DIFFERENTIAL ALLELOPATHIC POTENTIAL AMONG WHEAT ACCESSIONS TO ANNUAL RYEGRASS

H. Wu[1], J.E. Pratley[1], D. Lemerle[2], T. Haig[3] and B. Verbeek[2]

[1]Farrer Centre for Conservation Farming, Charles Sturt University, Wagga Wagga, NSW 2678
[2]NSW Agriculture, Agricultural Research Institute, Wagga Wagga, NSW 2650
[3]School of Science & Technology, Charles Sturt University, Wagga Wagga, NSW 2678

Abstract

Thirty-eight wheat cultivars (*Triticum aestivum* L.) and one durum wheat (*Triticum durum* Desf.) were used to evaluate the differential allelopathic potential against annual ryegrass (*Lolium rigidum* Gaud.) by an extract bioassay . Substantial variation in allelopathic potential to the ryegrass was detected in the collection. Both germination and radicle growth of ryegrass were significantly inhibited by the aqueous shoot extracts of wheat cultivars. The inhibition for radicle elongation ranged from 19.2% to 98.7% and for seed germination from 4.2% to 73.2%. Six wheat cultivars suppressed the radicle elongation of ryegrass by more than 90%. Among these six cultivars, three inhibited seed germination by 66-73%. The total pheno lic content in wheat tissues varied with the cultivar, ranging from 4.59 to 7.15 mg vanillic acid equivalents per gram of wheat residue. Allelopathic inhibition of ryegrass radicle elongation was positively correlated with total phenolic acids containe d in each wheat extract (R=0.846**).

Key words: Allelopathy, wheat (Triticum aestivum L.), annual ryegrass (Lolium rigidum Gaud.).

Research on wheat allelopathy has shown its potential for weed biological control. The phytotoxicity of water extract from wheat residues was found in the 1960s (3, 7). During the past two decades, the research has focused on the allelopathic effect s of wheat residues on target weed species. The aqueous extracts from wheat (*Triticum aestivum* L.) were allelopathic to germination and seedling growth of a number of noxious weed species (11, 16, 17). These results clearly showed that phytoto xins in wheat residues are water soluble. The toxins can be leached into the soil to influence the growth of weeds in the vicinity. The allelopathic nature of wheat residues has also been demonstrated in fields where residues are mulched on the soil su rface. Wheat straw mulch significantly inhibited emergence, seedling growth and dry matter accumulation of various weed species (6, 13, 15).

Annual ryegrass (*Lolium rigidum*) is an important grass weed of winter crops in southern Australia. An infestation of *L. rigidum* at 200 plants/m^2 resulted in 20-50% yield loss in wheat, costing $A100/ha with a strongly compet itive cultivar and $A250/ha with a poorly competitive one (9). Differential competitive abilities against *L. rigidum* were further detected in spring wheat (10), causing a variation in weed biomass from 0 to 500 g/m^2. Correspondingly , wheat yield reduction from weed competition ranged from 0-100% between genotypes. With the development of resistance of *L. rigidum* to herbicides (14), the breeding for more competitive crop cultivars should be included in any integrated weed control package (9).

The aims of this research were:

• to determine wheat varietal differences in allelopathic potential against *L. rigidum*; and,

• to determine correlation of allelopathic activity with total phenolic contents.

Material and methods

Collection of wheat material and seeds of ryegrass

Shoots of 39 wheat varieties near maturity were harvested from the field on the same day in November, 1996 at Wagga Wagga. Leaves and stems were combined after the removal of spikes. Susceptible seeds of annual ryegrass were obtained commercially.

Preparation of extracts from wheat residue

Wheat shoot residues were oven-dried at 40° C for 72 hours and were then ground to pass a sieve of 0.25 mm. Ten grams of residue powder from each wheat variety were extracted with 100 mL of distilled water in a glass jar for 48 hours at 20° ;C. The pulpy mixture was filtered through 4 layers of cheese cloth 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, 0.25mm). The sterilised filtrate, designated as full strength (100%), was collected in a plastic jar and stored in a freezer prior to use.

Bioassay with a concentration series from the extract of Mitchell wheat

A concentration series was made up from the extract of Mitchell (full strength, 100%) into 100%, 75%, 50%, 25% 12.5% and 0% (water control). Thirty seeds of ryegrass were sown onto 9 cm petri dishes lined with one layer of Whatman #1 filter paper. Five mL of each concentration were then delivered to each petri dish. To reduce evaporation, each petri dish was covered tightly with parafilm before pressing the top dish cover. All dishes were maintained in a tissue culture room at 23 °;C with fluor escent lights for 24 hours, and were arranged in a randomized complete block design with 3 replicates. Germinated seeds with > 1 mm radicle were recorded and radicle lengths measured after 7 days of incubation.

