ($P \leq 0.05$) are indicated with different letters according to Duncan's Multiple Range Test.

DAS was reduced by 33–52% compared with the control. Growth of common lambsquarters, pigweed, spiny sowthistle, white clove, barnyardgrass, and large crabgrass was controlled 23–95% and 25–80% by soil application of IRG and DB roots, respectively, compared with the control. There were no significant differences in growth inhibition of wheat and weeds between different plant species (IRG and DB), cultivars (Stephens and Tubbs 06), and application amounts (150 g m⁻² or 300 g m⁻²) except for plant parts (shoot and root). Shoot and root fresh weights of both wheat cultivars were 80–100% inhibited by fermentation extract of IRG and DB shoots or IRG roots at 2.5% and 5% concentrations. However, shoot and root fresh weights of both wheat cultivars were inhibited by less than 15% by fermentation extract of DB roots even at 5% concentration. The phenol compounds hydrocinnamic acid, caffeic acid, *p*-coumaric acid, and ferulic acid were confirmed in the fermentation extraction of IRG and DB shoot and roots by HPLC analysis. Shoot and root weight of both wheat cultivars were inhibited 42–69% by 0.5, 1, and 3 mM treatments of the phenol compounds *p*-coumaric acid, ferulic acid, and caffeic acid. Therefore, retarded growth of wheat and weed may have been caused by the phenol compounds of the DB and IRG.

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