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Variation within flax (*Linum usitatissimum*) and barley (*Hordeum vulgare*) in response to allelopathic chemicals

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Summary. A possible method of manipulating allelopathy would be to develop crop varieties showing an increased tolerance to allelopathic chemicals. We therefore examined four flax (*Linum usitatissimum*) varieties and two wild *Linum* species in the presence of *p*-coumaric acid and four barley (*Hordeum vulgare*) varieties in the presence of *p*-coumaric acid, scopoletin and wild oat (*Avena fatua*) extract. Analysis of variance indicates significant interaction between variety and treatment for shoot and root growth for seedling flax, shoot growth for older flax, and root growth for seedling barley. These differences in tolerance between varieties could be exploited to develop varieties with greater tolerances to the allelochemicals produced by weeds or in crop residues and therefore potentially more tolerant of the presence of weeds.

Key words: Flax – Barley – Wild oats – Allelopathy – Variation

Introduction

Performance in the presence of weeds varies between different crops. Fifty wild oat (*Avena fatua*) plants per square meter reduces the yield of flax (*Linum usitatissimum*) by 40%, wheat (*Triticum aestivum*) by 23%, and barley (*Hordeum vulgare*) by only 15% (O'Donovan and Sharma 1983). This variation in interference is generally ascribed to differences in the degree of competitiveness of the crop species. However, allelopathic chemicals, toxins which may be exuded by both the crop plant and the weed, may also affect the ability of the plant to perform well in the presence of weeds. Allelopathic potential has been identified in a large number of aggressive weed species (Putnam and Weston 1986). Commonly, allelopathic chemicals are phenolic compounds such as coumarins and phenolic acids. Wild oats may release the coumarin scopoletin, the phenolic acids *p*-coumaric acid, vanillic acid, ferulic acid, and possibly other phenolic compounds (Tinnin and Muller 1972; Schumacher et al. 1983).

It may be possible to manipulate the phenomenon of allelopathy either by increasing the toxicity of the crop plant to weeds, as suggested by Putnam and Duke (1974), or by increasing the tolerance of the crop plant to weeds. Either approach requires the existence of variation in toxicity or tolerance within the crop species.

Lockerman and Putnam (1979) found cucumber (*Cucumis sativum*) accessions of higher toxicity, as seedlings, towards several weeds. These accessions were able to reduce the germination and growth of adjacent weeds enough to substantially improve their own growth.

The existence of variation in tolerance of allelopathic chemicals has not, to our knowledge, been documented, although A. Kilvert in our laboratory has identified such variation in alfalfa (*Medicago sativa*) varieties (personal communication) and Huber and Abney (1986) noted that soybean (*Glycine max*) residue affected two cultivars of wheat differently. The existence of within-species variation in weed tolerance in the field has been identified in tall fescue (*Festuca arundinacea*) (Peters and Zam 1981), sorghum (*Sorghum bicolor*) (Alsaadawi et al. 1986), and wheat (Reeves and Brooke 1977). The latter found that they could not attribute the differences in yield reduction to cultivar height, tiller number, dry matter production, spikelet number or grain yield of controls. These cases might potentially be due to differences in allelochemical production or tolerance, or to other factors.

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We wished to establish whether variation in response to allelochemicals exists within crop species and consequently examined cultivars of barley and flax (including two wild *Linum* species) in the presence and absence of two common allelopathic chemicals, *p*-coumaric acid or scopoletin, and extract of wild oats shoots. In this article we report variation in response to these compounds.