Bioassay with extracts from 39 wheat varieties

Five mL of each extract (33.33% strength) from 39 wheat varieties were delivered to each petri dish and distilled water (5 mL) was used as control. Thirty seeds of ryegrass were sown onto 9 cm petri dishes lined with one layer of Whatman #1 filter paper . The management of petri dishes and the 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 (1) with vanillic acid as the standard, because it has been identified as an allelopathic agent in wheat residues (4, 5, 12). Ten mL of 1% strength of each aqueous extract were pipetted into 200 x 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 and mixed well, and allowed to stand for 1 hour 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 o btained from solutions of 0.5, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/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 van illic acid equivalents per mL of extract.

Statistical analysis

Data were statistically analyzed and treatment means were tested separately with least significant difference (LSD) at a 5% or 1% level of probability.

Results
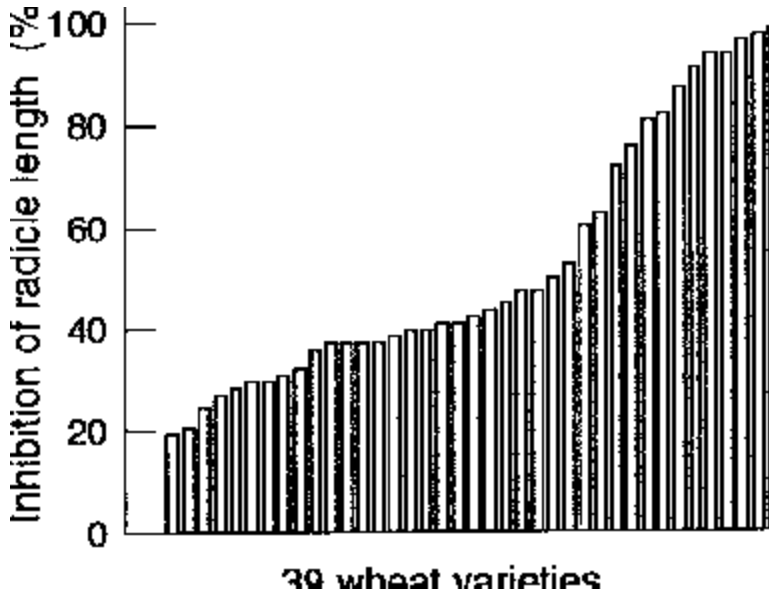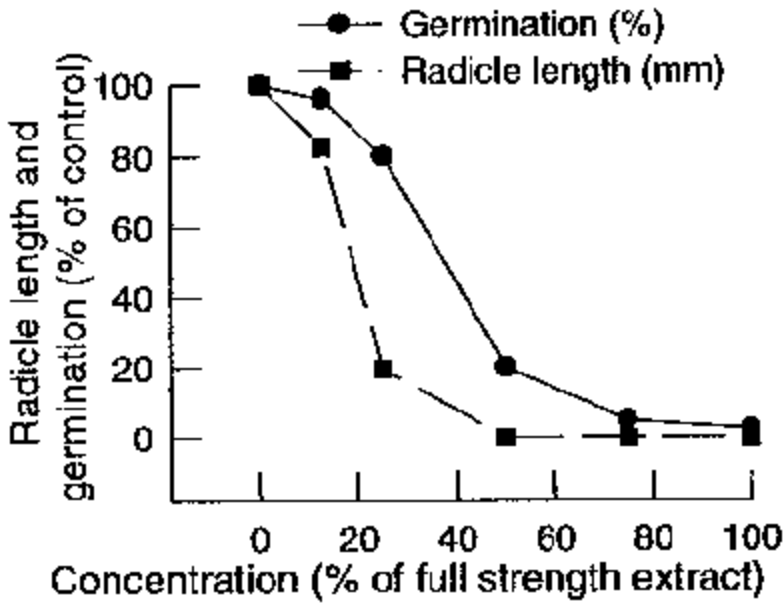
Dose effect of aqueous wheat shoot extract on root growth of susceptiannual ryegrass

