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Phytotoxic Effects of Wheat Extracts on a Herbicide-Resistant Biotype of Annual Ryegrass (*Lolium rigidum*)

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Thirty-nine wheat accessions were used to evaluate their extract phytotoxicity against annual ryegrass (*Lolium rigidum* Gaud.). Aqueous extracts of wheat shoot residues significantly inhibited the germination and root growth of a biotype of annual ryegrass resistant to herbicides of acetyl CoA carboxylase inhibitors (group A), acetolactate synthase inhibitors (B), photosystem II inhibitors (C), and tubulin formation inhibitors (D). The germination of the herbicide resistant (HR) biotype was inhibited by 3−100%, depending upon the wheat accession. The phytotoxic effects on ryegrass root growth ranged from 12% stimulation to 100% inhibition, compared to a control. The germination and root growth of a herbicide-susceptible (HS) biotype of annual ryegrass were also inhibited by the wheat extracts, with germination inhibited by 4−100%, and root growth by 19−100%. Bioassays with two known wheat allelochemicals showed that *p*-coumaric acid and propionic acid significantly inhibited the growth of both HR and HS biotypes of annual ryegrass. The two compounds completely inhibited the root growth of HR ryegrass at concentrations greater than 5.0 mM. In comparison with *p*-coumaric acid, propionic acid was more inhibitory to seed germination, shoot, and root growth of both ryegrass biotypes. The root growth of the HR biotype was more sensitive when exposed to wheat extracts, to *p*-coumaric acid, and to propionic acid. The results suggest that residues of certain wheat cultivars with strong allelopathic potential could provide a nonherbicidal alternative for the management of herbicide-resistant weed species.

KEYWORDS: Allelopathy; wheat (*Triticum aestivum* L.); *p*-coumaric acid; propionic acid; annual ryegrass (*Lolium rigidum* Gaud.); herbicide resistance

INTRODUCTION

Annual ryegrass (*Lolium rigidum* Gaud.) is an important grass weed of winter crops in Southern Australia. An infestation of *L. rigidum* at 200 plants/m^2 resulted in a 20−50% yield loss in wheat, costing $100/ha with a strongly competitive cultivar and $250/ha with a poorly competitive one (*1*). Biotypes of this weed have developed resistance to six major herbicide groups (*2*). High levels of herbicide resistance in *L. rigidum* were also found in the wheat belt of Western Australia (*3*). Of 264 randomly collected populations of *L. rigidum*, 46% exhibited resistance to diclofop-methyl and 64% to chlorsulfuron, with 37% exhibiting multiple resistance to both herbicides. The level of resistance to glyphosate, a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitor, has been quantitatively evaluated for annual ryegrass (*4*). The ineffectiveness of herbicides on resistant weed species and environmental imperatives have prompted the search for nonherbicidal innovations to manage weed populations (*5*).

Wheat straws and their rhizosphere soil have been reported to exhibit inhibitory effects on crops and weeds (*6*−*8*). It has been found that the aqueous extract of wheat residues is phytotoxic to a number of weeds and has consistently reduced weed emergence and growth. Under laboratory conditions, aqueous extracts from wheat straw are inhibitory to a broad spectrum of weed species (*6*, *9*, *10*). Steinsiek et al. (*9*) reported that weed species differed in their responses to the wheat extracts, with ivyleaf morning glory (*Ipomoea hederacea* (L.) Jacq.) and velvetleaf (*Abutilon theophrasti* Medic.) being inhibited the most, and Japanese barnyard millet (*Echinochloa crus-galli* var. *frumetaceae* (Roxb.) Link], pitted morning glory (*Ipomoea lacunosa* L.), and sicklepod (*Cassia obtudifolia* L.) the least. Liebl and Worsham (*6*) showed that an aqueous extract of wheat straw reduced the germination and root length of pitted morning glory (*I. lacunosa* L.) and common ragweed (*Ambrosia artemisiifolia* L.). Rambakudzibga (*10*) found that aqueous wheat extract inhibited the germination of *Amaranthus hybridus*, *Nicandra physalodes*, *Chenopodium album*, *Acanthospermum hispidum*, *Urochloa panicoides*, *Tagetes minuta*, and *Portulaca oleracea*. The phytotoxic effects of wheat straws on weeds have also been demonstrated in the field (*11*, *12*). Thilsted and Murray (*11*) found that the inhibition of *Amaranthus* spp. in wheat straw-

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mulched plots was approximately equivalent to that obtained with herbicides in straw-mulched and bare-soil plots. Banks and Robinson (*12*) also reported that straw mulch suppressed the growth of spiny amaranth (*Amaranthus spinosus* L.), tall morning glory (*Ipomoea purpurea* (L.) Roth), and volunteer wheat more than herbicides used on nonmulched areas.

