Allelopathic Potential of Various Plant Species on Downy Brome: Implications for Weed Control in Wheat Production

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ABSTRACT

Allelopathy, the ability of plants to inhibit germination of other plants, is an untapped resource for weed control in crops that could revolutionize organic crop production. The main objective of the study was to evaluate allelopathic potential of various plant species on downy brome (*Bromus tectorum* L.), a major pest of wheat (*Triticum aestivum* L.). To screen for potential allelopathy, plants were grown to flowering stage in a greenhouse, separated into shoots and roots, dried, and ground. Five percent aqueous extracts (w/v) were prepared by extracting 5 g of dried, ground plant samples with 100 mL of deionized water. Downy brome seeds were germinated on extract-amended sand. Extracts from most plant species tested inhibited downy brome and wheat seed germination. Extracts from broadleaf plants were more inhibitory than extracts from cereal plants. In most plant species, shoot extracts were more inhibitory to growth of the root and shoot of downy brome than root extracts. Meadowfoam seed meal (*Limnanthes alba* Hartw.), yard-long bean [*Vigna sesquipedalis* (L.) Fruw.], blue spruce (*Picea pungens pungens* Engelm), and pine (*Pinus* spp.) extracts, which completely inhibited the germination of downy brome seed, have the potential for use in the control of downy brome in wheat-based cropping systems. Meadowfoam seed meal extract inhibited wheat germination by 77% and root and shoot growth by 97 and 96%, respectively. Radishes reduced wheat germination by 75 to 100%, root growth by 54 to 80% and shoot growth by 45 to 81%. Plants evaluated in this study have the potential to be used for biologically based weed control methods in organic cropping systems.

Allelopathy, the ability of plants to inhibit germination of other plants, is so far, an untapped resource for weed control in crops. Yet, it shows considerable promise in both conventional and organic agriculture. The U.S. Pacific Northwest (PNW) agriculture has traditionally focused on conventional farming systems using synthesized pesticides. In conventional agriculture, weeds can and do develop resistance to pesticides being used to control them, making pesticides less and less effective (Putwain, 1982; Alizadeh et al., 1998; Tranel and Wright, 2002; De Prado and Franco, 2004). Concerns of ecological, environmental, and health problems possibly associated with synthesized pesticides has increased the interest in organic agriculture (Dayan et al., 1999; Walz, 1999). Under organic farming, no synthesized herbicides or fertilizers are tolerated (Wallace, 2001). Weeds are, however, controlled by tillage, a practice that is labor intensive. Furthermore, tillage to control weeds depletes organic matter and exposes the soil to wind and water erosion (Rasmussen and Parton, 1994). The development of biosynthesized herbicides to control weeds can permit the development of no-till (direct-seed) organic wheat production.

Many plant species have allelopathic effects on other plant species (Rice, 1974, 1984; Putman and Tang, 1986; Rice, 1995; Cutler and Cutler, 1999; Marcías et al., 2004; Vasilakoglou et al., 2005; Dhima et al., 2006). Information on the chemical nature and mode of action of allelochemicals is expanding (Rice, 1984; Cutler and Cutler, 1999; Dayan et al., 2000; Dayan, 2002; Inderjit and Duke, 2003; Marcías et al., 2004; Singh et al., 2005). Allelopathy may be used in several ways in weed control. Just as crop plants are bred for disease resistance, crop plants can be bred to be allelopathic to weeds common to specific regions (Rice, 1984, 1995; Jensen et al., 2001; Wu et al., 2000, 2003; Olofsdotter et al., 2002; He et al., 2004). The most practical and immediate way to use allelopathy in weed control is to use allelopathic cover crops in rotations, or apply residues of allelopathic weeds or crops as mulches (Rice, 1984; Caamal-Maldonaldo et al., 2001; Dhima et al., 2006). An equally promising way to use allelopathy in weed control is using extracts of allelopathic plants as herbicides (Dayan, 2002; Singh et al., 2005). Because biosynthesized herbicides are easily biodegradable, they are believed to be much safer than synthesized herbicides (Rice, 1984, 1995; Dayan et al., 1999; Duke et al., 2000).