Initial research with full-strength extract from wheat residue indicated that the 100% concentration was so toxic that varietal differences in the inhibition of the germination and growth of annual ryegrass could hardly be detected. At this 100% concent ration level, only 15 out of the 39 wheat genotypes actually germinated, with a maximum germination rate of 16%. The successfully e only able to grow to a radicle length ranging from 0.0 to 2.0 cm, compared to water control with a r adicle length of 6.6 cm. In order to find a concentration level that allows 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 radic le growth of ryegrass were inhibited by the extract of Mitchell wheat (Fig. 1). The extract toxicity was enhanced by increased concentration. The radicle elongation of ryegrass was completely inhibited at concentrations over 50%. Root growth of ryegras s was more sensitive to the extracts than seed germination. Twenty percent of ryegrass seeds were able to germinate at 50% concentration, while the radicle elongation was completely suppressed at the same concentration level. The I50 was calculated as 22% for radicle elongation and 38% for the germination, respectively.

Allelopathic inhibition of wheat extracts against susceptible annual ryegrass

The data indicated that the degree of phytotoxicity of the extracts differed among varieties. Of the 39 wheat cultivars tested, 15 wheat accessions significantly reduced ryegrass radicle elongation by more than 50%. However, only 6 cultivars gave more t han 90% radicle length reduction in ryegrass, with a length of 0.1-0.7 cm, compared to a control of 7.8 cm (Fig. 2 and 3). Seed germination of ryegrass was also inhibited by the aqueous wheat extracts. Three cultivars were able to inhibit seed germinati on by 66-73%, while the suppression of seed germination by the other 36 cultivars was less than 50% (Fig. 3). The three accessions were Kallalac, Currawong and Sunbri. These three accessions not only significantly inhibited seed germination, but also c oincided with the strongest inhibition over the radicle elongation of ryegrass, with a length of 0.2, 0.7 and 0.1 cm, respectively. The phytotoxicity of wheat extracts was more pronounced against radicle elongation than seed germination of ryegrass. Th e average inhibition was 52.4% against radicle elongation and 31.2% against seed germination.
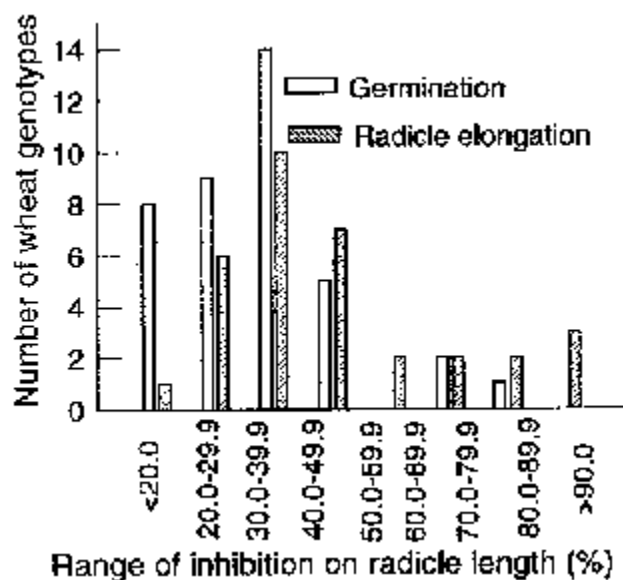

Relation between total phenolic content and allelo- pathic inhibition of wheat extract

Total phenolic contents varied significantly with wheat accessions, ranging from 4.59 to 7.15 mg vanillic acid equivalents per gram of wheat residue. The allelopathic behaviour of a particular wheat extract was highly correlated with its total phenolics . There was a significant linear relationship between the root growth of ryegrass and the total phenolic content contained in wheat extracts. Radicle elongation and seed germination of ryegrass were more inhibited by the extracts containing higher amou nts of total phenolics (Fig. 4). Results indicated that phenolics were active compounds responsible for the allelopathic effect of wheat residue against annual ryegrass.

Discussion and conclusions

Recent research has shown that six wheat accessions were strongly allelopathic against radicle growth of ryegrass and three against seed germination. The phytotoxicity of wheat extracts in inhibiting radicle elongation of ryegrass varied significantly w ith wheat cultivars, from 19.2% for Vulcan and 98.7% for Sunbri. Some wheat cultivars therefore produce more toxins than others; *eg.* Sunbri residue yielded a much more toxic extract than Vulcan. The differential phytotoxicity of wheat extracts was also reported by others. Guenzi *et al.* (5) detected differential phytotoxicity of wheat straw-water extracts of 9 varieties (*T. aestivum*). Wheat varieties differed significantly in inhibition of wheat seedling shoot growth, rangin g from 11% for "Nebred" to 36% for "Omaha", while "Ponca" significantly depressed germination of wheat. Kimber (7, 8) also demonstrated the varietal differences in the phytotoxicity of wheat residues with cv.