Bioassays with aqueous plant extracts have been employed to investigate cultivar differences in the phytotoxicity of rice (*Oryza sativa* L.) (*13*). These workers found a wide range of variation in the inhibitory effects of aqueous extracts in 100 rice accessions, using lettuce as a test species. In wheat, Guenzi et al. (*14*) detected differential phytotoxicity of wheat straw-water extracts of nine cultivars (*T. aestivum*). Wheat cultivars differed significantly in inhibition of wheat seedling shoot growth, ranging from 11% for cv. Nebred to 36% for cv. Omaha, while cv. Ponca significantly depressed germination of wheat. Wu et al. (*15*) found that the exudates of living wheat seedlings inhibited the root growth of *L. rigidum* and that this inhibition differed significantly within a worldwide collection of 453 wheat accessions, with root growth of ryegrass being inhibited from 9.7 to 90.9%, depending upon the accession. However, little information is available on wheat cultivar differences in extract phytotoxicity on weed species, especially on herbicide resistant (HR) weed biotypes.

The aims of this research were (1) to determine wheat cultivar differences in phytotoxic effects of shoot extracts against the germination and growth of a biotype of *L. rigidum* resistant to herbicides of acetyl CoA carboxylase inhibitors (group A), acetolactate synthase inhibitors (group B), photosystem II inhibitors (group C), and tubulin formation inhibitors (group D); (2) to determine the differential responses of HR and herbicide susceptible (HS) biotypes of *L. rigidum* to wheat extracts and two allelochemicals, (3) to determine any correlation of wheat extract allelopathy with wheat total phenolic contents, and (4) to study the effects of two allelochemicals identified from wheat on the germination and growth of the HR biotype of *L. rigidum*.

MATERIALS AND METHODS

Collection of Wheat Material and Seeds of Annual Ryegrass. A worldwide collection of wheat accessions has been found to differ significantly in their competitive abilities against the growth of annual ryegrass (*L. rigidum*) in the field (*16*). Thirty-nine wheat accessions from the collection with varied competitive abilities were chosen for the evaluation of phytotoxic effects of wheat straw on the growth of this weed. The experimental soil was a red brown earth of 30% clay, 25% silt, and 45% sand, with an effective cation exchange capacity of 6.5 meq (100 g soil)$^{-1}$, as determined using a $BaCl_2$ extractant, an aluminum content of 0.1 meq (100 g soil)$^{-1}$, 1.8% of organic matter, and a pH of 4.6 (1:5 soil/0.01 M $CaCl_2$) (*16*). Shoots of 39 wheat cultivars near maturity were collected from the field. Spikes were discarded, and leaves and stems were combined. Two biotypes of annual ryegrass were included. One was the HR biotype and the other was an HS biotype. Seeds of the HR biotype were obtained from Mr Peter Baines of the Farrer Centre for Conservation Farming, Charles Sturt University, Australia, and seeds of the HS biotype were obtained commercially.

Preparation of Aqueous Extracts from Wheat Residues. A bioassay procedure previously developed by An et al. (*17*) was employed with slight modification. Wheat shoot residues were oven-dried at 40° C for 72 h and ground to pass through a sieve of 0.25 mm. Ten grams of residue powder from each wheat cultivar were extracted with 100 mL of distilled water in a glass jar for 48 h at 20° C. The mixture was filtered through four layers of cheesecloth, and the resulting filtrate was centrifuged at 10 000 rpm for 15 min at 10° C. The supernatant was then vacuum-filtered through one layer of microfilter paper (Whatman, 0.25 μm). The sterilized filtrate, designated as full strength (100%), was collected and stored in a freezer prior to use.

Bioassay With a Concentration Series. Preliminary studies showed that extracts of full strength (100%) were too toxic to detect cultivar differences in phytotoxic effects. Wheat cv. Mitchell was then chosen for its moderate phytotoxicity found in preliminary studies. A concentration series was made up from the extract of cv. Mitchell (full strength, 100%) into 100, 75, 50, 25, 12.5, and 0% (water control). The HS biotype of annual ryegrass was used as a test species due to the limited availability of HR biotype seeds. Thirty seeds of HS ryegrass were sown onto 9-cm Petri dishes lined with one layer of Whatman No.1 filter paper. Five mL of each concentration were delivered to each Petri dish. Each Petri dish with its cover was sealed with a piece of Parafilm to reduce evaporation. All dishes were maintained in a tissue culture room at 23° C with fluorescent lights for 24 h and were arranged in a randomized complete block design with four replicates. Germinated seeds with > 1 mm radicle were recorded and root lengths measured after 7 days of incubation.