The use of biosynthesized herbicides in the control of weeds common to the PNW should be evaluated. The main objective of this research was to screen various plants for allelopathic effects on downy brome, a major weed in wheat cropping systems of the PNW. This information is a prerequisite for the development of biological weed control methods that can allow for no-till organic crop production in the PNW.

Materials and Methods

The screening of different plant species for allelopathic potential on downy brome and on wheat injury was conducted at the Columbia Basin Agricultural Research Center (CBARC) near Pendleton (45.7° N lat, 118.6° W long, with elevation of 438 m), OR. To screen for allelopathic potential, a rapid bioassay technique (Gliessman, 2000) was used.

Extract Preparations

Forty-two plant species were screened for allelopathic potential on downy brome and wheat. Plants, except meadowfoam seed meal, Austrian pine (*Pinus* spp.), and blue spruce, were grown in pots in a greenhouse. At the beginning of flowering, the plants were harvested, separated into leaves and roots, and air-dried for ~96 h. Needles of blue spruce and pine

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Published in Agron. J. 99:127–132 (2007).
Notes & Unique Phenomena
doi:10.2134/agronj2006.0122

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Abbreviations: CBARC, Columbia Basin Agricultural Research Center; PNW, U.S. Pacific Northwest.

trees were collected from nearby trees and dried. The dried plant material was then ground to pass a 2-mm sieve using a Thomas-Wiley laboratory mill (Model 4). Aqueous extracts (w/v) were prepared by extracting 5 g of dried, ground plant samples with 100 mL of deionized water in a shaker for 2 h using 200-mL Erlenmeyer flasks. The mixture was then filtered using filter paper (Whatman no. 1) to obtain 5% extracts. Extracts from Austrian pine and blue spruce were obtained by shaking 5 g of dried, ground needles and 5 g of pine tar in 20 mL of 90% ethanol in 125-mL flasks for 2 h at ~25°C at a moderate speed. The extracts were filtered under vacuum through Whatman no.1 filter paper and the leachates transferred to 100-mL test tubes. The test tubes were placed in a water bath at 80°C under a hood to evaporate the ethanol. The extracts were taken-up in distilled water as the ethanol evaporated, and the process was complete when the ethanol smell was no longer "humanly" detected. Extracts were then transferred to 125-mL flasks or beakers, and deionized water was added to reach the 100 mL mark to obtain 5% leachates.

Screening for Allelopathic Potential

About 45 g of clean sand was weighed into 9-cm diam. Petri dishes. A Whatman no.1 filter paper was then placed on the sand surface in each dish. Using volumetric pipettes, 10.0 mL of the extract was pipetted into the Petri dishes. Deionized water was used for the control. Using tweezers, 10 nondormant seeds (99.9% germination) of downy brome were placed, equally spaced, in a circle on the filter paper placed on the surface of each Petri dish. Three Whatman no.1 filter papers were then soaked in the same extract that was pipetted into the Petri dishes (or deionized water for the control) and placed over the seeds. Lids were placed over the Petri dishes and the Petri dishes were then placed in a dark incubator set at 25°C for 72 h. After 72 h, the Petri dishes were removed from the incubator and data on downy brome seed germination and root and shoot growth were taken. Germination was defined as root emergence. Root and shoot growth was determined by measuring root and shoot length to the nearest tenth of a millimeter. Extracts from selected plant species that showed strong phytotoxic effects on downy brome were evaluated on 'Stephens' wheat seed as well.

Data Collection and Statistical Analysis

The bioassay was conducted with four replications using a completely randomized design. Percentage growth inhibition (using all seed with seed that did not germinate being zero) was calculated by the following equation:

$$\% \text{ Reduction} = [(\text{Control} - \text{Extracts}) \div \text{Control}] \times 100$$

The analysis of variance was conducted using the general linear model (PROC GLM) procedure in Statistical Analysis System (SAS) program (SAS Institute, 1997). Means were separated using the LSD test and statistical significance was evaluated at $P = 0.05$. Simple correlations (Pearson) among variables were calculated using PROC CORR in SAS.