Materials and methods

Flax

Seed of four varieties of *Linum usitatissimum* ('Raja', 'McGregor', 'Indian Type 8', and 'Abyssinian Brown'), *Linum halogynum*, and *Linum pallescens* was drawn from the University of Alberta flax collection of the late Dr. W. E. Smith. Fifty seeds per treatment per variety were used. The seeds were surface sterilized by soaking them first for 1 min in 70% ethanol and then for approximately 20 min in 25% commercial bleach; this was followed by extensive rinsing in distilled water. Seeds were placed singly in a 10-cm-deep vermiculite layer in 15-cm test tubes. Ten milliliters of Hoagland's solution (Wetter and Constabel 1982) or Hoagland's solution containing 10^{-3} *M* *p*-coumaric acid were added to each tube. Vermiculite and solutions were autoclaved; the pH was adjusted to 5.9, previous trials having shown that pH had little effect on the results of phenolic acid treatments. The tubes were randomly placed in every second row in racks to help equalize light intensity. Seeds were grown in the dark for 4 days at 22 °C, then moved to a 23°–26 °C growth chamber under 16 h light, 8 h dark, under fluorescent lights. The seeds were handled as uniformly as possible.

At 3 weeks the shoot length of all seedlings was measured. For 10 seedlings per treatment per variety, root length was measured; the seedlings were then dried overnight at 55 °C, and shoot and root dry weight were measured. Twenty untreated and 20 treated seedlings of each variety were potted in 6:1 potting soil:vermiculite, 5 per 15-cm pot, and all pots randomized and frequently rotated. Seedlings shorter than 20 mm (late-germinating in previous trials) were omitted; otherwise assignment within a treatment and variety was random. To previously treated potted seedlings 10 ml of 10^{-2} *M* *p*-coumaric acid was added once weekly. Care was taken not to wet the leaves. Plant height was measured at 6, 9, 11, 14 and 19 weeks. At 19 weeks dry weight per top, stem number, seed number, and seed weight per plant were measured.

To examine the effects of a combination of phenolic compounds on flax cv 'McGregor', *p*-coumaric, ferulic, *p*-hydroxybenzoic and vanillic acids totalling 4×10^{-3} *M* were made up in half-strength Hoagland's solution. The pH of phenolic solutions varied from 3.3 to 4.4. Five centimeters of sterile solution was added to about 5 cm of sterile vermiculite. The seeds were not sterilized, but added to test tubes and grown as above. Seedlings were harvested at 14 days; shoot and root length and dry weight were determined.

Barley

Agriculture Alberta donated the barley varieties 'Conquest', 'Elrose', 'Galt', and 'Johnston'. Twenty seeds per treatment per variety were used. Seeds were surface sterilized by first soaking them for 2 min in 70% ethanol and then for 10 min in 25% bleach; this was followed by extensive rinsing. Single seeds were placed on a 5-cm-deep sterile vermiculite layer with 5 ml half-strength Hoagland's solution with or without 95% (vol/vol) of an overnight water extract of wild oats (about 30% fresh weight per volume water), 2×10^{-3} *M* *p*-coumaric acid, or 5×10^{-4} *M* scopoletin. The pH was adjusted to 5.9 in all cases. The tubes were randomized, spaced in racks and grown as were the flax seedlings until 10 days, when shoot height, root length, and shoot dry weight were measured. For root length only the 3rd to 10th tallest plants were measured, a procedure that is likely to underestimate root damage to some degree.

Results

Flax

A previous trial involving 21 varieties and species of flax treated with wild oat extract showed considerable variation in response among varieties (H. Ray unpublished data). From this previous trial we selected the species and varieties used in the experiment detailed here. We examined two wild *Linum* species, two Canadian cultivars, and two randomly selected others for tolerance of *p*-coumaric acid. Table 1 shows several parameters of these varieties at 3, 6, 9 and 19 weeks. At 3 weeks seedlings treated with *p*-coumaric acid showed substantially decreased shoot height, root length, and shoot and root dry weight. Analysis of variance (ANOVA) showed significant effects ($P < 0.05$ or 0.01) of treatment, variety, and the interaction between them, for shoot height and root length (Table 1). Untreated seedlings continued to be taller than treated ones for several weeks; at flowering, height increase stopped and the treated plants caught up. The delay in height increase produced by *p*-coumaric acid varied from 1 to 8 days. Two varieties set seed very poorly in the growth chamber so harvest data are omitted. ANOVA showed significant interaction between treatment and variety at 6 and 9 weeks for shoot height (Table 1). Overall, 'McGregor' appears most tolerant, and 'Raja' least tolerant, but rankings vary somewhat with measurement. By harvest each pot had received the equivalent of 100 kg/ha *p*-coumaric acid on a surface area basis; sorghum residue incorporated into soil releases this amount of *p*-coumaric acid (Guenzi and McCalla 1966).