Bioassay With Extracts from 39 Wheat Cultivars. The phytotoxic effects of aqueous wheat extracts were evaluated using the HR biotype of *L. rigidum* as a bioassay species. The HS biotype of *L. rigidum* was included for comparison. Five mL of each extract (1/3 of the full strength) from 39 wheat cultivars was delivered to each Petri dish, and distilled water (5 mL) was used as a control. Thirty seeds of *L. rigidum* were sown onto 9-cm Petri dishes lined with one layer of Whatman No. 1 filter paper. The management of Petri dishes and measurements were as previously described.

Determination of Total Phenolics. The total phenolic contents of each wheat extract were determined by the Folin-Ciocalteu method as described by An (*18*), with vanillic acid as the standard. Ten mL of 1% strength of each aqueous extract was pipetted into 200 mm × 25 mm test tubes, followed by the addition of 1.5 mL of 20% Na_2CO_3 and 0.5 mL of Folin-Ciocalteu reagent. The solutions were shaken immediately, mixed well, and allowed to stand for 1 h at room temperature (20−25°C) for the reaction to complete. The absorbance of each solution was determined at 750 nm against a blank (distilled water) on a HITACHI U-1100 spectrophotometer. A standard calibration curve was obtained from solutions of 0.5, 2.0, 4.0, 6.0, 8.0, and 10.0 μg/mL vanillic acid. The total phenolics in each wheat extract was then calculated from the calibration curve. Units of total phenolic contents were expressed in micrograms of vanillic acid equivalents per mL of extract.

Bioassay with Two Known Allelochemicals from Wheat. Phenolic acids and short chain fatty acids have been reported as allelochemicals associated with wheat allelopathy (*19*−*21*). Because of their dominance and relative toxicities, *p*-coumaric acid and propionic acid from the two chemical groups, respectively, were chosen to investigate their phytotoxicity on the germination and growth of both HR- and HS-biotypes of *L. rigidum*. Concentrations of standard compounds at 0.1, 1, 2.5, 5, and 10 mM were prepared with methanol (HPLC grade) for *p*-coumaric acid, and with distilled water for propionic acid. To assess the phytotoxicity of *p*-coumaric acid, 5 mL of varying concentrations of *p*-coumaric acid in methanol solution was applied to each Petri dish lined with one layer of Whatman No. 1 filter paper. After the solvent was evaporated, 5 mL of distilled water was added to the Petri dish prior to the sowing of 30 seeds of each biotype onto each Petri dish. To assess the phytotoxicity of propionic acid, 30 seeds of each biotype were sown on the filter paper in the Petri dish, and 5 mL of each propionic acid solution was delivered to each Petri dish. Distilled water (5 mL) was included as a control, along with the standard compounds. The management of Petri dishes and measurements were the same as those mentioned above.

Statistical Analysis. Experimental data were subjected to analysis of variance using Genstat 5 (Release 3.2), and treatment means were tested separately with least significant difference (lsd) at a 5% level of probability where appropriate. The I_{50} value was determined as the concentration of allelochemicals required to cause 50% inhibition in germination and growth of annual ryegrass. Percentage of inhibition on seed germination and root growth was calculated as ((control − raw data)/control) × 100.

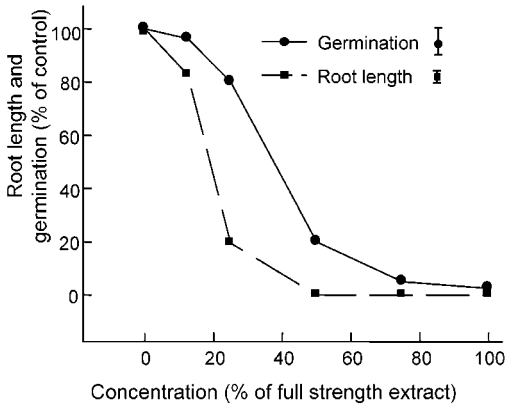

Figure 1. Dose effects of aqueous wheat extracts (cv. Mitchell) on the germination and root growth of HS ryegrass biotype. Bars are the lsds at 5% level of probability for germination and root length, respectively.