Results

Phytotoxic Effects of Shoot Extracts on Downy Brome Seed Germination and Root and Shoot Growth

Plant shoot extracts effects on downy brome seed germination and root and shoot growth are shown in Table 1. Downy brome seed germination was significantly reduced by most, except six, plant extracts when compared to the control. Germination percentage ranged from 0 to 90. Meadowfoam seed meal and shoot extracts of yard-long bean, blue spruce, Austrian pine needles, Austrian pine bark, and pine tar completely inhibited the germination of downy brome. Shoot extracts of all plant species evaluated were phytotoxic to downy brome root and shoot growth when compared to the control. Shoot extracts reduced downy brome root growth by 33 to 99% and shoot growth by 25 to 99% . Radishes [white icicle (*Raphanus sativus* L.), daikon long (*R. sativus* L.), French breakfast (*R. sativus* L.), gourmet blend (*R. sativus* L.), cherry belle (*R. sativus* L.), sparkler (*R. sativus* L.), early scarlet (*R. sativus* L.), and shogoin giant (*Raphanus sativus* L.)], grain amaranth (*Amaranthus cruentus* L.), mustard (*Sinapis alba* L.), marigold (*Tagetes* spp.), brown flax (*Linum usitatissimum* L.), sugar pea (*Pisum sativum* L.), and pigeon pea [*Cajanus cajan* (L.) Millsp.] were more phytotoxic than the other plant species. Riser oat (*Avena sativa* L.), annual ryegrass (*Lolium multiflorum* Lam.), dent corn (*Zea mays* L.), and winged bean (*Psophocarpus tetragonolobus* L.) were the least effective in reducing downy brome root growth; and buckwheat (*Fagopyrum esculentum* Moench), dent corn, riser oat, annual ryegrass and winged bean were the least effective in reducing downy brome shoot growth. Shoot extracts from legumes and broadleaves tended to inhibit both root and shoot growth of downy brome more than cereals. There was a strong correlation between percentage germination and phytotoxicity of root ($r^2 = 0.75$, $P < 0.01$) and shoot ($r^2 = 0.70$, $P < 0.01$). In addition, root growth inhibition was highly correlated with shoot growth inhibition ($r^2 = 0.96$, $P < 0.01$).

Phytotoxic Effects of Root Extracts on Downy Brome Seed Germination and Root and Shoot Growth

The effects of plant root extracts on downy brome seed germination and root and shoot growth are shown in Table 2. Only data from plant species with adequate root biomass (5 g) for the assay are shown. Downy brome seed germination was significantly affected by 43% of the plant extracts and ranged from 5 to 98%. Root extracts reduced downy brome root growth by 7 to 99% and shoot growth by 6 to 99%. Extracts from lab lab rongai (*Dolichos lablab* L.), tepary bean (*Phaseolus acutifolius* A. Gray), grain sorghum [*Sorghum bicolor* (L.) Moench], grain amaranth (*Amaranthus cruentus*), sparkler radish, and hairy vetch (*Vicia villosa* Roth) were most effective, but none completely inhibited downy brome seed germination. Annual ryegrass, and robust barley (*Hordeum vulgare* L.) were the least effective. Seed germination was significantly correlated with root ($r^2 = 0.87$, $P < 0.01$) and shoot ($r^2 = 0.75$, $P < 0.01$) growth. Root growth inhibition was also highly correlated with shoot growth inhibition ($r^2 = 0.84$, $P < 0.01$).

Phytotoxic Effects of Plant Extracts on Wheat Seed Germination and Root and Shoot Growth

Extracts with the greatest potential to inhibit downy brome germination and growth (meadowfoam seed meal and six radish species) were evaluated for phyto-

Table 1. Phytotoxic effects of plant shoot extracts (5%) on downy brome germination, and root and shoot growth.