Flax seedlings treated with combinations of phenolic acids showed additive degrees of damage in most cases; that is, the damage produced by a combination of phenolic acids totalling 4×10^{-3} *M* is approximately equal to the average of the damage produced by this concentration of the components when considered individually (Table 2). ANOVA found no significant difference between the values for mixed treatments and the averages of single-compound treatments except for root length in seedlings treated with *p*-coumaric and vanillic acids.

Barley

Each variety of barley was subjected to three allelopathic treatments, none identical to those experienced by flax:

Table 1. Effects of 10^{-3} *M* *p*-coumaric acid on flax at 3, 6, 9, and 19 weeks[b]

Variety		3 weeks[a]							
		Shoot height (mm)		Root length (mm)		Shoot dry weight (mg)		Root dry weight (mg)	
Raja	Un	85.4		187		20		22	
	Tr	67.3 a	79%	148 ab	79%	11	55%	15	68%
McGregor	Un	90.3		141		19		15	
	Tr	79.6 b	88%	126 ab	89%	12	63%	10	67%
Indian Type 8	Un	93.4		142		20		21	
	Tr	72.8 a	78%	129 a	91%	10	50%	12	57%
Abyssinian Brown	Un	81.2		150		14		13	
	Tr	61.4 c	73%	99 b	66%	6	43%	5	38%
L. halogynum	Un	87.8		159		17		16	
	Tr	69.6 a	79%	133 a	84%	9	53%	10	63%
L. pallescens	Un	99.4		147		20		18	
	Tr	85.1 b	86%	131 a	89%	13	65%	14	78%
Average			81%		83%		54%		63%
Interaction component from ANOVA		*		**		n.a.		n.a.	

Variety		6 weeks		9 weeks		19 weeks			
		Shoot height (mm)		Shoot height (mm)		Shoot height (mm)		Top dry weight (mg)	
Raja	Un	210		376		499		467	
	Tr	148 a	71%	302 a	80%	500	100%	382	82%
McGregor	Un	159		261		658		863	
	Tr	139 b	87%	264 c	101%	687	104%	704	82%
Indian Type 8	Un	204		345		688		802	
	Tr	156 ab	76%	295 ab	86%	669	97%	671	84%
Abyssinian Brown	Un	116		203		416		458	
	Tr	91 ab	79%	170 ab	83%	428	103%	369	81%
L. halogynum	Un	160		297		543		534	
	Tr	127 ab	79%	248 ab	83%	522	96%	501	94%
L. pallescens	Un	185		284		600		724	
	Tr	153 ab	83%	266 bc	94%	564	94%	634	88%
Average			79%		88%		99%		85%
Interaction component from ANOVA		**		**		n.d.		n.a.	

* Indicates significant treatment × variety interaction at the 0.05 level; ** at the 0.01 level; n.a. indicates not applicable; n.d. not done; Un, untreated; Tr treated

[a] At 3 weeks, seedlings <20 mm are not considered

[b] Responses which are not significantly different at the 0.05 level by Newman-Keuls' test are indicated by the same letter, where applicable

wild oat shoot extract, 2×10^{-3} *M* *p*-coumaric acid, or 5×10^{-4} *M* scopoletin. Barley shoot height and weight were little affected by any treatment, but root length was severely affected (Table 3). For all three treatments ANOVA showed significant effect of treatment, variety, and their interaction for root length at 10 days ($P<0.05$ or 0.01; Table 3). 'Elrose' appears to be significantly more tolerant overall than 'Conquest' by the Newman-Keuls' Multiple Range Test.