RESULTS

Dose Effect of Aqueous Wheat Extract on Root Growth of Annual Ryegrass. Initial research with full-strength extract from wheat residue indicated that the 100% concentration was so toxic that cultivar differences in the inhibition of the germination and growth of HS annual ryegrass could hardly be detected. At this 100% concentration level, 24 out of the 39 wheat genotypes completely suppressed the germination and growth of HS ryegrass, and the remaining 15 wheat genotypes significantly inhibited the germination and growth, with an average germination of 7%, compared to a control of 71%. The successfully germinated seeds of ryegrass were only able to grow to an average root length of 4 mm, compared to the control length of 75 mm. To find a concentration level that allowed sufficient germination and growth of ryegrass, a concentration series was made from the full strength extract of Mitchell wheat. Results revealed that both germination and root growth of ryegrass were inhibited by the extract of wheat cv. Mitchell (**Figure 1**). The extract phytotoxicity was enhanced by increased extract concentration. The root growth of ryegrass was completely inhibited at concentrations over 50% of the initial full strength. Root growth of ryegrass was more sensitive to the extracts than seed germination. Twenty percent of ryegrass seeds were able to germinate at 50% concentration, while the root growth was completely suppressed at the same concentration level. The I_{50} was calculated as 22% for root elongation and 38% for the germination.

Phytotoxic Effects of Wheat Extracts Against Annual Ryegrass. Phytotoxicity of wheat aqueous extracts (1/3 of the full strength) differed significantly among cultivars. Of the 39 wheat cultivars tested, 16 accessions significantly reduced HR ryegrass root growth by more than 80%, and five accessions by less than 50%. Two accessions, Angus (#38 in **Figure 2**) and Jing Hong (#39), stimulated the root growth, with a length of 47.7 and 49.0 mm, respectively, compared to a water control of 43.7 mm (**Figure 2**). Seed germination of HR ryegrass was inhibited by 3−100% depending upon the accession (data not shown). The phytotoxicity of wheat extracts was more pronounced against root growth than seed germination of ryegrass.

To determine the differential responses of HR- and HS-biotypes of annual ryegrass to wheat extracts, the HS biotype was included as a reference. Results showed that 10 wheat accessions significantly reduced HS ryegrass root elongation by more than 80%, while 24 accessions gave less than 50% root length reduction in ryegrass (**Figure 2**). The aqueous extracts of Sunbri (#3) and Kallalac (#14) not only significantly inhibited seed germination but also coincided with the strong inhibition over the root elongation of HS ryegrass, with a length of 2 and 1 mm, respectively, in comparison with a water control of 77 mm.

The root growth of HR and HS biotypes of annual ryegrass had differential responses to the aqueous extracts of wheat straw. Twenty-three wheat accessions produced an aqueous extract that was more inhibitory to the HR biotype than the HS biotype. However, twelve accessions were more inhibitory to HS biotype than the HR one. For example, the accessions Kharchia (#33) and Heron (#37) significantly inhibited the root growth of HS ryegrass by 96 and 94%, respectively, while the inhibition on HR ryegrass was only 47 and 16%, respectively. On the other hand, both HR and HS biotypes had similar responses to the aqueous extracts of four wheat accessions (i.e., Yallaroi (#1), Purplestraw (#25), Janz (#34), and Swift (#35)). The aqueous

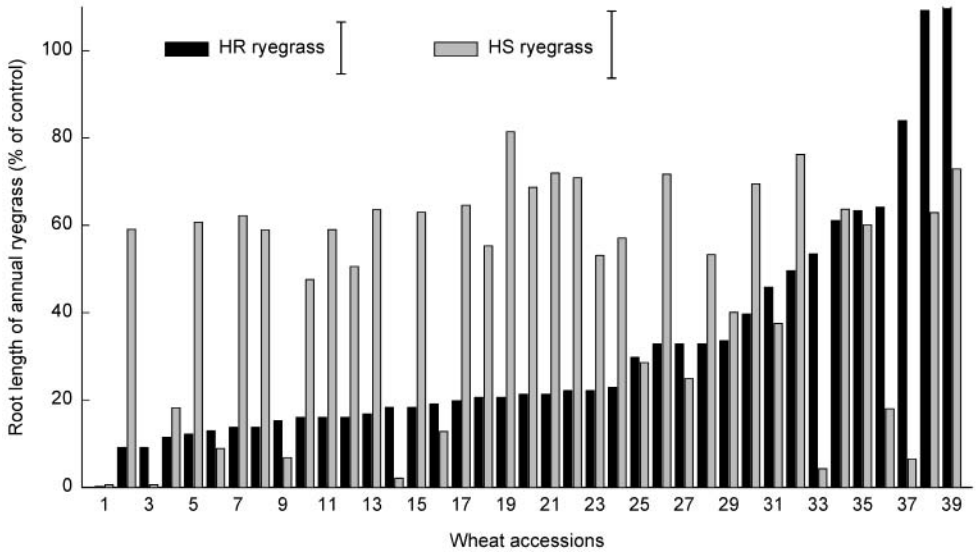

Figure 2. Root response of annual ryegrass to the aqueous shoot extracts of 39 wheat accessions. The wheat accessions from 1 to 39 were Yallaroi, V743, Sunbri, Mitchell, Sunelg, Currawong, Hartog, Peck, Rosella, BD 9–1, Warbler, Batavia, Triller, Kallalac, Co 2532–904, Pulsar, C-Millewa, Shrike, Vulcan, Oxley, Robin, Federation, LH 31, Wyuna, Purplestraw, Dollarbird, Katunga, Sunco, 22917, Rac 710, Sunstate, Gutha, Kharchia, Janz, Swift, Owlet, Heron, Angus, and Jing Hong, respectively. Bars are the lsds at 5% level of probability for HR and HS ryegrass, respectively.