Plant name	Scientific name	Germination	Root		Shoot	
			Length	Reduction†	Length	Reduction†
		%	mm	%	mm	%
Meadowfoam seed meal	*Limnanthes alba* Hartw.	0	0.0	100	0.0	100
Yard-long bean	*Vigna sesquipedalis* (L.) Fruw.	0	0.0	100	0.0	100
Blue spruce (needles)	*Picea pungens pungens* Engelm	0	0.0	100	0.0	100
Austrian pine (needles)	*Pinus* spp.	0	0.0	100	0.0	100
Austrian pine (bark)	*Pinus* spp.	0	0.0	100	0.0	100
Pine (oil)	*Pinus* spp.	0	0.0	100	0.0	100
White icicle radish	*Raphanus sativus* L.	10	0.1	100	0.0	100
Daikon long radish	*Raphanus sativus* L.	23	0.2	99	0.1	99
Grain amaranth	*Amaranthus cruentus* L.	5	0.2	99	0.1	100
French breakfast radish	*Raphanus sativus* L.	8	0.3	99	0.0	100
Gourmet blend radish	*Raphanus sativus* L.	15	0.3	99	0.0	100
Cherry belle radish	*Raphanus sativus* L.	20	0.5	99	0.2	99
Sparkler radish	*Raphanus sativus* L.	5	0.5	99	0.6	96
Early scarlet radish	*Raphanus sativus* L.	53	1.5	96	0.0	100
Mustard	*Sinapis alba* L.	53	1.6	96	0.1	99
Brown flax	*Linum usitatissimum* L.	63	1.8	95	0.5	96
Shogoin giant radish	*Raphanus sativus* L.	58	1.9	95	0.3	98
Sugar pea	*Pisum sativum* L.	25	2.4	94	0.4	97
Pigeon pea	*Cajanus cajan* (L.) Millsp.	25	2.4	93	0.4	97
Marigold Petite mix	*Tagetes* spp.	43	2.8	93	0.6	95
Marigold Cracker Jack mix	*Tagetes* spp.	35	2.8	92	0.2	99
Bush bean	*Phaseolus vulgaris* L.	43	3.8	90	0.4	97
Velvet bean	*Mucuna deeringiana* (Bort.) Merr.	60	4.1	89	0.7	95
Wax bean	*Phaseolus vulgaris* L.	48	4.2	89	0.6	95
Hairy vetch	*Vicia villosa* Roth	53	4.4	88	1.0	92
Bean (Mark Siemens)	*Phaseolus vulgaris* L.	48	4.5	88	0.8	93
Marigold Sparky mix	*Tagetes* spp.	50	4.6	88	1.1	91
Tepary bean tohono	*Phaseolus acutifolius* A. Gray	40	4.9	87	0.9	93
Tepary bean	*Phaseolus acutifolius* A. Gray	28	5.0	86	1.2	91
Safflower	*Carthamus tinctorius* L.	68	6.1	83	1.7	87
Soybean	*Glycine max* (L.) Merr.	45	7.9	79	1.1	92
Buckwheat	*Fagopyrum esculentum* Moench	83	8.6	77	4.2	66
Lab lab rongai	*Dolichos lablab* L.	55	8.7	77	2.6	79
Chickpea myles	*Cicer arietinum* L.	90	10.9	71	2.2	83
Grain sorghum	*Sorghum bicolor* (L.) Moench	60	11.6	69	3.0	76
Robust barley	*Hordeum vulgare* L.	48	11.9	68	3.8	69
Barley	*Hordeum vulgare* L.	68	12.3	67	3.5	72
Sunflower (oil type)	*Helianthus annuus* L.	75	15.5	58	3.8	70
Riser oat	*Avena sativa* L.	80	15.5	58	5.8	54
Annual ryegrass	*Lolium multiflorum* Lam.	83	22.0	40	6.3	50
Dent corn	*Zea mays* L.	70	22.3	40	4.3	66
Winged bean	*Psophocarpus tetragonolobus* (L.)	88	24.6	33	9.4	25
Water (deionized)	H_2O	89	36.9	0	12.5	0
LSD(0.05)		25	0.7		0.3	

† Percentage reduction compared with control.

toxicity on wheat seed germination, and root and shoot growth (Table 3). While none of the extracts completely inhibited wheat seed germination, all extracts significantly reduced wheat seed germination and root and shoot growth compared to control. Meadowfoam seed meal extract inhibited wheat germination by 77% and root and shoot growth by 97 and 96%, respectively. Although wheat germination was 75 to 100% using radish extracts, these extracts reduced root growth by 54 to 80% and shoot growth by 45 to 81%. There was a positive correlation between germination response and root ($r = 0.89$, $P < 0.01$) and shoot growth ($r = 0.80$, $P < 0.05$) and between root and shoot growth ($r = 0.98$, $P < 0.01$).