Discussion

Weeds interact with crop performance by three main routes: competition for resources, production of allelochemicals by both crop and weed, generally to the injury of the other, and tolerance on the part of either to those allelochemicals. Changes in any of these factors may affect the others. For example, plants that have been injured by allelopathy as seedlings are less competitive long after this stress is removed (H. Ray, unpublished data).

Different varieties of flax and barley respond significantly differently to allelopathic stress as indicated by the treatment × variety component of ANOVA and by Newman-Keuls' Multiple Range Test. Flax is the more sensitive species both to interference by wild oats in the field

(O'Donovan and Sharma 1983), wild oat extract (H. Ray unpublished data), and pure allelochemicals produced by wild oats. Barley is more tolerant of wild oat interference in the field (O'Donovan and Sharma 1983), wild oat extract, and pure allelochemicals.

Table 2. Effects of phenolic acids, added either alone or in combination to a total of 4×10^{-3} *M* on "McGregor" flax root and shoot length

	Shoot length as % of control	Root length as % of control
Control	100	100
Ferulic acid (4 m*M*)	39.6	31
p-Coumaric acid (4 m*M*)	36.9	19
p-Hydroxybenzoic acid (4 m*M*)	43.7	48
Vanillic acid (4 m*M*)	46.2	25
Fer. + Cou. (2 m*M* each)	42.1 n.s.	34 n.s.
Fer. + Van. (2 m*M* each)	41.8 n.s.	28 n.s.
Cou. + Van. (2 m*M* each)	42.4 n.s.	27 *
Fer. + Cou. + Van. + Benz. (1 m*M* each)	43.1 n.s.	30 n.s.

* Indicates significant difference by ANOVA at the 0.05 level from average of components added singly to same total molarity; n.s. indicates no significant difference by ANOVA;
All the treatments are significantly different from the untreated control

The phenolic compounds studied here are produced in various amounts by wild oats. Wild oat hay contains *p*-coumaric acid, ferulic acid, vanillic acid and scopoletin (Tinnin and Muller 1972). Wild oat root exudate from 1- to 4-leaf plants contains vanillic and/or *p*-hydroxybenzoic acid and scopoletin and/or umbelliferone (Schumacher et al. 1983). Unfortunately these were not quantified. Guenzi and McCalla (1966) found *p*-coumaric acid to be the chief phenolic acid in domestic oat hay, which contained the equivalent of 26 kg/ha *p*-coumaric acid as well as small amounts of ferulic, vanillic, and *p*-hydroxybenzoic acid. (Domestic and wild oats may be considered to be the same biological species; Baum 1968.) While there may be other, unidentified components, it is probable that those identified – *p*-coumaric, ferulic, and vanillic acids, and scopoletin – account for the bulk of the allelopathic activity of wild oats and wild oat extracts. The effect of these compounds in combination on flax or wheat was additive in all but one combination, a finding compatible with the results of Rasmussen and Einhellig (Rasmussen and Einhellig 1977; Einhellig and Rasmussen 1978) on grain sorghum. The additivity may indicate that they act by a similar mechanism and therefore that the effect of mixtures may be estimated by using a single chemical, adjusted by a factor for relative toxicity. However, the specific types of injury produced by each are sometimes distinguishable. There is strong similarity

Table 3. Effects of 2×10^{-3} *M* *p*-coumaric acid, 5×10^{-4} *M* scopoletin, or 95% wild oat extract on barley at 10 days[a]