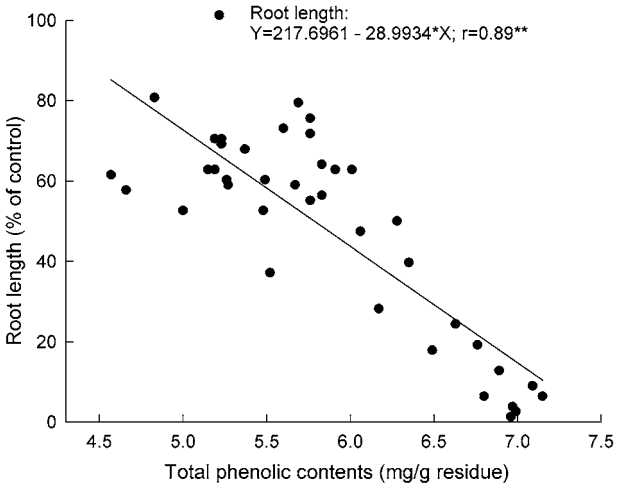

Figure 3. Effects of total phenolic contents in wheat extracts on the germination and root growth of HS ryegrass.

extracts from accession Yallaroi completely inhibited germination and root growth of both HR and HS biotypes of annual ryegrass. The average inhibition of wheat extracts on root growth was 68 and 54% for the HR and HS biotypes, respectively.

Total Phenolic Content and Phytotoxicity of Wheat Extract. Total phenolic contents varied significantly with wheat accessions, ranging from 4.59 to 7.15 mg of vanillic acid equivalents per gram of wheat residues. The phytotoxicity of a particular wheat extract was significantly correlated with its total phenolics when HS ryegrass was tested (**Figure 3**). There was a significant linear relationship between the total phenolic content contained in wheat extracts and the root growth of HS ryegrass ($Y = 217.6961 - 28.9934 \times X$; $r = 0.89$), although no significant correlation was found with the HR ryegrass biotype. Root growth of HS ryegrass was more inhibited by the extracts containing higher amounts of total phenolics. These results indicate that phenolic compounds might be involved in the phytotoxicity of wheat residue against the growth of HS annual ryegrass.

Phytotoxic Effects of *p*-Coumaric and Propionic Acids on Annual Ryegrass. The phytotoxic effects of *p*-coumaric acid on the HR and HS ryegrass biotypes are illustrated in **Figure 4**. *p*-Coumaric acid stimulated the germination of HR ryegrass at the lowest concentration of 0.1 mM, while germination inhibition was found at concentrations greater than 1.0 mM (**Figure 4a**). This compound significantly inhibited the growth of HR ryegrass, with root growth being inhibited from 22 to 100%, and shoot growth from 8 to 84%, depending upon the concentration. The root growth was completely inhibited at concentrations in excess of 5.0 mM. Similar results were also obtained when HS ryegrass was tested with *p*-coumaric acid. The germination and growth of the HS ryegrass biotype were