Discussion

Results clearly demonstrated that most of the plant species tested inhibited seed germination and root and shoot growth of downy brome and wheat. The high and positive correlation between downy brome germination and root and shoot growth may indicate that extracts that allowed rapid germination also allowed more time for root and shoot growth compared to extracts that delayed germination. This suggests that the reduction in root and shoot growth may have been a reflection of delayed germination rather than due to a direct effect of an allelochemical. These results indicate that most of the plant extracts evaluated in this study act by inhibiting seed germination and may have potential for preemergence weed control. However, there were exceptions that suggested that reduction in root and shoot lengths were the direct effect of allelochemicals. These exceptions involved results where germination responses to different extracts were not significantly different yet root and shoot lengths were significantly different (Table 1 and 2). These results suggest that some extracts targeted root and shoot growth and may have potential for postemergence weed control. But without information on rate of germination, it is impossible to firmly conclude that the reduction in root and shoot growth was due to a direct extract effect or due to delayed germination.

Table 2. Phytotoxic effects of plant root extracts (5%) on downy brome germination, and root and shoot growth.

Common name	Scientific name	Germination	Root		Shoot	
			Length	Reduction	Length	Reduction
		%	mm	%	mm	%
Lab lab rongai	*Dolichos lablab* L.	5	0.2	99	0.1	100
Tepary bean	*Phaseolus acutifolius* A. Gray	10	0.7	98	0.1	99
Grain sorghum	*Sorghum bicolor* (L.) Moench	28	4.2	88	1.2	91
Grain amaranth	*Amaranthus cruentus* L.	63	4.3	87	2.8	80
Sparkler radish	*Raphanus sativus* L.	45	4.6	86	1.3	91
Hairy vetch	*Vicia villosa* Roth	33	5.8	83	2.1	84
Winged bean	*Psophocarpus tetragonolobus* (L.)	33	9.5	72	3.8	72
Yard-long bean	*Vigna sesquipedalis* (L.) Fruw.	53	11.0	67	6.0	56
Sunflower (oil type)	*Helianthus annuus* L.	58	12.5	63	3.3	76
French breakfast radish	*Raphanus sativus* L.	75	14.3	58	7.9	42
Bean (Mark Siemens)	*Phaseolus vulgaris* L	75	16.6	51	7.7	44
Buckwheat	*Fagopyrum esculentum* Moench	78	17.7	48	4.8	65
Bush bean	*Phaseolus vulgaris* L.	73	22.0	35	8.7	36
Wax bean	*Phaseolus vulgaris* L.	85	23.8	29	10.9	20
Velvet bean	*Mucuna deeringiana* (Bort.) Merr.	90	26.5	21	9.7	29
Soybean	*Glycine max* (L.) Merr.	70	30.6	9	10.2	25
Dent corn	*Zea mays* L.	78	30.8	9	10.2	26
Barley	*Hordeum vulgare* L.	88	31.8	6	13.7	0
Mustard	*Sinapis alba* L.	98	32.8	3	10.7	21
Annual ryegrass	*Lolium multiflorum* Lam.	93	33.7	0	12.8	6
Robust barley	*Hordeum vulgare* L.	83	35.9	−7	12.7	7
Water (deionized)	H_2O	92	33.6	0	13.6	0
LSD(0.05)		26	11.0		5.0	

Extracts from broadleaf plants were generally more phytotoxic than those from cereal plants, information that has important implications for weed control in cereal-based cropping systems. Despite beneficial effects of broadleaves in crop rotations, most small grain farmers in the PNW practice wheat monoculture. While poor markets and low prices discourage broadleaf rotations, this new information makes a good case for including them in wheat-based systems. Allelopathic broadleaves could be used as cover crops that could be mowed and undercut at flowering to provide mulch that controls weeds during the fallow period of the wheat–fallow cropping system that is predominant in the PNW. In particular, broadleaf legumes with allelopathic potential, would not only have the potential to reduce weeds, but also add N to the soil (Power, 1987), thereby reducing herbicide and N input costs.