Gabo being the most allelopa thic. These results showed that wheat extracts inhibited not only wheat itself but also other species.
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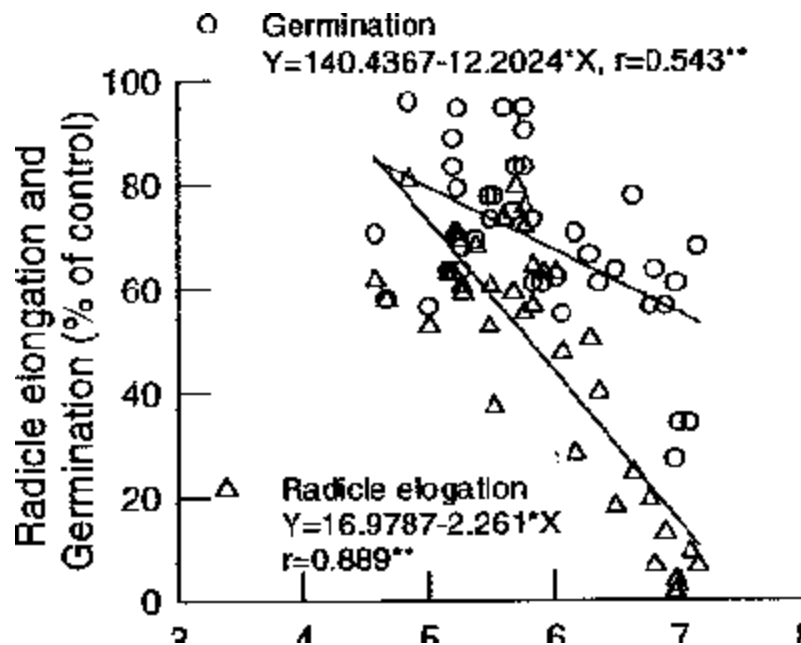
Some wheat accessions therefore possess stronger allelopathic activity than others, suggesting that allelopathy is an inherited trait. However, further research on the genetic behaviour of this allelopathic trait is needed before any development of alle lopathic wheat cultivars occurs. < lopathic wheat cultivars occurs.

Phenolic compounds are among many allelopathic agents in wheat. The research presented here has shown that seed germination and radicle elongation of ryegrass were more inhibited by those extracts with higher amounts of phenolics. Ben-Hammouda et al. (2) also found that the allelopathic potential of sorghum plant parts was positively correlated with total phenolic content. An (1) reported that phenolics were the responsible agents for the allelopathic effects of vulpia residue on tested species. With advanced HPLC, GC and GC/MS techniques, bioactive phenolic compounds, ferulic, p-coumaric, syringic, vanillic and p-hydroxybenzoic, were isolated and identified from wheat residues (4, 12, 18). The total amount of phenolic acids from wheat residues lef t on the field was estimated as 1.5 t/ac under no-tillage systems (19) and the amount of individual phenolic acid isolated from wheat residue was calculated as 941 kg/ha for ferulic acid and 684 kg/ha for p-coumaric acid (12). However, although there w as a strong correlation between wheat extract toxicity 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 radicle elongation. This resul t implies that phenolics are not the only category of chemicals involved in the phytotoxicity of wheat residue. The regression model for seed germination only explained 30% for the variance, which may be due to variable germination of the ryegrass.

Research from Guenzi *et al.* (5) and Kimber (7, 8) has revealed that autotoxicity of wheat straw and extract phytotoxicity varied between wheat varieties. In the present study, annual ryegrass was used as a test weed species to successfully scre en the differential phytotoxicity of wheat straw extracts. The phytotoxicity was found to correlate with the total phenolic contents in the extract. It may be concluded that different wheat cultivars have different allelopathic potentials, and that the exploitation of allelochemicals in the residues from strongly allelopathic cultivars would be of particular value for weed control under no-tillage farming systems. However, before any possibility of utilising wheat allelopathy in weed control, further screening of different wheat varieties and their effects on different weeds species is needed. The wheat autotoxicity could be studied in some depth so that a better residue management

package might be released to alleviate the negative impacts of whe at residue on the following crops, including wheat itself.

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