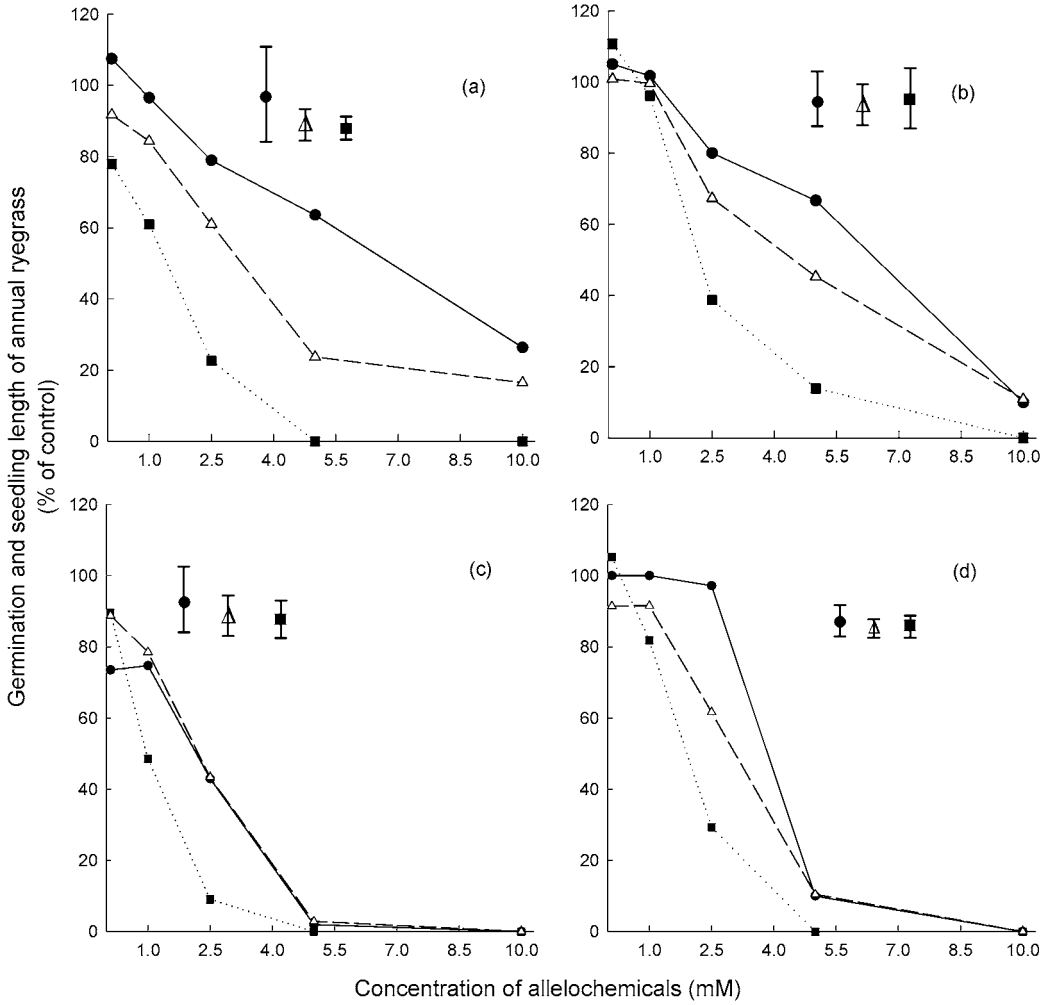

Figure 4. Responses of HR- and HS biotypes of annual ryegrass to the treatments of two known allelochemicals. (a) HR biotype and *p*-coumaric acid, (b) HS biotype and *p*-coumaric acid, (c) HR biotype and propionic acid, (d) HS biotype and propionic acid. Bars are the lsds at 5% level of probability for germination, shoot, and root length, respectively. –●– = germination, –△– = shoot length, ···■··· = root length.

Table 1. I_{50} of *p*-Coumaric and Propionic Acids for the Germination and Growth of Both Biotypes of Annual Ryegrass

	p-coumaric acid			propionic acid		
biotypes	germination	shoot	root	germination	shoot	root
HR	6.9	3.3	1.5	2.2	2.3	0.9
HS	6.5	3.1	2.2	3.8	3.1	1.9

stimulated in the presence of *p*-coumaric acid at a concentration of 0.1 mM, and were inhibited at concentrations greater than 1.0 mM (**Figure 4b**). Seed germination of both biotypes of annual ryegrass was the least sensitive to *p*-coumaric acid, while the root growth was the most sensitive.

Propionic acid, one of the fatty acids identified in wheat residues, also significantly inhibited the germination and growth of both HR- and HS-biotypes of annual ryegrass (**Figure 4c**,**d**). The root growth of both biotypes was significantly inhibited at concentrations over 1.0 mM in comparison with a water control. Complete suppressions were found at concentrations greater than 5.0 mM.

The concentrations of the two acids required to cause 50% inhibition on germination, root, and shoot growth of both biotypes of annual ryegrass are presented in **Table 1**. Higher values of I_{50} were obtained with *p*-coumaric acid than with propionic acid, demonstrating that propionic acid was more inhibitory to the germination, root, and shoot growth of both HR and HS biotypes. On the other hand, the I_{50} of *p*-coumaric acid was higher for the root growth of the HS biotype than that of the HR biotype. Similarly, a higher I_{50} of propionic acid was required to inhibit the germination, root, and shoot growth of the HS biotype than those of the HR biotype. These results indicated that the HR biotype of ryegrass was more sensitive to these allelochemicals than the HS biotype.