Treatment	Variety	Shoot height (mm)		Root length (mm)		Shoot dry weight (mg)	
Control	Conquest	176	100%	132	100%	21.6	100%
	Elrose	136	100%	121	100%	19.1	100%
	Galt	159	100%	137	100%	18.3	100%
	Johnston	171	100%	119	100%	14.8	100%
p-Coumaric acid	Conquest	158	90%	82 a	62%	19	88%
	Elrose	130	96%	107 b	89%	17.7	93%
	Galt	136	85%	91 a	66%	16.2	89%
	Johnston	156	91%	83 a	70%	13.2	89%
	Average		91%		72%		90%
	ANOVA interaction	n.s.		**		n.a.	
Scopoletin	Conquest	176 a	100%	64 a	48%	22.7	105%
	Elrose	143 a	105%	69 ab	57%	18.3	96%
	Galt	150 a	94%	73 ab	53%	18.3	100%
	Johnston	175 a	102%	72 b	60%	15.7	106%
	Average		100%		55%		102%
	ANOVA interaction	**		*		n.a.	
Wild oat extract	Conquest	167	95%	82 a	62%	19.8	92%
	Elrose	137	100%	117 b	97%	20.2	106%
	Galt	150	95%	125 b	91%	18.3	100%
	Johnston	175	102%	109 b	91%	16.8	114%
	Average		98%		85%		103%
	ANOVA interaction	n.s.		**		n.a.	

* Indicates significant treatment × variety interaction at the 0.05 level; ** at the 0.01 level; n.s. not significant; n.a. not applicable
[a] Responses which are not significant different at the 0.05 level by Newman-Keuls' test are indicated by the same letter, where applicable

between the rank of each variety at 3 weeks and at 9 weeks, suggesting that the seedlings may be used to estimate the response of the older plants.

The existence of variation in response to allelochemicals among flax and barley varieties grown under uniform conditions indicates a probable genetic component to this response. If so, it should be possible to manipulate plant responses to allelochemicals. This suggests that it should be possible to identify or select for crop varieties with an increased tolerance to the allelochemicals produced by weeds. Such an increased tolerance might be expected to allow the crop plant better growth and productivity in the presence of weeds. Identification of tolerant and susceptible varieties would also allow genetic methods to be used in the study of allelopathy, possibly allowing more accurate estimates of the relative contributions of allelopathy and competition to weed interference with crop plants, a long-standing problem in this area (Putnam and Tang 1983; Qasem and Hill 1989).

Several factors might be manipulated to increase crop tolerance of weeds and decrease the demand for use of often toxic herbicides. Some do not involve allelopathy. Plant architecture, rate of early growth, general growth habit, and competitiveness all differ between varieties and should be susceptible to selective breeding.

Two genetic approaches to the manipulation of allelopathy may be used (for various other approaches, see Einhellig and Leather 1988). One is to increase the toxicity of the crop plant to weeds, as successfully demonstrated by Putnam and Duke (1974) and Lockerman and Putnam (1979). Several cucumber accessions which as seedlings reduced the germination and growth of several weeds were identified (Putnam and Duke 1974). Leachate of these accessions reduced the germination and fresh weight of proso millet (*Panicum miliaceum*) by up to 66% and 79%, respectively (Lockerman and Putnam 1979). In the field, accession PI169391 reduced the total number of weeds by 50% and their fresh weight by 69%, relative to a control cv 'Pioneer'. 'PI169391' was more successful in the presence of weeds, although not superior in relative growth, net assimilation rates or leaf area ratio (Lockerman and Putnam 1981). Genetic variation in allelochemical production has also been identified in soybean (Massantini et al. 1977) and oats (Fay and Duke 1977). The difficulties of this approach are that it might make edible parts of the plant distasteful or toxic or that allelochemicals left in or on the soil might injure the following crop, as frequently occurs with crops such as rye (*Secale cereale*) (Barnes et al. 1986) and soybean (Huber and Abney 1986), particularly with no-till methods.

The use of crop varieties with increased tolerances of weed allelopathy avoids these difficulties. It might introduce others such as decreased general tolerance to pathogens. No study has been made of these or other possible disadvantages. However, it appears to us that this approach may have utility in the development of crop varieties that are more tolerant of the allelochemicals produced by weeds or residues of previous crops. It avoids injury to following crops and is assayable on small numbers of seedlings in the absence of weeds.

It would also be possible for plant breeders to take advantage of any of these factors, without discriminating among them, by growing and examining cultivars under development in the presence of a select company of their commonest weeds.

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