Of the plant species evaluated, shoot extracts, comprising mostly of leaves, were more effective in reducing seed germination and root and shoot length of downy brome than root extracts. Oueslati (2003), Turk et al. (2003), Tawaha and Turk (2003) made similar observations on other plant species. However some plants, such as winged bean, lab lab rongai, tepary bean, grain sorghum, and sunflower (*Carthamus tinctorius* L.), showed more phytotoxic effects in root than in shoot extracts. Differences in shoot and root extract effects may indicate the presence of different allelochemicals or concentrations of allelochemicals in roots and shoots. For example, sorgoleone, an allelochemical of sorghum, constituted more than 80% of root exudate composition (Nimbal et al., 1996; Czarnota et al., 2003) but none was found in immature and mature leaves and stems of sorghum (Yang et al., 2004). In contrast, sorghum shoots produce higher amounts of cynogenic glucosides whose phenolic breakdown products inhibit plant growth (Einhellig and Rasmussen, 1989; Weston et al., 1989; Séne et al., 2001). The relative effectiveness of shoot and root extracts is important in formulating weed control strategies.

Plant extracts that were phytotoxic to downy brome also reduced seed germination and root and shoot growth of wheat. Meadowfoam seed meal extract, in particular, was very effective on both downy brome and wheat. However, wheat was less sensitive than downy brome to various plant extracts probably because the wheat seed was relatively larger. Pérez (1990) found that smaller seeds were generally more sensitive to allelochemicals and that seed size influenced the concentration of allelochemicals necessary to produce an effect on

Table 3. Phytotoxic effects of plant root and shoot extracts (5%) on wheat germination, and root and shoot growth.

Plant name	Scientific name	Germination	Root		Shoot	
			Length	Reduction	Length	Reduction
		%	mm	%	mm	%
Meadowfoam (meal)	*Limnanthes alba* Hartw.	23	1.0	97	0.9	96
Daikon long radish (leaves)	*Raphanus sativus* L.	80	6.6	80	4.2	81
Shogoin giant radish (leaves)	*Raphanus sativus* L.	85	7.4	78	4.6	79
Early scarlet radish (leaves)	*Raphanus sativus* L.	75	8.3	75	5.0	77
Sparkler radish (leaves)	*Raphanus sativus* L.	85	8.7	74	5.5	75
White icicle radish (leaves)	*Raphanus sativus* L.	78	9.0	73	7.0	68
French breakfast radish (root)	*Raphanus sativus* L.	100	15.2	54	11.9	45
DI Water	H_2O	90	33.3	0	21.8	0
LSD(0.05)		41	2.1		2.1	

seed germination. Even though wheat was less sensitive to various plant extracts, care should be taken to minimize any such effect. In practice, wheat seeding could be delayed to avoid the inhibitory period of extracts. To this end, breakdown patterns of allelochemicals of various plant species under field conditions require investigations.

Allelochemicals in some of the crops evaluated in this study have been revealed in other studies. Allelopathy in meadowfoam, radishes, and mustard was attributed to glucosinolates (Vaughn et al., 1996; Vaughn, 1999). Quercetin and its derivatives such as rutin were responsible for allelopathy in buckwheat (Golisz et al., 2004; Kalinová et al., 2004). Most legumes (bean, pea, vetch) produced quinolizidine alkaloids that may be responsible for allelopathy (Wink, 2004). In velvet bean [*Mucuna deeringiana* (Bort.) Merr.], L-3,4-dihydroxyphenylalanine (L-DOPA), an intermediate of many alkaloids, was determined to be the allelochemical (Fujii, 1999). Allelopathy in cereals (cultivated and wild plants of the Gramineae family) was attributed mostly to hydroxamic acids (Sánchez-Moreiras et al., 2004). Sorgoleone was identified as the allelochemical from sorghum roots and heliannuols, annuolides, tambulin, and heliannones were identified as allelochemicals from sunflower (Vyvyan, 2002).

Conclusions

Most plant species screened in this study, in particular, meadowfoam seed meal, yard-long bean, blue spruce, pine, and radishes, which completely inhibited the germination of downy brome seed, have great potential for downy brome control in wheat-based cropping systems. Additional work is required to test the efficacy of residues or extracts from these plants on weed control under field conditions and to isolate and identify allelochemicals involved. This information may allow the development of biosynthesized herbicides and other biologically based weed control methods that could lead to no-till organic wheat production in the PNW.

Acknowledgments

The authors would like to thank the USEPA for providing the grant to conduct this research and Christopher Humphreys for assistance in data collection.

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