DISCUSSION

Over-reliance on synthetic herbicides for weed control in various farming systems has resulted in the rapid development of herbicide resistance in weeds. Globally, at least 276 weed biotypes, 166 weeds species, including 99 dicots and 67 monocots, have now been reported to have acquired resistance to important herbicides (*22*). Exploitation of allelopathy in integrated weed management might reduce the dependence on herbicides and extend the commercial life of valuable chemicals (*23*). In this study, it was also found that aqueous extracts of wheat shoot tissues significantly inhibited the germination and root growth of both HR and HS biotypes of annual ryegrass and that the levels of inhibition varied with the cultivar. In a similar study, Przepiorkowski and Gorski (*24*) also demonstrated that shoot extracts of rye (*Secale cereale*) and soils containing rye roots inhibited not only the germination and growth of triazine susceptible biotype, but also the resistant biotypes of barnyard grass (*Echinochloa crus-galli* L.), horseweed (*Conyza canadensis* L.) and willowherb (*Epilobium ciliatum* Rafin).

Osmotic pressure, pH and conductivity have been reported to partly contribute to the observed phytotoxicity of aqueous plant extracts, particularly when a highly concentrated extract was used (*25*). However, Kimber (*26*) reported that extract toxicity was not due to toxic levels of salts being leached from the straw, as maximum toxicity was observed where the conductivity scarcely exceeded 3 mmhos/cm. Guenzi and McCalla (*27*) estimated that only 3−8% of the inhibiting effects could be ascribed to salts. Although the osmotic pressure was not measured in this study, the diluted 1:30 solutions had an electrical conductivity over a range of 1.4−2.5 mS/cm, and a pH value between 4.7 and 7.4 across the wheat accessions. These values are within the acceptable limits for normal germination and seedling growth (*26*, *28*). In addition, it was found that the phytotoxicity of straw extracts did not correlate well with either the values of conductivity or the pH. Lodhi et al. (*20*) demonstrated that germination and seedling growth of cotton and wheat were significantly affected by the buffered and nutrient-rich solutions added with extracts of wheat mulch and soils collected from the wheat field, which clearly indicated the presence of allelochemicals.

There are reports that wheat straw contains phenolic compounds, and these compounds have shown phytotoxic effects on crops and weeds (*14*, *20*, *21*, *29*). Guenzi et al. (*30*) reported that *p*-coumaric acid at a range of concentrations between 3.8 and 30.5 mM inhibited root growth of wheat seedlings over a range of 87−41%. Lodhi et al. (*20*) reported that *p*-coumaric acid inhibited the radicle growth of radish (*Raphanus sativa* L.) at concentrations of 0.1−1.0 mM. Individual phenolic acids also reduced radicle and hypocotyl length of crimson clover (*Trifolium incarnatum* L.) and ivyleaf morning glory (*Ipomoea bederacea* L.) (*31*). The present study showed that *p*-coumaric acid inhibited the growth of HR and HS biotypes of annual ryegrass at concentrations over 1.0 mM.

The research presented here has shown that seed germination and root elongation of HS ryegrass were more inhibited by those wheat extracts with higher amounts of phenolics. Ben-Hammouda et al. (*32*) also found that the allelopathic potential of sorghum plant parts was positively correlated with total phenolic content. An (*18*) reported that phenolics were the responsible agents for the allelopathic effects of *Vulpia myuros* residue on tested species. However, although there was a strong correlation between wheat extract phytotoxicity and its total phenolic content, care must be taken in interpretation. The present study showed that the regression equation only accounted for 79% of the variance for root elongation. In addition, no correlation was found between the total phenolic content and the allelopathic effects of wheat extracts on root growth of HR ryegrass. These results imply that phenolics are not the only category of chemicals involved in the phytotoxicity of wheat residue. Fatty acids, hydroxamic acids, coumarins, and other bioactive compounds have also been reported as responsible allelochemicals in wheat (*21*, *33*, *34*). Phytotoxic effects of wheat straw on annual ryegrass may therefore involve other compounds than those examined in this study.

The phytotoxicity of fermented suspensions of wheat straw was associated with the formation of propionic, acetic, and butyric acids under anaerobic conditions (*19*, *35*). The relative toxicity of propionic and butyric acids was about 5−6 times that of acetic acid (*36*). Propionic acids at concentrations over 1.0 mM significantly inhibited the root growth of wheat. Synergistic effects among these fatty acids, such as propionic, acetic, and butyric acids were reported (*37*). The present study showed that propionic acid significantly inhibited the root growth of HR biotype of annual ryegrass over a range of 11−100%, depending on the concentration. The root growth of HS biotype was also affected, ranging from 5% stimulation to 100% inhibition. In comparison with *p*-coumaric acid, propionic acid was more inhibitory to germination, shoot, and root growth of the two biotypes. Little information is available on the real concentration of fatty acids in the field. Tang and Waiss (*19*) found that the amounts of acetic, propionic, and butyric acids were 20.3, 1.3, and 5.9 μmol/mL straw extract at 12 days of incubation in water. Lynch et al. (*35*) reported that the acetic

acid concentration of freshly harvested straw was 384 mM before absorption of soil moisture, and the acid was rapidly diluted within 6 h of incorporation into the soil, where the concentration fell to about 10 mM.

Allelochemical content in the soil varies greatly from place to place and year to year (*7*). The amounts of phenolic compounds in the soil depend on many biotic and abiotic factors, such as cultivation, climatic condition, timing of assessment, quantity of wheat straw retained, cultivars, placement and degree of decomposition, microbial activity, nutrient status, and other physical parameters (*25*). *p*-Coumaric acid in the field occurs in the highest amount in comparison with other phenolic acids under wheat no-till systems, and its concentration in the top 2.5 cm of soil is about 4 mg/kg soil (*38*). The amount of *p*-coumaric acid has been estimated at 684 kg/ha in the field, where wheat stubbles were retained (*20*). Siqueira et al. (*39*) estimated that as much as 100−120 kg/ha of phenolics can be added into the soil every year. The concentration of total phenolic acid in the top 2.5 cm of soil under a wheat no-till system was 12.3 mg/kg soil (*38*). Opoku et al. (*8*) also reported that the total amount of phenolic compounds was 11.5 mg/kg of soil after 42 days of surface straw application.

The concentration of each individual chemical required to produce phytotoxic effects in this study appears to be relatively high compared with the concentrations found in the soil. It is unlikely that any one particular compound would be responsible for reduced weed growth in the field. However, higher plants and microorganisms produce not only phenolic compounds and fatty acids, but also other chemically distinct compounds. These compounds might act additively or synergistically in the complex mixture (*40*, *41*). If these compounds are present in the right combination and concentration at a given locality, phytotoxic effects may be observed (*6*). In addition, phytotoxic substances are not evenly distributed in the soil, and they might reach the toxic levels in localized pockets of decomposing residue fragments (*6*, *42*). It is possible that relatively high concentrations of allelochemicals may concentrate at or near the soil surface along with the stubble retention, as such chemicals would be continually leached from the surface straw into the soil by rainfall to affect the growth of weeds in the vicinity, especially on poorly drained soils (*9*). The surface straw might also be used as substrates by soil microbes. Microbial decomposition of wheat straw might produce a variety of secondary metabolites. It has been reported that microbially transformed chemicals can be more potent than their precursors (*6*, *43*).

Crop residue allelopathy could provide a nonherbicidal option for the integrated management of herbicide resistant weed species. It would normally be anticipated that the HS biotype is more sensitive to herbicide applications when compared to a HR biotype. However, this study has demonstrated that the root growth of the HR biotype was the more sensitive when exposed to wheat extracts, to *p*-coumaric acid, and to propionic acid. About 59% of the wheat accessions inhibited the root growth of HR biotype more than the HS biotype, while 31% of the accessions were more inhibitory to the HS biotype than that of HR. The average inhibition of 39 wheat accessions on root growth of annual ryegrass was 68% for the HR biotype and 52% for the HS biotype. Similarly, Hensley and Counselman (*44*) reported that seedling growth of a triazine resistant biotype of redroot pigweed (*Amaranthus retroflexus* L.) was more suppressed in the presence of allelochemicals than its susceptible counterpart. However, no significant difference in sensitivity of germination and growth was found between triazine-resistant and susceptible biotypes of *E. crus-galli*, *C. Canadensis*, and *E. ciliatum* when exposed to rye shoot extracts or to the soil mixed with rye roots (*24*). It appears that use of wheat residue allelopathy could be effective in managing both HR and HS biotypes. In addition, the exploitation of allelochemicals in the residues from strongly allelopathic cultivars could be of particular value for the management of the HR biotype because of its high sensitivity to allelochemicals.

The accumulation of phytotoxic compounds near the soil surface coincides with the fact that most weed seedlings emerge from the top soil layer. The phytotoxic substances leached out from retained crop stubbles might not be lethal, however, they may retard and suppress weed seedling growth, especially when weed seedlings are small and young roots are in the vicinity of clumps of straw. The weed growth inhibition at this seedling stage would reduce weed competitiveness against crop seedlings. As a result, the new addition of seeds into the soil by the weed plants of reduced competitiveness would be minimal, thereby decreasing the size of the soil seedbank and reducing weed infestation to the following crops. Retained stubbles may also have impact on seedbank dynamics via altered microbial activities. Increased microbial activities associated with stubble retention might accelerate the aging of the soil seedbank. Long-term studies and simulation modeling are underway to understand how weed growth, seedbank, and weed species distribution might be affected by the common practice of retaining crop stubbles under conservation farming systems. The phytotoxic effects of other phenolic compounds and short chain fatty acids are being investigated.

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